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Oxytocin Analogues Containing No Disulfide Bond. I. Synthesis of the Lactam of L-Tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide*1

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The synthesis of an analogue of deamino-oxytocin, which has an ethylene linkage in place of the disulfide bond, was studied in order to establish a general route for the synthesis of such cyclic peptides. L-α-Aminosuberic acid (Asu) was converted to the *N*-benzyloxycarbonyl-ω-*t*-butyl ester and was used as the starting substance for the synthesis. A protected octapeptide was prepared by the step-by-step elongation method with the respective benzyloxycarbonylamino acid active esters, and finally the cyclization of the ω-carboxyl group of the Asu-residue to the amino group of the tyrosyl-residue was achieved by the trifluoroacetate method. The final product showed obviously higher oxytocin-like activities than those reported previously. The conditions for the syntheses of *p*-nitrophenyl and 2,4,5-trichlorophenyl trifluoroacetates were improved during the investigation.

The twenty-membered ring in oxytocin is known to be essential for biological activities. This was demonstrated by comparing the biological activities of [mercaptoacetyl[1]]-oxytocin,[1] which has a 19-membered ring, and of [γ-mercaptobutyl[1]]-oxytocin,[1,2] (21-membered ring), with the activity of [β-mercaptopropionyl[1]]-oxytocin[3] (20-membered ring). Although [β-mercaptopropionyl[1]]-oxytocin, which is called deamino-oxytocin, is well known to show rather higher biological activities than the natural oxytocin, both the 19- and 21-membered derivatives have almost no biological activity. Furthermore, the hydrogenation of oxytocin to linear dihydro-oxytocin results in the disappearance of hormonal activities.[4]

Recently, two analogues of deamino-oxytocin (deamino-carba[1]-oxytocin and deamino-dicarba-oxytocin), in which the disulfide bonds are replaced by a methylene-thio and an ethylene linkage respectively, were synthesized by Rudinger and Jošt.[5,6] These compounds were found to exhibit weaker but definite oxytocin-like activities. These facts can be interpreted by supposing that the disulfide linkage in the 20-membered ring of oxytocin is important as a structural element, but is not essential for biological activities.

Studies along the same lines have been carried out independently in our laboratory to elucidate the relationship between the steric structure of the hormone molecule and its biological activities.[7] In the present paper, a new method for the synthesis of deamino-dicarba-oxytocin will be described. This synthesis was investigated in order to establish a widely-applicable procedure for the syntheses of such series of cyclic peptides.[8] Although Jošt and Rudinger had already succeeded in synthesizing this compound,[6] their method is not considered to be generally applicable.

Before starting the synthesis of this peptide, the *N*-benzyloxycarbonyl-L-α-aminosuberic acid ω-*t*-butyl ester (III) was synthesized from *N*-benzyloxycarbonyl-L-α-aminosuberic acid (I)[9] as is shown in Scheme 1. The first step from I to II represents an application of the Nefkens method.[10] The compound, III, thus obtained was coupled with L-prolyl-L-leucylglycine amide[11] by the tri-

*1 This work was presented at the 5th Symposium on Peptide Chemistry in Japan, Kyoto, November 23, 1967.

1) D. Jarvis and V. du Vigneaud, *Science*, **143**, 545 (1964).

2) D. Jarvis, B. M. Ferrier and V. du Vigneaud, *J. Biol. Chem.*, **240**, 3553 (1965).

3) D. B. Hope, V. V. S. Murti and V. du Vigneaud, *ibid.*, **237**, 1563 (1962).

4) E. Schröder and K. Lübke, "The Peptides," Vol. II, Academic Press, New York (1966), p. 315.

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

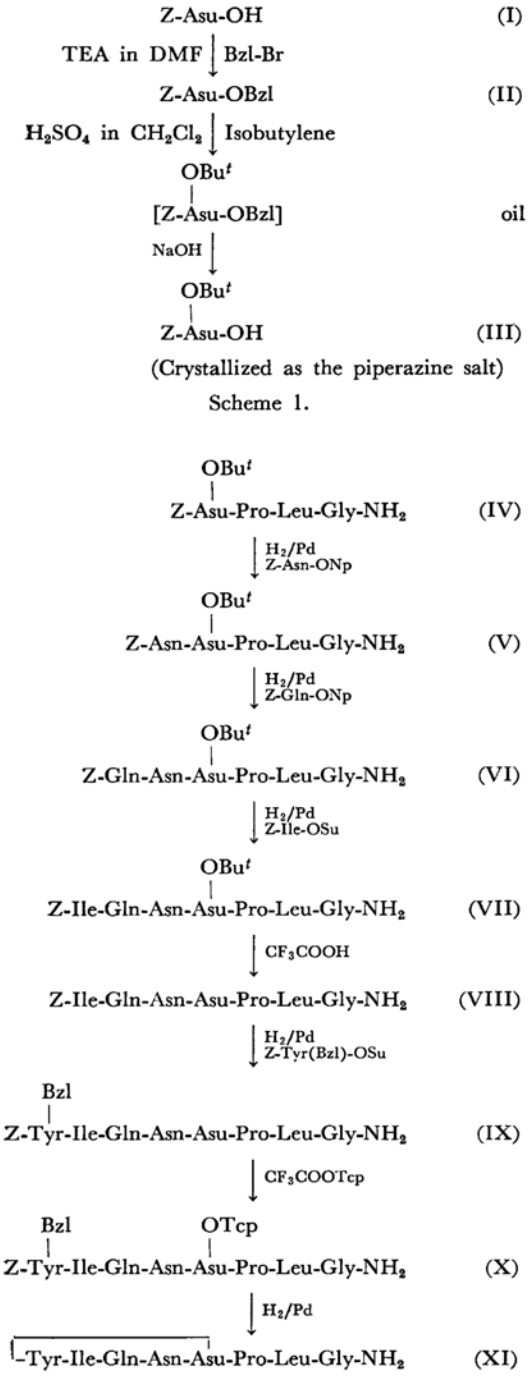
6) K. Jošt and J. Rudinger, *Collect. Czech. Chem. Commun.*, **32**, 1229 (1967).

7) S. Sakakibara, R. Kiyoi and Y. Shimonishi, Proc. 3rd Symposium on Peptide Chemistry (Japan), Inst. for Protein Research, Osaka Univ. (1964), p. 52.

8) A. Kobayashi, S. Hase and S. Sakakibara, Proc. 5th Symposium on Peptide Chemistry (Japan), Faculty of Pharmaceutical Sci., Kyoto Univ. (1967), p. 20.

9) S. Hase, R. Kiyoi and S. Sakakibara, This Bulletin, **41**, 1266 (1968).

10) G. H. L. Nefkens and R. J. F. Nivard, *Rec. trav. chim.*, **83**, 199 (1964).



Scheme 1.

Scheme 2.

fluoroacetate method[12] because the *p*-nitrophenyl ester of compound III was not crystallizable and the application of the dicyclohexylcarbodiimide method seemed undesirable. The benzyloxycarbonyl group in the tetrapeptide amide (IV) was removed by catalytic hydrogenolysis, and the peptide chain was elongated by the step-by-step addition of the respective benzyloxycarbonylamino acids using the active-ester method, as is shown in Scheme 2. The *t*-butyl ester group in the side chain of α-aminosuberic acid was removed by trifluoroacetic acid before the removal of the benzyloxycarbonyl group of isoleucyl heptapeptide amide (VIII). The protected octapeptide amide (IX) was converted to its 2,4,5-trichlorophenyl ester (X) in a good yield using the trifluoroacetate method.[12] The cyclized compound, XI, was then obtained smoothly by the catalytic hydrogenolysis of a dilute solution of X in dimethylformamide. The unchanged compound was removed by passing the aqueous solution successively through Dowex 50 and Amberlite IR-45 columns; the neutral fraction obtained was then purified by passing it through a column of Sephadex G-25, giving a homogeneous product. No dimer or polymer was detectable in the eluate after gel-filtration, as is shown in Fig. 1. The homogeneity of the final product was confirmed by paper chromatography, paper electrophoresis, amino acid analysis, and elemental analysis of the lyophilizate. The molecular weight of this compound was estimated by sedimentation equilibrium methods to be about 850, which indicates that this is a monomeric cyclic peptide.

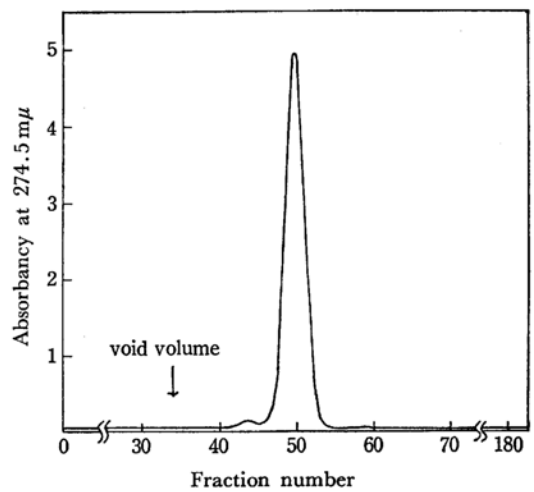


Fig. 1. Gel-filtration of the final product on Sephadex G-25. See Experimental part for details of conditions.

The biological activities of the product, XI, were determined as shown in Table 1; these values were compared with those reported by Rudinger and Jošt for the same compound[6] and with those of oxytocin and of deamino-oxytocin. This compound was found, contrary to the reported values, to

11) M. Zaoral and J. Rudinger, *Collect. Czech. Chem. Commun.*, **20**, 1183 (1955).

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

Table 1. Biological activities of compound XI[a]

Compound	Rat Uterus *in vitro*	Avian depressor	Rat pressor	Rat antidiuretic
Oxytocin[b]	486±5	507±23	3.1±0.1	2.7±0.2
Deamino-oxytocin[c]	803±36	975±25	1.44±0.06	19
Compound XI	96[d]	52[e]	<0.25[d]	1.78±0.2[e]
Compound XI[f]	9	2.5	No response with up to 20 μg/kg	0.7

a) International units per mg. b) W. Y. Chan and V. du Vigneaud, *Endocrinology*, **71**, 977 (1962). c) B. M. Ferrier, D. Jarvis and V. du Vigneaud, *J. Biol. Chem.*, **240**, 4264 (1965). d) Determined by Dr. T. Okada, Yoshitomi Pharmaceutical Ind., Ltd., Yoshitomi, Fukuoka. e) Determined by Dr. S. Yoshida, Faculty of Medicine, the University of Tokyo, Bunkyo-ku, Tokyo. f) Reported by Jošt and Rudinger: cited as Ref 6.

show about one-tenth of the biological activities of deamino-oxytocin and to show a faint rat-pressor activity, as has been reported previously.

The syntheses of *p*-nitrophenyl and the 2,4,5-trichlorophenyl trifluoroacetates used as reagents in the trifluoroacetate method were improved during the investigation. Formerly these compounds were synthesized by refluxing the respective hydroxy compounds with trifluoroacetic anhydride for a long time,[12] but there was a danger that the reaction was incomplete. In the present study the reaction was carried out under pressure in a sealed bottle and at a higher temperature. This improved procedure should be useful in routine work in the preparation of all other trifluoroacetate reagents.

Experimental[13]

Removal of the Benzyloxycarbonyl Group by Catalytic Hydrogenolysis. Hydrogen was gently bubbled through a solution of the benzyloxycarbonyl compound at room temperature, using palladium black as the catalyst. The completion of the reaction was checked by thin-layer chromatography using Merck's silica-gel G. The solvent systems used for the chromatography were chloroform: methanol: acetic acid (95: 5 : 3 v/v) for IV and V, and *n*-butanol : acetic acid: water (4 : 1 : 1 v/v) for VI, VIII, and X. After the reaction, the catalyst was removed by filtration, the filtrate was concentrated under reduced pressure, and the residue was subjected to the next reaction.

***p*-Nitrophenyl Trifluoroacetate.** A mixture of *p*-nitrophenol (75 g, 0.538 mol), benzene (130 m*l*), and trifluoroacetic anhydride (190 g, 0.904 mol) was placed in a glass pressure-vessel which had been sealed by a screw cap equipped with a Teflone stopper. The mixture was heated to 80—90°C in an oil bath for about 8 hr. Then the yellow solution was concentrated to a residue under reduced pressure, taking care to exclude moist air from the mixture. The trace of trifluoroacetic acid present was removed in a desiccator over phosphorus pentoxide *in vacuo*, and the residue was used without further purification. The yield of the reagent was quantitative.

2,4,5-Trichlorophenyl Trifluoroacetate. A mixture of 2,4,5-trichlorophenol (1.98 g, 0.01 mol), benzene (2 m*l*), and trifluoroacetic anhydride (4.20 g, 0.02 mol) was heated for 8 hr in a sealed bottle as has been described above. The reaction mixture was then concentrated under reduced pressure, and the residue was used without further purification.

Benzyloxycarbonyl-L-α-aminosuberic Acid α-Benzyl Ester (II). A solution of benzyloxycarbonyl-L-α-aminosuberic acid (I)[9] (32.3 g, 0.1 mol) and triethylamine (14 m*l*, 0.1 mol) in dimethylformamide (20 m*l*) was treated with benzyl bromide (18.8 g, 0.11 mol) at room temperature. After 20 hr, water (200 m*l*) was added to the reaction mixture, and the oil which separated was extracted twice with ethyl acetate. The combined extract was washed with water and then with a 5 % sodium bicarbonate solution. (The unchanged I was extractable from the ethyl acetate solution with a sodium bicarbonate solution, but the main product, II, remained in the ethyl acetate under the present conditions. The unchanged I was recovered from the sodium bicarbonate solution after acidification with hydrochloric acid; wt, 8.5 g). The ethyl acetate solution, which should contain the sodium salt of II, was converted to the ether solution, and the main product was extracted three times with a sodium carbonate solution. The acidification of the carbonate solution with 6 N hydrochloric acid afforded an oily material which was extracted with ethyl acetate. After washing with water and drying over sodium sulfate, the solution was concentrated under reduced pressure; the oily residue was crystallized by the addition of petroleum ether; yield of crude material, 24.8 g, mp 66.5—69°C. Recrystallization from ethyl acetate and petroleum ether (1 : 2 v/v) gave 19.5 g of needles (yield, 47 %). When a correction is made for the unchanged I, the yield is 64 %; mp 71—72°C, $[\alpha]_D^{25}$ −19.1° (*c* 3, dimethylformamide).

Found: C, 66.64; H, 6.82; N, 3.40 %. Calcd for $C_{23}H_{27}O_6N$: C, 66.81; H, 6.66; N, 3.39 %.

Bis(benzyloxycarbonyl-L-α-aminosuberic Acid ω-*t*-Butyl Ester) Piprazine Salt (III). A solution of compound II (20.7 g, 0.05 mol) in methylene chloride (100 m*l*) was allowed to react with isobutylene (50 m*l*) in the presence of sulfuric acid (0.5 m*l*) as the catalyst. After 2 days at room temperature, the reaction mixture was poured into 5 % sodium carbonate, and then the methylene chloride and the excess isobutylene were removed by distillation under reduced pressure. The oily material which appeared was extracted into ether,

13) All the melting points were determined by the capillary method, and all are given as uncorrected values. All products were dried over phosphorus pentoxide *in vacuo* at room temperature unless otherwise noted.

and the ether solution was washed successively with 5 % sodium carbonate, water, 0.2N hydrochloric acid, and water. The concentration of the solution under reduced pressure yielded an oily material (α-benzyl ω-*t*-butyl esters of L-α-aminosuberic acid), which was then dissolved in acetone (200 m*l*) and saponified with 1 N sodium hydroxide (50 m*l*) for 3 hr at 0—5°C. The reaction mixture was neutralized with 1 N hydrochloric acid, the acetone was removed under reduced pressure, ether was added to the residual solution, and the main product was transferred to the aqueous layer by the addition of 5 % sodium carbonate. The neutral material was removed with ether, and the aqueous layer was acidified with 1 N hydrochloric acid; then the oil which separated was extracted with ethyl acetate. The concentration of the extract gave an oily material, which was then neutralized with piperazine hexahydrate (4.4 g, 0.0225 mol) in acetone. The solution was concentrated under reduced pressure, and the residue was flushed twice with toluene to remove the water. The trituration of the dried residue with ethyl acetate yielded crude crystals which were recrystallized from ethyl acetate; yield, 16.0 g (76 %); mp 130—131°C, $[\alpha]_D^{28}$ −2.7° (*c* 2, acetic acid). This material was dried over phosphorus pentoxide *in vacuo* at 80°C for 10 hr for analysis.

Found: C, 62.28; H, 8.17; N, 6.57 %. Calcd for $C_{41}H_{68}O_{12}N_4$: C, 62.54; H, 8.11; N, 6.63 %.

Benzyloxycarbonyl-ω-*t*-butyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide (IV). The benzyloxycarbonyl-L-α-aminosuberic acid ω-*t*-butyl ester (7 mmol) was extracted from III (2.96 g) with ethyl acetate by shaking with 1N hydrochloric acid; the ethyl acetate layer was then dried over sodium sulfate. The dried solution was concentrated to a syrup, which was dissolved in pyridine (4 m*l*). The solution was then treated with *p*-nitrophenyl trifluoroacetate (1.97 g, 8.4 mmol) at room terperature for 3 hr. A large amount of water was added to the reaction mixture, and the oily material thus precipitated was extracted with ethyl acetate; the ethyl acetate extract was washed with 0.5N hydrochloric acid and water successively, and dried over anhydrous sodium sulfate. The concentration of the dried solution left an oily residue which corresponded to the *p*-nitrophenyl ester of the starting material; the oily residue was allowed to react with L-prolyl-L-leucylglycine amide[11] (2.39 g, 8.2 mmol as the hemihydrate) in dimethylformamide (7 m*l*) for 2 days at room temperature. The addition of water gave a precipitate which was taken into ethyl acetate. The organic layer was washed successively with 1N aqueous ammonia, water, 1N hydrochloric acid, and water. Finally the solution was dried over sodium sulfate and concentrated to a residue which was crystallized by the addition of ether; yield, 4.08 g (90 %), mp 108—110°C, $[\alpha]_D^{13}$ −66.8° (*c* 2, ethanol). This material was dried for analysis at 80°C for 2 hr over phosphorus pentoxide *in vacuo*.

Found: C, 61.19; H, 8.37; N, 10.82%. Calcd for $C_{33}H_{51}O_8N_5$: C, 61.37; H, 7.96; N, 10.85 %.

Benzyloxycarbonyl-L-asparaginyl-ω-*t*-butyl-L-α-aminosuberyl-L-prolyl-L-luecylglycine Amide (V). The benzyloxycarbonyl group in compound IV (5.83 g, 9 mmol) was removed in ethanol by catalytic hydrogenolysis. The catalyst was then removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was allowed to react with the benzyloxycarbonyl-L-asparagine *p*-nitrophenyl ester[14] (3.48 g, 9 mmol) for 2 days in dimethylformamide at room temperature. The product was then precipitated by the addition of a large amount of ethyl acetate, and the precipitate was collected by filtration. Recrystallization from 90 % ethanol yielded an analytically-pure product; yield, 6.10 g (89 %), mp 212°C (decomp.), $[\alpha]_D^{27}$ −47.7° (*c* 1, dimethylformamide). This material was dried for analysis at 80°C for 2 hr over phosphorus pentoxide *in vacuo*.

Found: C, 58.70; H, 7.70; N, 12.92%. Calcd for $C_{37}H_{57}O_{10}N_7$: C, 58.55; H, 7.56; N, 12.90%.

Benzyloxycarbonyl-L-glutaminyl-L-asparaginyl-ω-*t*-butyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide Monohydrate (VI). The benzyloxycarbonyl group of compound V (6.20 g, 8.2 mmol) was removed by catalytic hydrogenation in a mixture of dioxane (70 m*l*) and water (35 m*l*). After the reaction, the catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was flushed several times with toluene, and then dissolved in dimethylformamide (18 m*l*). The benzyloxycarbonyl-L-glutamine *p*-nitrophenyl ester[14] (3.93 g, 9.8 mmol) was added to the solution, and the mixture was allowed to react overnight at room temperature. The reaction product was precipitated by the addition of excess ethyl acetate, and the precipitate was collected by filtration and recrystallized from 90% ethanol; yield, 5.85 g (81 %), mp 207—208°C (decomp.), $[\alpha]_D^{26}$ −45.7° (*c* 1, dimethylformamide). This material was dried for analysis at 80°C *in vacuo* for 3 hr over phosphorus pentoxide.

Found: C, 55.71; H, 7.51; N, 13.88 %. Calcd for $C_{42}H_{65}O_{12}N_9 \cdot H_2O$: C, 55.68; H, 7.45; N, 13.90%.

Benzyloxycarbonyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-ω-*t*-butyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide Monohydrate (VII). The benzyloxycarbonyl group was removed from compound VI (5.70 g, 6.3 mmol) by catalytic hydrogenolysis in a mixture of dioxane and water (50 m*l*+50 m*l*). The catalyst was then removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was flushed several times with toluene, and the oil which remained was allowed to react with the benzyloxycarbonyl-L-isoleucine *N*-hydroxysuccinimide ester[15] (3.44 g, 9.5 mmol) for one day at room temperature in dimethylformamide (25 m*l*). The product was precipitated by the addition of ethyl acetate; the precipitate was recrystallized from hot 90 % ethanol. The yield was 5.66 g (88 %); mp 225°C (decomp.), $[\alpha]_D^{27}$ −41° (*c* 1, dimethylformamide). This material was dried for analysis at 80°C *in vacuo* for 10 hr over phosphorus pentoxide.

Found: C, 56.93; H, 7.63; N, 13.71 %. Calcd for $C_{48}H_{76}O_{17}N_{10} \cdot H_2O$: C, 56.56; H, 7.71; N, 13.74 %.

Benzyloxycarbonyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide Monohydrate (VIII). Compound VII (1.00 g, 0.98 mmol) was dissolved in trifluoroacetic acid (20 m*l*), and the solution was allowed to stand for 3 hr at room temperature. Then the solvent was removed by evaporation under reduced pressure, and the

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

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

residue was triturated with ether and reprecipitated from hot 90 % ethanol; yield, 0.75 g (80 %), mp 212—214° (decomp.), $[\alpha]_D^{17}$ −44.2° (*c* 1, dimethylformamide). This material was dried for analysis at 80°C *in vacuo* for 10 hr over phosphorus pentoxide.

Found: C, 54.99; H, 7.36; N, 14.64 %. Calcd for $C_{44}H_{68}O_{13}N_{10}\cdot H_2O$: C, 54.87; H, 7.33; N, 14.55%.

Benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide Monohydrate (IX) The benzyloxycarbonyl group was removed from VIII (710 mg, 0.74 mmol) by catalytic hydrogenation in a mixture of dioxane (30 m*l*) and water (10 m*l*). The catalyst was removed by filtration; the filtrate was concentrated under reduced pressure, and the residue was dried by flushing with toluene. This residue was dissolved in dimethylformamide (40 m*l*), and the *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine *N*-hydroxysuccinimide ester[16] (557 mg, 1.1 mmol) was added together with *N*-ethylmorphorine (0.1 m*l*, 0.98 mmol). The mixture was allowed to react for 2 days at room temperature, and then the product was precipitated by the addition of excess ethyl acetate and collected by filtration. Reprecipitation from 90 % ethanol afforded an amorphous powder; yield, 660 mg (73 %), mp 231—232°C (decomp.), $[\alpha]_D^{28}$ −36.4° (*c* 0.74, dimethylformamide). This material was dried over phosphorus pentoxide *in vacuo* at 80°C for 10 hr before analysis.

Found: C, 59.42; H, 7.28; N, 12.78%. Calcd for $C_{60}H_{83}O_{15}N_{11}\cdot H_2O$: C, 59.24; H, 7.04; N, 12.66%.

Benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-ω-2, 4, 5-trichlorophenyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide Monohydrate (X). 2,4,5-Trichlorophenyl trifluoroacetate (733 mg, 2.5 mmol) was added to a suspension of IX (304 mg, 0.25 mmol) in a mixture of dimethylformamide (4 m*l*) and pyridine (2 m*l*), and the mixture was allowed to react for 3 hr at 50°C; gradually the suspension changed to a clear solution during the reaction. A large amount of ether was added to the solution to precipitate the product, which was then collected by filtration. Reprecipitation from dimethylformamide and ether yielded 327 mg of the product; yield 95 %, mp 231—232°C (decomp.), $[\alpha]_D^{23}$ −27.8° (*c* 0.75, dimethylformamide). This material was dried over phosphorus pentoxide at 60°C *in vacuo* for 10 hr before analysis.

Found: C, 56.70; H, 6.27; N, 11.12; Cl, 7.79 %. Calcd for $C_{66}H_{84}O_{15}N_{11}Cl_3\cdot H_2O$: C, 56.79; H, 6.21; N, 11.04; Cl, 7.62 %.

16) M. Löw and L. Kisfaludy, *Acta chim. Hung.*, **44**, 61 (1965).

Lactam of L-Tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine Amide Monohydrate (XI). The benzyloxycarbonyl and benzyl ether groups were simultaneously revomed from X (419 mg, 0.3 mmol) by catalytic hydrogenolysis in dimethylformamide (300 m*l*). The catalyst was removed by filtration, the filtrate was concentrated under reduced pressure, and the residue was dissolved in water (27 m*l*). The trichlorophenol formed was removed by extraction with ether. The aqueous solution was passed successively through columns (1.0 × 10 cm) of Dowex-50 X2 (100—200 mesh, H^+ form) and of Amberlite IR-45 (100—200 mesh, OH^- form). These columns were washed with water, and the effluent and washing were combined and lyophilized to obtain 97.4 mg (33 %) of the cyclized product. A portion (75 mg) of this product was dissolved in 1N acetic acid (1.5 m*l*), and the solution was applied to a column of Sephadex G-25 (fine, 1.8 × 170 cm). The flow rate of the solvent, 1N acetic acid, was 25 m*l* per hr. Fractions of 5 g each were collected, and peptide was detected by the UV-absorption at 274.5 mμ, as is shown in Fig. 1. The effluents in tubes No. 48 to 52 were pooled and lyophilized; wt, 62 mg (recovery 83 %). This material was found to be homogeneous, as judged by paper electrophoresis and by paper chromatography. Rf: 0.66 (*n*-butanol : acetic acid : water, 4 : 1 : 1 v/v) and 0.74 (*n*-butanol : acetic acid : pyridine : water, 15 : 3 : 10 : 6 v/v). These spots were located with Pauli's reagent. Reported Rf's: 0.50 and 0.70 respectively with the above solvent systems.[6] $[\alpha]_D^{18}$ −98.4° (*c* 0.46, water).

Found for sample dried at 60°C for 10 hr *in vacuo*: C, 54.39; H, 7.13; N, 15.26 %. Calcd for $C_{45}H_{69}O_{12}N_{11}\cdot 2H_2O$: C, 54.45; H, 7.42; N, 15.53 %.

Found for sample dried at 90°C for 18 hr *in vacuo*: C, 55.01; H, 7.12; N, 15.95 %. Calcd for $C_{45}H_{69}O_{12}N_{11}\cdot H_2O$: C, 55.48; H, 7.35; N, 15.82 %. Ratio of amino acids after acid hydrolysis: $Tyr_{1.00}$, $Ile_{1.01}$, $Glu_{0.97}$, $Asp_{1.00}$, $Asu_{1.01}$, $Pro_{1.02}$, $Leu_{1.00}$, $Gly_{0.97}$. A mol wt of about 850 was estimated by the sedimentation equilibrium method: 0.1 M NaCl solution was used as the solvent, and a partial specific volume of 0.73 was assumed for the calculation. Calcd mol wt: 956.

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