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96. Haruaki Yajima, Yoshio Okada, Takao Oshima,*1 and Saul Lande*2 : Studies on Peptides. Ⅷ.*3,*4 Synthesis of Two Heptapeptides Isolated from Pituitary Glands.

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Recently Lande, *et al.*[1] have engaged in the fractionation of porcine pituitary glands and isolated, besides α-, β-melanocyte-stimulating hormone (MSH), a number of new peptides including a fraction which exhibited an *in vitro* melanotropic activity[2] equivalent to nearly 10^8 MSH U/g. Partial sequence analysis of this component has indicated that the structure is phenylalanylarginyltryptophylglycylserylprolylproline (I), a heptapeptide sequence corresponding to positions 10 to 16 of porcine β-MSH.[3,4]

Independently Gros and Leygues[5] have recently isolated from porcine posterior hypophysis the heptapeptide amide, phenylalanylarginyltryptophylglycyllysylprolylvaline amide (Ⅱ), which corresponds to positions 7 to 13 of α-MSH.[6] They reported that the peptide amide exhibited an *in vitro* activity of 8×10^6 MSH U/g.

	Phe. Arg. Try. Gly. Ser. Pro. Pro.	(Ⅰ)
Asp. Glu. Gly. Pro. Tyr. Lys. Met. Glu. His. Phe. Arg. Try. Gly. Ser. Pro. Pro. Lys. Asp.		β-MSH (porcine)
	Phe. Arg. Try. Gly. Lys. Pro. Val. NH_2	(Ⅱ)
Ac. Ser. Tyr. Ser. Met. Glu. His. Phe. Arg. Try. Gly. Lys. Pro. Val. NH_2		α-MSH

Fig. 1.

These two heptapeptides, as shown in Fig. 1, differ from each other at positions 5 and 7; the latter also possesses an amide group at the C-terminus. Whether the presence in the pituitary of the two heptapeptides, representing partial structures of α- and β-MSH, is significant, is not known. However the observation that these peptides possess an intrinsic melanotropic activity can be verified only by assay of synthetic analogues, in which case contamination with biologically active impurities is impossible. We have synthesized these two heptapeptides, I and Ⅱ, and observed that neither exhibited *in vitro* MSH activity in contrast to the observations reported by Lande, *et al.*[1] on native I, by Gros, *et al.*[5] on native Ⅱ and Ney, *et al.*[7] on synthetic Ⅱ prepared by Schwyzer.

For the synthesis of I, as shown in Chart 1, the protected heptapeptide,

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*3 Part Ⅶ : This Bulletin, **14**, 775 (1966).

*4 The peptides and peptide derivatives mentioned in this communication are of the L-configuration. The customary L-designation for individual amino acid residues has been eliminated.

1) S. Lande, A. B. Lerner, G. V. Upton : J. Biol. Chem., **240**, 4259 (1965).

2) MSH activities were determined according to the procedure of A. B. Lerner, M. R. Wright : Methods of Biochem. Anal., **8**, 295 (1960).

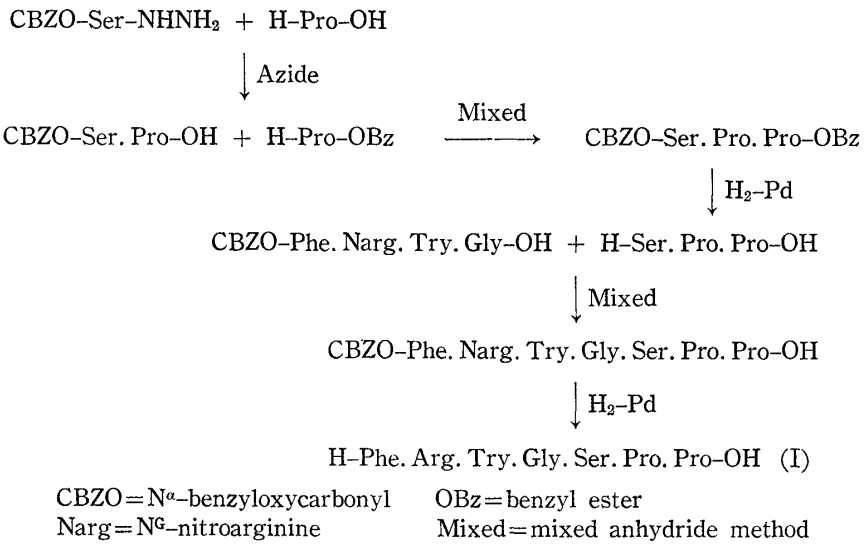
3) J. I. Harris, P. Roos : Nature, **178**, 90 (1956); Biochem. J., **71**, 434 (1959).

4) I. I. Geschwind, C. H. Li, L. Barnafi : J. Am. Chem. Soc., **78**, 4494 (1956); *Ibid.*, **79**, 620 (1957); I. I. Geschwind, C. H. Li : *Ibid.*, **79**, 615 (1957).

5) C. Gros, C. Leygues : Bull. soc. chim. France, **1964**, 2840.

6) J. I. Harris, A. B. Lerner : Nature, **179**, 1346 (1957).

7) R. L. Ney, E. Ogata, N. Shimizu, W. E. Mcholson, G. W. Liddle : Proc. 2nd Int. Congr. Endocrinology, London, 1964. Exerpta. Medica Int. Congr. Series, **83**, 1184 (1965).



CBZO=N^{α}-benzyloxycarbonyl OBz=benzyl ester
Narg=N^{G}-nitroarginine Mixed=mixed anhydride method

Chart 1. Synthetic Scheme of I

benzyloxycarbonylphenylalanyl-N^{G}-nitroarginyltryptophylglycylserylprolylproline was prepared by coupling benzyloxycarbonylphenylalanyl-N^{G}-nitroarginyltryptophylglycine with the tripeptide, serylprolylproline by the mixed anhydride procedure.[8] The benzyloxycarbonyltetrapeptide was prepared as previously described.[9] Free serylprolylproline was obtained by the hydrogenolysis of benzyloxycarbonylserylprolylproline benzyl ester which had been prepared from the reaction of benzyloxycarbonylserylproline with proline benzyl ester[10] by the mixed anhydride procedure. The strong tendency of proline or prolylproline alkyl esters to form diketopiperazines, is a well documented phenomenon.[11] To eliminate this possibility, benzyloxycarbonylserylproline was prepared by the reaction of benzyloxycarbonylserine azide[12] with the triethylammonium salt of proline. Coupling the dipeptide to proline benzyl ester avoided the possibility of diketopiperazine formation during the preparation of the desired tripeptide. Activation of a C-terminal proline residue, as in the above described reaction, usually proceeds without racemization.[13]

Serylprolylproline, prepared through hydrogenolysis of the above protected tripeptide, exhibits a yellow color with ninhydrin on paper which shortly turns to a blue more typical of the ninhydrin reaction of peptides. The tripeptide was homogeneous on paper chromatography in two different solvent systems. Amino acid ratios of an acid hydrolysate were identical with those predicted by theory. One mole of serine and two moles of proline were also found on amino acid analysis after treatment of the synthetic tripeptide with leucine amino peptidase (LAP).[14] The resistance of prolyl peptide bonds to the action of highly purified LAP was reported by Meienhofer and Li[15] but partially purified LAP digested the tripeptide completely, presumably through the action of prolidase,[16] a contaminant in the enzyme preparation. A similar effect

8) J.R. Vaughan Jr., R.L. Osato: J. Am. Chem. Soc., **73**, 3547 (1951).
9) K. Hofmann, S. Lande: *Ibid.*, **83**, 2286 (1961).
10) R.E. Neuman, E.L. Smith: J. Biol. Chem., **193**, 97 (1951).
11) J.S. Fruton: Advan. in Protein Chem., **5**, 1 (1949).
12) R.W. Holly, E. Sondheimer: J. Am. Chem. Soc., **76**, 1326 (1954).
13) K. Hofmann, P.G. Katsoyannis: "The Protein," Vol. **I**, 53, H. Neurath Ed. Academic Press, N. Y. (1963).
14) Partially purified (through second ammonium sulfate fractionation) LAP was prepared according to the method of D.H. Spackman, E.L. Smith, D.M. Brown: J. Biol. Chem., **212**, 255 (1955).
15) J. Meienhofer, C.H. Li: J. Am. Chem. Soc., **84**, 2434 (1962).
16) S. Sarid, A. Berger, E. Katchalski: J. Biol. Chem., **237**, 2207 (1962).

was described by Hofmann, *et al.*,[17] with regard to arginylarginylprolylvaline amide.

Benzyloxycarbonylphenylalanyl-N^G-nitroarginyltryptophylglycylserylprolylproline was subjected to hydrogenolysis without prior purification. The resultant crude free heptapeptide was purified by ion exchange chromatography on carboxymethylcellulose (CMC)[18] and the desired compound was eluted with 0.025*M* pyridine acetate buffer. The synthetic peptide (I) revealed a sharp single spot by ninhydrin, Sakaguchi and Ehrlich tests after chromatography on paper in two different solvent systems. Acid hydrolysis and LAP digestion gave the ratios of the constituent amino acids predicted by theory except for tryptophan which was destroyed during the acid hydrolysis.

Synthetic and natural I were compared by thin-layer chromatography on cellulose and silica and by electrophoresis in a pyridine acetate buffer at pH 5.6. Both compounds behaved identically in all cases.[1]

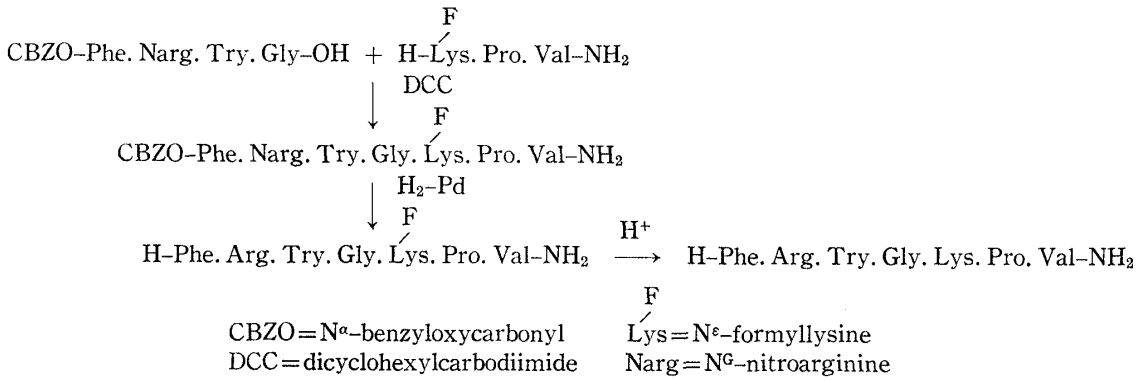


Chart 2. Synthetic Scheme of II

For the synthesis of II, as indicated Chart 2, benzyloxycarbonylphenylalanyl-N^G-nitroarginyltryptophylglycine[9] and N^ε-formyllysylprolylvaline amide[19] were condensed by the dicyclohexylcarbodiimide procedure[20] to form benzyloxycarbonylphenylalanyl-N^G-nitroarginyltryptophylglycyl-N^ε-formyllysylprolylvaline amide which was then hydrogenated over a palladium catalyst. The resulting product was purified by chromatography on CMC. The desired, partially protected heptapeptide amide, phenylalanylarginyltryptophylglycyl-N^ε-formyllysylprolylvaline amide was eluted with 0.075 *M* pyridine acetate buffer.

The synthesis of the N^ε-formyllysine heptapeptide amide was reported by Hofmann, *et al.*;[21] experimental details were not given. We treated the peptide amide with 0.5*N* hydrochloric acid to remove the formyl group attached to ε-amino group of lysine residue. The product, phenylalanylarginyltryptophylglycyllysylprolylvaline amide (II), was purified by column chromatography on CMC and 0.15*M* pyridine acetate buffer served to elute the desired compound. The purified material appeared homogeneous on paper chromatogram and the acid hydrolysate contained the constituent amino acids in the ratios predicted by theory except for tryptophan which was destroyed during acid hydrolysis. Treatment of this product with carboxypeptidase*5 did not release any valine, indicating that the amide group at the C-terminal valine of the heptapeptide is stable to treatment with hot aqueous hydrochloric acid as previously observed by

*5 Carboxypeptidase A was purchased from Sigma Chem. Co.

17) K. Hofmann, H. Yajima, T. Liu, N. Yanaihara, C. Yanaihara, J. L. Humes: J. Am. Chem. Soc., **84**, 4475 (1962).
18) E. A. Peterson, H. A. Sober: *Ibid.*, **78**, 751 (1956).
19) K. Hofmann, E. Stütz, G. Spühler, H. Yajima, E. T. Schwarts: *Ibid.*, **82**, 3727 (1960).
20) J. C. Sheehan, G. P. Hess: *Ibid.*, **77**, 1067 (1955).
21) K. Hofmann, H. Yajima: "Recent Prog. in Hormone Res.", **18**, 41 (1962), Academic Press, N. Y.

Hofmann and Yajima.[22] Although comparison between the synthetic and natural heptapeptide amide was not made, the above experimental findings indicate that the synthetic product is homogeneous and possesses the desired structure.

Three different batches of the synthetic heptapeptide (I) were assayed by the *in vitro* method[2] using frog skin from *Rana Pipiens*. It was found that none exhibited MSH activity. Since N-acetyl peptides with melanotropic activity are more potent than the corresponding unsubstituted peptides,[23] I was acetylated with acetic anhydride. It was found, however, that the acetyl heptapeptide was also devoid of MSH activity. From the above experimental results, it may be concluded that the MSH activity of native I resulted from contamination with traces of highly active material.

Hofmann, *et al.*[21] reported previously that phenylalanylarginyltryptopyhylglycyl-N^{ε}-formyllysylprolylvaline amide was inactive. Gros and Leygues[5] reported that the peptide (II) was active, indicating the possible requirement of a free lysyl ε-amino group for melanotropic activity. However, when synthetic (II) and the N^{ε}-blocked lysine analogue amide were assayed in our laboratories, neither exhibited MSH activity. By the present time, any experimental data of II has not been presented by Schwyzer, *et al.* Until some reason for this lack of agreement can be found, the determination of the minimal structural requirements for activity must still be considered inconclusive.

Experimental

The general experimental methods are essentially the same as described in the part IV[24] of this series. Amino acid compositions of the acid and enzymatic hydrolysates were determined according to the method of Moore, Spackman and Stein.[25] The following abbreviations for amino acids were used : Phe=phenylalanine, Arg=ariginine, Try=tryptophan, Gly=glycine, Ser=serine, Pro=proline, Lys=lysine and Val=valine. Rf^1 values refer to the system of Partridge.[26] Rf^2 values refer to the system of 2-butanol-ammonia[27] and are expressed as a multiple of the distance traveled by phenylalanine under identical conditions.

N^{α}-Benzyloxycarbonylserylproline——The entire operation was performed in a cold room at 4°. An ether solution of N^{α}-benzyloxycarbonylserine azide (prepared from 10.12 g. of the corresponding hydrazide according to the method of Holly and Sondheimer[12]) was added to a solution of proline (6.90 g.) and triethylamine (8.3 ml.) in H_2O (30 ml.). The reaction mixture was stirred vigorously at 4° for 48 hr. The aqueous phase was separated from the ether layer which was extracted with three 10 ml. portions of 5% NH_4OH. The aqueous phase and the extracts were combined and the solution was acidified with 6*N* HCl. The resulting oily product was extracted into AcOEt, which was washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent gave a solid which was recrystallized from AcOEt; yield 9.00 g. (67%), m.p. 107~109°, $[\alpha]_D^{27}$ −70.2°(c=0.7, MeOH). *Anal.* Calcd. for $C_{16}H_{20}O_6N_2$: C, 57.1; H, 6.0; N, 8.3. Found : C, 56.9; H, 6.2; N, 8.1.

N^{α}-Benzyloxycarbonylserylprolylproline Benzyl Ester——A mixed anhydride was prepared in the usual manner from N^{α}-benzyloxycarbonylserylproline (5.04 g.) in anhydrous tetrahydrofuran (25 ml.) with tri-*n*-butylamine (3.6 ml.) and ethyl chloroformate (1.4 ml.). This solution was added to a chilled solution of benzyl prolinate hydrochloride[10] (3.61 g.) and triethylamine (2.1 ml.) in tetrahydrofuran (20 ml.). The mixture was stirred in an ice-bath for 2.5 hr. The solvent was evaporated and the oily residue was dissolved in AcOEt, which was successively washed with 5% NH_4OH, 1*N* HCl and H_2O. The organic phase was dried over Na_2SO_4 and the solvent was removed by evaporation to give an oily residue which solidified on trituration with ether. The product was recrystallized from MeOH to give white needles; yield 4.12 g. (53%), m.p. 129~131°, $[\alpha]_D^{18}$ −134.5°(c=1.0, MeOH); amino acid ratios in an acid hydrolysate : $Ser_{1.00}Pro_{2.11}$(average recovery 89.7%). *Anal.* Calcd. for $C_{28}H_{33}O_7N_3$: C, 64.2; H, 6.4; N, 8.0. Found : C, 64.5; H, 6.6; N, 8.0.

Serylprolylproline——N^{α}-Benzyloxycarbonylserylprolylproline benzyl ester (3.80 g.) in 95% MeOH (50 ml.) containing glacial AcOH (0.5 ml.) was hydrogenated over a Pd catalyst for 6 hr. A crystalline product separated from the solution during hydrogenation. The solvent was first removed by evaporation *in vacuo*. H_2O was added to the residue and the catalyst was removed by filtration. The filtrate was condensed to a small

22) K. Hofmann, H. Yajima : J. Am. Chem. Soc., **83**, 2289 (1961).
23) St. Guttmann, R. A. Boissonnas : Experientia, **17**, 265 (1961).
24) H. Yajima, K. Kubo : This Bulletin, **13**, 759 (1965).
25) S. Moore, D. H. Spackman, W. H. Stein : Anal. Chem., **30**, 1185 (1958).
26) S. M. Partridge : Biochem. J., **42**, 238 (1948).
27) J. F. Roland, A. M. Gross : Anal. Chem., **26**, 502 (1954).

volume and EtOH was added. The crystalline compound which formed on standing was collected by filtration and recrystallized from H_2O by addition of EtOH; yield 1.91 g. (88%), m.p. 177~181° (decomp.), $[\alpha]_D^{20}$ −186.5° (c=1.0, H_2O), Rf^1 0.32, Rf^2 0.22, amino acid ratios in an acid hydrolysate : $Ser_{1.00}Pro_{2.23}$ (average recovery 86.3%); amino acid ratios in a LAP digest : $Ser_{1.00}Pro_{2.10}$ (average recovery 75%). *Anal.* Calcd. for $C_{13}H_{21}O_5N_3$: C, 52.2; H, 7.1; N, 14.0. Found : C, 52.4; H, 7.3; N, 13.9.

Phenylalanylarginyltryptophylglycylserylprolylproline——Ethyl chloroformate (0.05 ml.) was added to a cold solution of N^{α}-benzyloxycarbonylphenylalanyl-N^{G}-nitroarginyltryptophylglycine[9] (0.40 g.) and tri-*n*-butylamine (0.13 ml.) in anhydrous dimethylformamide (DMF) (3 ml.). The mixture was stirred at −15° for 15 min. This solution was added with stirring to an ice-cold solution of serylprolylproline (0.15 g.) and triethylamine (0.18 ml.) in H_2O (1 ml.) and DMF (2 ml.). The reaction mixture was stirred at 0° for 2 hr. The solvent was evaporated and the residue was treated with H_2O to form a solid powder, which was collected by filtration. The product was dissolved in 50% AcOH and was hydrogenated over a Pd catalyst. Hydrogenation was continued until no further change was observed on the pattern obtained on paper chromatogram of aliquots of the reaction mixture. Examination of the final solution revealed the presence of two components (Rf^2 0.93 and 1.02), both positive to the ninhydrin, Sakaguchi and Ehrlich tests. The catalyst was removed by filtration. The solvent was removed first by evaporation and finally by lyophilization.

The lyophilizate was dissolved in H_2O (200 ml.) and applied to a CMC column (3×16 cm.) which was then developed with the following pH 5.0 pyridine acetate buffers : 0.025*M* (660 ml.) and 0.05*M* (825 ml.). Measurement of the absorbancy at 280 mμ served to locate the desired compound in the eluates. Two main peaks, one in the 0.025*M* eluate and the other in the 0.05*M* were found. The latter was identical with authentic phenylalanylarginyltryptophylglycine on paper chromatography. The 0.025*M* eluates were pooled, and the solvent was evaporated to dryness. The residue was lyophilized to give a white fluffy powder; yield 0.22 g. (49%), $[\alpha]_D^{24}$ −64.9° (c=0.9, H_2O), Rf^1 0.45, Rf^2 0.93, single ninhydrin, Sakaguchi and Ehrlich positive spot; amino acid ratios in an acid hydrolysate : $Phe_{1.00}Arg_{0.97}Gly_{1.00}Ser_{0.93}Pro_{1.99}$ (average recovery 83.5%); amino acid ratios in a LAP digest : $Phe_{1.03}Arg_{0.91}Try_{1.00}Gly_{1.00}Ser_{1.08}Pro_{2.08}$ (average recovery 85%). *Anal.* Calcd. for $C_{41}H_{55}O_9N_{11}\cdot CH_3COOH\cdot 3H_2O$: C, 53.8; H, 6.8; N, 16.1. Found : C, 53.6; H, 6.9; N, 15.9.

Acetylphenylalanylarginyltryptophylglycylserylprolylproline——Acetic anhydride (0.01 ml.) was added with stirring to an ice-cold solution of phenylalanylarginyltryptophylglycylserylprolylproline hydrochloride (0.05 g.) and $NaHCO_3$ (0.026 g.) in H_2O (1.5 ml.). Following the addition, stirring was continued at ice-bath temperature for 2 hr. The solvent was evaporated to dryness and the residue was dissolved in H_2O (170 ml.). The solution was applied to a column of CMC (2×10 cm.) which was eluted with H_2O. Ten ml. aliquots were collected at a flow rate of 5 to 6 ml. per min. and absorbancy at 280 mμ was determined in each fraction. The material present at tube 11 to 40 was pooled and the solvent was removed first by evaporation to a small volume then by lyophilization; yield 0.046 g. (87%), $[\alpha]_D^{15}$ −42.2° (c=0.4, H_2O), Rf^1 0.66, ninhydrin negative, single Ehrlich and Sakaguchi positive spot; amino acid ratios in an acid hydrolysate, $Phe_{1.08}Arg_{1.00}Gly_{1.12}Ser_{0.97}Pro_{2.08}$ (average recovery 93%).

Phenylalanylarginyltryptophylglycyl-N^{ε}-formyllysylprolylvaline Amide——N^{ε}-Formyllysylprolylvaline amide hydrochloride (0.44 g.) was dissolved in MeOH (20 ml.) containing triethylamine (0.16 ml.). The solvent was removed *in vacuo* at a bath temperature of 30° and the residue was dissolved in DMF (20 ml.). This solution was mixed with a solution of N^{α}-benzyloxycarbonylphenylalanyl-N^{G}-nitroarginyltryptophylglycine (0.80 g.) in DMF (25 ml.), and dicyclohexylcarbodiimide (0.30 g.) was added. The solution was kept at room temperature for 24 hr. Dicyclohexylurea which formed during the reaction, was removed by filtration and the solvent was removed *in vacuo* leaving a semi-solid material. H_2O was added to the residue and the resultant precipitate was collected, washed with ether and dried over P_2O_5; yield 1.02 g. This material was hydrogenated in 40% AcOH (60 ml.) over a Pd catalyst in a stream of hydrogen for 12 hr. The catalyst was removed by filtration, the filtrate was concentrated to dryness *in vacuo* and the residue was lyophilized to give a fluffy material; yield 0.70 g. The product was dissolved in H_2O (800 ml.) and applied to a CMC column (3×30 cm.) which was eluted with the following pyridine acetate buffers at pH 5.0 : 0.05*M* (1500 ml.), 0.075*M* (1200 ml.) and 0.1*M* (1000 ml.). Individual aliquots (15 ml. each) were collected at a flow rate of 3 to 4 ml. per min., absorbancy at 280 mμ served to locate peptides in the various chromatographic fractions. Tubes 151~205 (0.075*M* eluate) contained the desired peptide. The contents of the tubes were pooled and the solvent removed first by evaporation, then by lyophilization to give a fluffy white powder; yield 0.26 g. (36%), $[\alpha]_D^{27}$ −35.3° (c=1.0, H_2O), Rf^1 0.58, Rf^2 1.36, single ninhydrin, Sakaguchi and Ehrlich positive spot, amino acid ratios in an acid hydrolysate : $Phe_{1.04}Arg_{1.00}Gly_{1.08}Lys_{1.00}Pro_{0.98}Val_{0.99}$ (average recovery 95%), amino acid ratios in a LAP digest : $Phe_{1.09}Arg_{0.99}Try_{1.00}Gly_{1.00}N^{\varepsilon}$-formyllys$_{1.00}Pro_{1.00}Val_{0.87}$ (average recovery 86%). *Anal.* Calcd. for $C_{45}H_{65}O_8N_{13}\cdot 2CH_3COOH\cdot 5H_2O$: C, 52.3; H, 7.4; N, 16.2. Found : C, 52.2; H, 7.9; N, 16.4.

Phenylalanylarginyltryptophylglycyllysylprolylvaline Amide——Phenylalanylarginyltryptophylglycyl-N^{ε}-formyllysylprolylvaline amide diacetate (0.05 g.) was dissolved in 0.5*N* HCl (4 ml.) and the mixture was heated in a boiling water-bath for 30 min. The hydrolysate was cooled in an ice-bath, 2*N* NH_4OH was added until the pH reached 7 and the mixture was diluted with H_2O (500 ml.). The solution was applied to a CMC column (2×10 cm.) which was eluted with the following pyridine acetate buffers at pH 5.0 : 0.075*M*

(900 ml.), 0.1*M* (750 ml.), 0.15*M* (750 ml.) and 0.3*M* (500 ml.). Individual fractions (15 ml. each) were collected at a flow rate of 3 to 4 ml. per min. Absorbancy at 280 mμ served to locate the peptides in the various eluates. The desired peptide amide was located in the 0.15*M* buffer eluate. The contents of these tubes were pooled and lyophilized to give a white powder; yield 0.02 g. (39%), $[\alpha]_D^{27}$ −29.7°(c=0.6, H_2O), Rf^1 0.41, Rf^2 0.87, single ninhydrin, Sakaguchi and Ehrlich positive spot; amino acid ratios in an acid hydrolysate: $Phe_{1.00}Arg_{0.98}Gly_{1.05}Lys_{1.00}Pro_{0.89}Val_{1.00}$ (average recovery 93%); amino acid ratios in a LAP digest: $Phe_{1.00}Arg_{0.90}Try_{1.06}Gly_{1.00}Lys_{0.99}Pro_{0.97}Val_{0.91}$ (average recovery 88%). Treatment of the peptide with carboxypeptidase*5 in 0.01*M* sodium bicarbonate buffer at pH 8.0 with an enzyme-substrate ratio of 1:50 produced no free valine. *Anal.* Calcd. for $C_{44}H_{65}O_7N_{13}\cdot 3CH_3COOH\cdot 5H_2O$: C, 51.9; H, 7.6; N, 15.7. Found: C, 51.8; H, 7.5; N, 16.1.

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Summary

Two heptapeptides, phenylalanylarginyltryptophylglycylserylprolylproline (I) and phenylalanylarginyltryptophylglycyllysylprolylvaline amide (II) have been synthesized. It was found that neither exhibited *in vitro* melanotropic activity in contrast to the observations reported on native I and II isolated from porcine pituitary glands.

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97. Hiroshi Mitsuhashi,*1 Kensuke Sakurai,*2 Taro Nomura, and Norio Kawahara*1 : Constituents of Asclepiadaceae Plants. XVII.*3 Components of *Cynanchum wilfordi* HEMSLEY.*4

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It has been shown in preceding papers of this series that the plant family *Asclepiadaceae* contains a series of polyhydroxypregnane derivative. The present study was initiated in order to find further pregnane compounds. *Cynanchum wilfordi* HEMSLEY (Japanese name "Koikema" Asclepiadaceae) is widely distributed in the southern part of Japan and Korea. The powdered roots of this plant, collected in Korea by Prof. Hahn, was treated as shown in Fig. 1 and as described in the experimental part. The hexane-insoluble part showed a strong Keller-Kiliani reaction (bluish violet), and Liebermann-Burchard reaction, but an active methylene reaction was negative. This crude glycoside was hydrolysed with 0.05*N* sulfuric acid in 50% methanol, the condition usually employed for the hydrolysis of glycosides containing 2-deoxysugars.[1~4]

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*3 Part XVI : This Bulletin, **13**, 1332 (1965).
*4 Part of this work was reported at the 82nd Annual Meeting of the Pharmaceutical Society of Japan, Shizuoka, November 3, 1962.

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