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99. Haruaki Yajima and Kazuo Kubo : Studies on Peptides. IV.*1 The Synthesis of D-Histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine and L-Histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine and Their Physiological Properties in Frog Melanocyte *in vitro*.

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The elucidation of the structure of α-melanocyte-stimulating hormone (α-MSH) by Harris and Lerner[1] was a landmark in the studies on hormonal control of cell pigmentation. Systematic synthesis of this hormone led to valuable observations on the relationship between the structure and its biological function.

Among those, Hofmann, *et al.*[2] have observed that the octapeptide, L-seryl-L-tyrosyl-L-seryl-L-methionyl-L-glutaminyl-L-histidyl-L-phenylalanyl-L-arginine which relates to the N-terminus of α-MSH was inactive but the addition of L-tryptophylglycine resulted in a decapeptide with biological activity (2.9×10^6 MSH U/g.). The partially protected C-terminal heptapeptide, L-phenylalanyl-L-arginyl-L-tryptophylglycyl-N^{ε}-formyl-L-lysyl-L-prolyl-L-valine amide was inert but biological activity (8×10^6 MSH U/g.) ensued when a single L-histidine was attached to the N-terminus of this heptapeptide amide. Consequently Hofmann, *et al.*[2] suggested key functions for histidine and tryptophan in relation to melanocyte-expanding potency.

Furthermore Hofmann, *et al.*[3] and Schwyzer, *et al.*[4] have shown independently that the synthetic pentapeptide, L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine (I) which corresponds to positions 6 to 10 of the α-MSH sequence and contains both histidine and tryptophan within the sequence exhibited 3×10^4 MSH U/g., about one millionth of the native MSH activity. Recently Otsuka, *et al.*[5] found the same level of activity in their synthetic tetrapeptide L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophan as that of I.

It is not known whether the mechanism of melanine granule dispersion caused by such fragments is exactly the same as that produced by the native hormone but definite darkening effects of these fragments were reported.[3~5] Thus there is a posibility that both histidine and tryptophan residues still play an important role for the melanocyte-expanding activities of these fragments.

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*1 A preliminary communication of this paper (Part III) : Biochem. Biophys. Acta, **97**, 596 (1965).

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1) J. I. Harris, A. B. Lerner : Nature, **179**, 1346 (1957).
2) K. Hofmann, H. Yajima : "Recent Progress in Hormone Research," Ed. by G. Pincus, **18**, 41 (1962), Academic Press, New York, N. Y.
3) K. Hofmann, M. E. Woolner, H. Yajima, G. Spühler, T. A. Thompson, E. T. Schwartz : J. Am. Chem. Soc., **80**, 6458 (1958).
4) R. Schwyzer, C. H. Li : Nature, **182**, 1669 (1958).
5) H. Otsuka, K. Inouye : Bull. Chem. Soc. Japan, **37**, 289, 1465 (1964).

In connection with the interesting observations that L-histidyl-D-phenylalanyl-L-arginyl-L-tryptophylglycine (D-Phe-isomer) synthesized by Li, *et al.*[6,7] and L-histidyl-L-phenylalanyl-D-arginyl-L-tryptophylglycine (D-Arg-isomer) prepared by Hofmann, *et al.*[8] exhibit the same level of activity as that of I, evaluation of the physiological activities of the pentapeptides substituted with either D-histidine or D-tryptophan for the corresponding L-isomer was made in our present investigation.

We have now completed the synthesis of the two stereoisomeric pentapeptides, D-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine (II) and L-histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine (III) and have observed that II, in contrast to I, was biologically inert while III exhibited 1×10^5 MSH U/g. in the isolated frog-skin assay.[9]

The schemes which we employed for the preparation of II and III are essentially identical to those described by Hofmann, *et al.*[8] and Geiger, *et al.*[10] except that D-histidine*3 and D-tryptophan*4 were used instead of their L-isomers. The purification procedure of the final products has been improved.

For the preparation of II, N^{α}-benzyloxycarbonyl-D-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine which was obtained by coupling N^{α}-benzyloxycarbonyl-D-histidine azide[11] with L-phenylalanyl-L-arginyl-L-tryptophylglycine,[8] was hydrogenated over a palladium catalyst. In order to remove any contamination of the undesired all L-isomer, the hydrogenated product was exposed to the action of leucine aminopeptidase (LAP)[12] and the undigested peptide was purified by column chromatography on carboxymethylcellulose (CMC).[13] The desired pentapeptide was eluted with 0.1*M* pyridine acetate buffer. The synthetic pentapeptide (II) was homogeneous on paper chromatogram in two different solvent systems. Acid hydrolysates of this material gave the amino acid ratios (minus tryptophan) predicted by theory. Histidine released by the action of LAP was negligible. This evidence indicates that the histidine residue of our pentapeptide (II) has a well established D-configuration.

For the synthesis of III, D-tryptophylglycine methyl ester[11], an intermediate of our synthesis of D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycine[11], was reacted with the mixed anhydride[14] of N^{α}-benzyloxycarbonyl-N^{G}-nitro-L-arginine[15] to form N^{α}-benzyloxycarbonyl-N^{G}-nitro-L-arginyl-D-tryptophylglycine methyl ester, which was then decarbobenzoxylated with hydrogen bromide in acetic acid. The resulting tripeptide ester was reacted with the mixed anhydride of N-benzyloxycarbonyl-L-phenylalanine to give N-benzyloxycarbonyl-L-phenylalanyl-N^{G}-nitro-L-arginyl-D-tryptophylglycine methyl ester. This tetrapeptide ester was also obtained by coupling N-benzyloxycarbonyl-L-phenylalanyl-N^{G}-nitro-L-arginine[15] with D-tryptophylglycine methyl ester *via*

*3 D-Histidine was purchased from Nutritional Biochemicals Corporation, Cleveland Ohio, Lot 1391.
*4 D-Tryptophan was purchased from Tanabe Pharmaceutical Co.

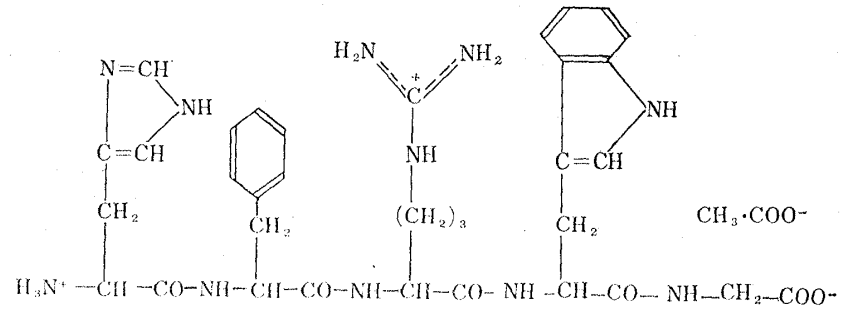
6) C. H. Li, E. Schnabel, D. Chung : J. Am. Chem. Soc., **82**, 2062 (1960).
7) E. Schnabel, C. H. Li : *Ibid.*, **82**, 4576 (1960).
8) K. Hofmann, S. Lande : *Ibid.*, **83**, 2286 (1961).
9) We wish to express our thanks to Dr. K. Hano and Mr. M. Koida of Osaka University for the MSH assays which were performed according to the method of K. Shizume, A. B. Lerner, T. B. Fitzpatrick : Endocrinol., **54**, 553 (1954).
10) R. Geiger, K. Sturm, W. Siedel : Ber., **96**, 1080 (1963).
11) The preliminary communication : K. Hano, M. Koida, K. Kubo, H. Yajima : Biochem. Biophys. Acta, **90**, 201 (1964). The full paper : in preparation.
12) Partially purified LAP was prepared according to the method of D. H. Spackman, E. L. Smith, D. M. Brown : J. Biol. Chem., **212**, 255 (1955). This was treated with diisopropyl phosphofluoridate according to the method of R. L. Hill, E. L. Smith : *Ibid.*, **228**, 577 (1957).
13) E. A. Peterson, H. A. Sober : J. Am. Chem. Soc., **78**, 751 (1956). Commercial preparation was purchased from Bio. Rad Laboratories.
14) J. R. Vaughan, Jr., R. L. Osato : *Ibid.*, **73**, 3547 (1951).
15) K. Hofmann, W. D. Peckham, A. Rheiner : *Ibid.*, **78**, 238 (1956).

the mixed anhydride procedure as described by Hofmann and Lande.[8] Treatment of the protected tetrapeptide ester with 1*N* sodium hydroxide in methanol followed by hydrogenolysis gave the free tetrapeptide, L-phenylalanyl-L-arginyl-D-tryptophylglycine. The N^{α}-benzyloxycarbonyl-L-histidine azide[16] was then reacted in dimethylformamide with the triethylammonium salt of the tetrapeptide to give N^{α}-benzyloxycarbonyl-L-histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine. After hydrogenolysis, the ensuing crude pentapeptide was purified by column chromatography on CMC. The desired pentapeptide (Ⅲ) was eluted as described in the preparation of Ⅱ.

The pentapeptide (Ⅲ) thus obtained was homogeneous on paper chromatogram in two different solvent systems. The amino acid ratios in an acid hydrolysate were identical with that predicted by theory. LAP treatment of the synthetic pentapeptide (Ⅲ) gave equimolar amounts of histidine and phenylalanine but the recovery of arginine was low. It is known that LAP does not cleave L-D peptide bonds quantitatively.[17] Examination by paper chromatography revealed that trypsin*5 cleaved the arginyl-tryptophyl bond of our pentapeptide (Ⅲ) completely. Treatment by trypsin followed by LAP resulted in the release of histidine, phenylalanine and arginine in nearly equal amounts. In addition, 0.16 μmoles each of tryptophan and glycine were present in the hydrolysate of 3.41 μmoles of the pentapeptide. The above evidence indicates that about 5% of L-tryptophan residue is present in our preparation of Ⅲ.

The synthetic pentapeptide (Ⅲ) has been judged to possess a slightly higher MSH activity than I. Since it is improbable that the MSH potency of our pentapeptide (Ⅲ) (1×10^5 MSH U/g.) can result from such a small contamination of L-tryptophan residue in the sequence, it seems reasonable to judge that the tryptophan residue in this pentapeptide moiety is not stereospecific for melanocyte-expanding activity at the same level as that of the D-Phe-isomer and D-Arg-isomer. Experiments *in vitro* demonstrated that our peptide (Ⅲ) failed to exhibit the effect of retardation or prolongation of MSH activity, which is typical of alkali treated α-MSH. These effects have recently been described as the result of racemization of the intact peptide chain of α-MSH.[18]

A very high concentration of the synthetic peptide (Ⅱ) (greater than 3 mg./ml.) showed slight darkening in the isolated frog-skin but this darkening value was far less than that produced by 4 MSH units of standard α-MSH or by a solution of I (33 μg./ml.). We conclude that this value is not significant. On the other hand, a very weak



	His.	Phe.	Arg.	Try.	Gly.	MSH U/g.
I[3,4]	L	L	L	L	gly	3×10^4
Ⅱ	D	L	L	L	gly	0
Ⅲ	L	L	L	D	gly	1×10^5

*5 Trypsin was purchased from Sigma Chem. Co. Lot T62 B-232.

16) R. W. Holley, E. Sondheimer : J. Am. Chem. Soc., **76**, 1326 (1954).
17) E. L. Smith, D. H. Spackman, W. J. Polglase : J. Biol. Chem., **199**, 801 (1952).
18) T. H. Lee, V. B.-Janusch : *Ibid.*, **238**, 2012 (1963).

inhibitory action of II toward α-MSH was noted. The potency was about one millionth of that of melatonin.[19]

From the above experimental results it can be concluded that the histidine residue in histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine must be in the L-configuration in order for the pentapeptide (I) to exert MSH activity.

Following the procedure described by Tanaka, *et al.*,[20] lipolytic activity of these pentapeptides was examined.*6 It was found that II is active on lipolysis in rabbit perirenal adipose tissue but this activity was lower than that of I. While the synthetic peptide (III) does not possess any lipolytic activity.

Experimental

The melting points are uncorrected. Rotations were determined in a Rex Photoelectric Polarimeter Model NEP-2. The amino acid composition of the acid and enzymatic hydrolysates was determined with a Hitachi Amino Acid Analyzer, Model KLA-2 according to the method of S. Moore, D. H. Spackman, W. H. Stein: Anal. Chem., **30**, 1185 (1958) and the following abbreviations for amino acids were used; His=histidine, Phe=phenylalanine, Arg=arginine, Try=tryptophan, Gly=glycine. Toyo filter paper No. 51 was used for paper chromatography. R_f^1 values refer to the Partridge system (S. M. Partridge: Biochem. J., **42**, 238 (1948)); R_f^2 values refer to the 2-butanol-ammonia system (J. F. Roland, A. M. Gross: Anal. Chem., **26**, 502 (1954)) and are expressed as a multiple of distance traveled by phenylalanine under identical conditions. Unless stated otherwise, solvents were evaporated *in vacuo* at a bath temperature of 40~50° in a rotatory evaporator. For column chromatography, Toyo Fraction Collector Model SF-200-A was used.

N^{α}-Benzyloxycarbonyl-D-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine Dihydrate——The entire operation was performed in a cold room (4°). N^{α}-Benzyloxycarbonyl-D-histidine hydrazide[11] (0.91 g.) was converted to its azide according to the method of Holley and Sondheimer.[16] An AcOEt solution (15 ml.) containing the above azide was added to a solution of L-phenylalanyl-L-arginyl-L-tryptophylglycine[8] (1.28 g.) and triethylamine (0.27 ml.) in dimethylformamide (30 ml.). The reaction was continued for 48 hr. The solvent was evaporated to one third of the volume and H_2O was added. The precipitated material was collected by filtration and recrystallized from a mixture of dimethylformamide and H_2O (1:1v/v); yield 1.37 g. (83%), m.p. 214~217° (decomp.), $[\alpha]_D^{28}$ +3.3° (c=1.0, 50% AcOH), R_f^1 0.70, R_f^2 2.0, ninhydrin negative, Pauly, Sakaguchi and Ehrlich positive spot. *Anal.* Calcd. for $C_{42}H_{49}O_8N_{11}\cdot 2H_2O$: C, 57.8; H, 6.1; N, 17.7. Found: C, 57.8; H, 6.3; N, 17.8.

D-Histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine Monoacetate Trihydrate——N^{α}-Benzyloxycarbonyl-D-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine (0.65 g.) was hydrogenated over a Pd catalyst in 50% AcOH (65 ml.) untill evolution of CO_2 ceased. The catalyst was removed by filtration and the filtrate evaporated *in vacuo*. The residue was lyophilized. A preliminary test indicated that LAP released 0.02 μmole of histidine from 1.0 μmole of this product. The lyophilized material (0.54 g.) was dissolved in H_2O (30 ml.). To this solution was added 0.1*M* $MgCl_2$ (3 ml.) and 0.1*M* "tris" buffer (1 ml.) and the pH of the solution was adjusted to 8 with 5% NH_4OH. The pre-incubated LAP[12] solution (5.6 ml.) was added. After the solution was incubated at 37° for 18 hr., the pH of the solution was adjusted to 6 with AcOH. The enzyme was denatured by heating for 2 min. in a boiling water-bath and the coagulum was removed by filtration. The filtrate was diluted with H_2O (100 ml.) and the solution was applied to a CMC[13] column (3×25 cm.) which was eluted with the following pyridine acetate buffers (pH 5.0); 0.05*M* (500 ml.) and 0.1*M* (1500 ml.). Individual fractions (15 ml. each) were collected at a flow rate of 3~4 ml. per minute. Absorbancy at 280 mμ served to locate the desired product (tube No. 92~160) in the 0.1*M* eluate. The fractions were pooled, the solvent was evaporated and the residue was lyophilized; yield 0.49 g. (80%), $[\alpha]_D^{23}$ −26.8° (c=0.5, 1*N* HCl), R_f^1 0.49, R_f^2 1.02, sharp ninhydrin, Pauly, Sakaguchi and Ehrlich positive spot, amino acid ratios in acid hydrolysate $His_{1.02}$ $Phe_{0.97}$ $Arg_{1.00}$ $Gly_{1.00}$ (average recovery 92%). LAP released less than 0.02 μmole of histidine from 2.47 μmoles of the product (II). *Anal.* Calcd. for $C_{34}H_{43}O_6N_{11}\cdot CH_3COOH\cdot 3H_2O$: C, 53.0; H, 6.6; N, 18.9. Found: C, 53.1; H, 6.8; N, 18.8.

N^{α}-Benzyloxycarbonyl-N^{G}-nitro-L-arginyl-D-tryptophylglycine Methyl Ester——A mixed anhydride[14] was prepared in the usual manner from N^{α}-benzyloxycarbonyl-N^{G}-nitro-L-arginine[15] (4.41 g.) in

*6 We are indebted to Drs. Tanaka and Otsuka of Shionogi Research Laboratory, Shionogi & Co., Ltd. for these assays.

19) A. B. Lerner, J. D. Case, R. V. Heinzelman: J. Am. Chem. Soc., **81**, 6084 (1959); A. B. Lerner, J. D. Case, Y. Takahashi: J. Biol. Chem., **235**, 1992 (1960). The authors express their sincere appreciation to Dr. S. Lande of School of Medicine, Yale University for determination of this potency.

20) A. Tanaka, B. T. Pickering, C. H. Li: Arch. Biochem. Biophys., **99**, 294 (1962).

ice-cold dioxane (20 ml.) with tributylamine (2.9 ml.) and ethyl chloroformate (1.2 ml.) and this solution was added to a chilled solution of methyl D-tryptophylglycinate hydrochloride[11] (3.86 g.) and triethylamine (5 ml.) in dimethylformamide (25 ml.). The mixture was stirred at 0° for 20 min. and at room temperature for 2 hr. The solvent was evaporated and the resulting oil was dissolved in AcOEt. The solution was washed successively with 5% NH_4OH, 2*N* HCl and a saturated solution of NaCl. The AcOEt phase was dried over Na_2SO_4 and the solvent was evaporated. The resulting product was recrystallized from EtOH; yield 5.55 g. (74%), m.p. 124~126°, $[\alpha]_D^{17}$ +23.6° (c=0.5, MeOH). *Anal.* Calcd. for $C_{28}H_{34}O_8N_8$: C, 55.1; H, 5.6; N, 18.4. Found: C, 54.8; H, 5.7; N, 18.5.

N^G-Nitro-L-arginyl-D-tryptophylglycine Methyl Ester Dihydrobromide Trihydrate——N^α-Benzyloxycarbonyl-N^G-nitro-L-arginyl-D-tryptophylglycine methyl ester (4.49 g.) was treated with 2.5*N* HBr in AcOH (54 ml.) at room temperature for 1 hr. The product precipitated by addition of anhydrous ether, was collected by filtration and dried over KOH pellets and P_2O_5 *in vacuo*; yield 5.18 g. (99%), $[\alpha]_D^{15}$ +28.0° (c=1.0, MeOH), R_f^1 0.56, R_f^2 0.70, single ninhydrin, Ehrlich positive spot. *Anal.* Calcd. for $C_{20}H_{28}O_6N_8 \cdot 2HBr \cdot 3H_2O$: C, 34.7; H, 5.2; N, 16.2. Found: C, 34.5; H, 5.0; N, 16.5.

N-Benzyloxycarbonyl-L-phenylalanyl-N^G-nitro-L-arginyl-D-tryptophylglycine Methyl Ester——(a) A mixed anhydride was prepared in the usual manner from N-benzyloxycarbonyl-L-phenylalanyl-N^G-nitro-L-arginine[15] (5.50 g.) in cold (−10°) tetrahydrofuran (50 ml.) with tributylamine (2.6 ml.) and ethyl chloroformate (1.0 ml.) and this solution was added to an ice-cold solution of D-tryptophylglycine methyl ester hydrochloride (3.13 g.) and triethylamine (1.4 ml.) in dimethylformamide (25 ml.). The mixture was stirred at 0° for 30 min. and at room temperature for 2 hr. After evaporation of the solvents, the oily residue was dissolved in AcOEt, which was washed successively with 5% NH_4OH, 2*N* HCl and H_2O. The AcOEt solution was dried over Na_2SO_4, and the solvent was removed by evaporation. The residue was dissolved in a small volume of MeOH and the solution was treated with AcOEt until precipitation occurred; yield 6.45 g. (85%), m.p. 199~202°, $[\alpha]_D^{15}$ +5.8° (c=0.5, dimethylformamide). *Anal.* Calcd. for $C_{37}H_{43}O_9N_9$: C, 58.6; H, 5.7; N, 16.6. Found: C, 58.5; H, 6.0; N, 16.4.

(b) Ethyl chloroformate (0.77 ml.) was added to an ice-cold solution of N-benzyloxycarbonyl-L-phenylalanine (2.40 g.) in anhydrous dioxane (15 ml.) containing tributylamine (1.9 ml.). The mixture was stirred in an ice-bath for 20 min. The above mixture was added with stirring to a cold dimethylformamide solution (15 ml.) containing N^G-nitro-L-arginyl-D-tryptophylglycine methyl ester (prepared from 4.92 g. of the hydrochloride with 2.2 ml. of triethylamine). The reaction was continued at 0° for 30 min. and at room temperature for 2 hr. The product was isolated as stated in (a); yield 1.34 g. (24%), m.p. 199~202°, $[\alpha]_D^{15}$ +5.3° (c=1.0, dimethylformamide). The product showed no melting point depression on admixture with the compound prepared by (a).

N-Benzyloxycarbonyl-L-phenylalanyl-N^G-nitro-L-arginyl-D-tryptophylglycine Monohydrate——N-Benzyloxycarbonyl-L-phenylalanyl-N^G-nitro-L-arginyl-D-tryptophylglycine methyl ester (2.27 g.) was dissolved in a mixture of MeOH (25 ml.) and dimethylformamide (5 ml.), 1*N* NaOH (6 ml.) was added and the mixture was stirred at 20° for 1.5 hr. The solution was neutralized with AcOH and the solvent was evaporated. The residue was acidified with 1*N* HCl and the precipitate was collected by filtration and recrystallized from MeOH; yield 1.29 g. (56%), m.p. 158~160°, $[\alpha]_D^{17}$ +5.1° (c=1.2, dimethylformamide). *Anal.* Calcd. for $C_{36}H_{41}O_9N_9 \cdot H_2O$: C, 56.8; H, 5.7; N, 16.5. Found: C, 57.3; H, 6.0; N, 16.5.

L-Phenylalanyl-L-arginyl-D-tryptophylglycine Monoacetate Dihydrate——N-Benzyloxycarbonyl-L-phenylalanyl-N^G-nitro-L-arginyl-D-tryptophylglycine (1.52 g.) was hydrogenated over a Pd catalyst in a mixture of MeOH (15 ml.) and 75% AcOH (20 ml.). The catalyst was removed by filtration and the solvent evaporated. The residue was lyophilized. The product was dissolved in H_2O and the solution was applied to a CMC[13] columm (3×25 cm.). The tetrapeptide was eluted with 0.025*M* ammonium acetate buffer (pH 6.9). The solvent was evaporated and ammonium acetate was removed by repeated lyophilization; yield 0.85 g. (63%), $[\alpha]_D^{23}$ +34.0° (c=1.0, 1*N* HCl), R_f^1 0.56, R_f^2 1.0, sharp ninhydrin, Sakaguchi and Ehrlich positive spot. *Anal.* Calcd. for $C_{28}H_{36}O_5N_8 \cdot CH_3COOH \cdot 2H_2O$: C, 54.5; H, 6.9; N, 16.9. Found: C, 54.0; H, 6.7; N, 17.4.

N^α-Benzyloxycarbonyl-L-histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine Dihydrate——An ice-cold AcOEt solution (15 ml.) of N^α-benzyloxycarbonyl-L-histidine azide[16] (prepared from 0.57 g. of the corresponding hydrazide) was added to an ice-cold mixture of dimethylformamide (15 ml.) and H_2O (1 ml.) containing L-phenylalanyl-L-arginyl-D-tryptophylglycine (0.80 g.) and triethylamine (0.2 ml.). The reaction mixture was stirred at 4° for 24 hr., then the additional azide (prepared from 0.28 g. of the hydrazide) was added. The reaction was continued for an additional 24 hr. The solvent was evaporated to one-half volume. The product precipitated by addition of H_2O was collected and recrystallized from MeOH; yield 0.43 g. (40%), m.p. 206~210°, $[\alpha]_D^{30}$ −10.0° (c=1.0, dimethylformamide), R_f^1 0.70, R_f^2 1.7, ninhydrin negative, Pauly, Sakaguchi and Ehrlich positive spot. *Anal.* Calcd. for $C_{42}H_{49}O_8N_{11} \cdot 2H_2O$: C, 57.8; H, 6.1; N, 17.7. Found: C, 57.5; H, 6.4; N, 17.3.

L-Histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine Monoacetate Trihydrate——N^α-Benzyloxycarbonyl-L-histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine (0.34 g.) was hydrogenated over a Pd catalyst in 50% AcOH (15 ml.). After filtration the solvent was evaporated and the residue lyophilized. The product was dissolved in H_2O (200 ml.) and the solution was applied to a CMC[13] column (3×20 cm.) which

was eluted with the following pyridine acetate buffers (pH 5.0); 0.05*M* (400 ml.) and 0.1*M* (500 ml.). Individual fractions (10 ml.) were collected at a flow rate of 3~4 ml. per minute and absorbancy at 280 mμ was determined for each fraction. The desired material was present in the 0.1*M* eluate (tube No. 96~137) which were pooled, evaporated to a small volume and lyophilized; yield 0.12 g. (38%), $[\alpha]_D^{23}$ +16.5° (c=0.5, 1*N* HCl), R_f^1 0.49, R_f^2 1.02, ninhydrin, Pauly, Sakaguchi and Ehrlich positive spot, amino acid ratios in acid hydrolysate $His_{1.03}$ $Phe_{0.99}$ $Arg_{1.00}$ $Gly_{1.00}$ (average recovery 90%). Amino acid ratios in LAP[12] digest; $His_{1.00}$ (80% recovery) $Phe_{0.80}$ $Arg_{0.46}$. Treatment of the product (Ⅲ) (3.41 μmoles) with trypsin*5 followed by LAP[12] gave His (3.03 μmoles), Phe (2.76 μmoles), Arg (2.81 μmoles), Try (0.16 μmoles), and Gly (0.16 μmoles). *Anal.* Calcd. for $C_{34}H_{43}O_6N_{11}\cdot CH_3COOH\cdot 3H_2O$: C, 53.0; H, 6.6; N, 18.9. Found: C, 52.9; H, 6.8; N, 18.9.

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Summary

The synthesis of two steroisomeric pentapeptides, D-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine and L-histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine was described and their melanocyte-expanding activities have been assayed *in vitro*. It was observed that D-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine is biologically inert while L-histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine possesses 1×10^5 MSH U/g.

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100. Takao Maki, Hiroshi Nakamura, Setsuzo Tejima, and Masuo Akagi: Thiosugars. VII.*1 Synthesis of 1-Thio-2-deoxy-β-D-glucose Derivatives.

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Since several years ago, we have been working on the syntheses of sulfur containing sugar derivatives in order to find some physiologically active material among them. The antitumor activity of our preparations has been tested, and found the intraperitoneal administration of β-D-mannopyranosyl ethylxanthate[1] increases the survival time of *dd* strain of mouse inoculated with Ehrlich ascites carcinoma cells.[2]

In the meanwhile, comparatively a large amount of papers has been reported on the physiological activities of 2-deoxy-D-glucose and it is noteworthy to mention that 2-deoxy-D-glucose is an inhibitor of cancerous growth in animals,[3] antagonists in experimental cancer,[4] and an inhibitor of anaerobic bacteria[5] and influenza virus.[6]

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