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119. Haruaki Yajima, Koichi Kawasaki,*1 Masao Koida,*2 and Saul Lande*3 : Studies on Peptides. X.*4~*6 Synthesis of N-(Histidylphenylalanylarginyl)-5-methoxytryptamine, an Inhibitor of α-Melanocyte-stimulating Hormone *in vitro*.

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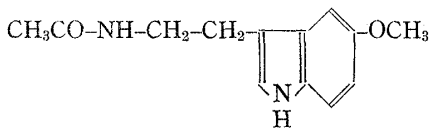
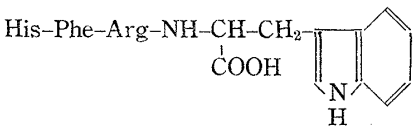
In 1959, Lerner, Case and Heinzelman[1] isolated N-acetyl-5-methoxytryptamine (melatonin) from pineal glands and reported that this compound lightens the color of frog-skin melanocytes by causing melanin granules to aggregate about the nuclei of the cells. These investigators further noted that melatonin reverses the darkening action on frog-skin of melanocyte-stimulating hormone (MSH), adrenocoriticotropic hormone and caffeine.[2]

The major structural feature of melatonin, the tryptamine skeleton, is similar to tryptophan, a constituent of MSH and all MSH-active peptides such as histidylphenylalanylarginyltryptophan[3] which corresponds to positions 6 to 9 in the α-MSH molecule.[4] We have replaced the acetyl moiety of melatonin with a histidylphenylalanylarginyl group in order to compare the potency and specificity of such a compound with those of melatonin and the above stated tetrapeptide. Synthetic N-(histidylphenylalanylarginyl)-5-methoxytrypamine (I) appeares to be a more specific

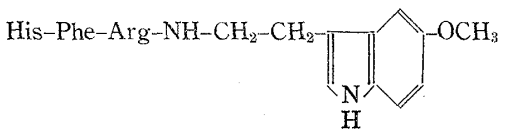
Melatonin :



His-Phe-Arg-Try :



N-(His-Phe-Arg)-5-methoxytryptamine (I) :



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*4 Part Ⅷ : This Bulletin, **14**, 707 (1966).
*5 A preliminary communication reported herein has appeared in Biochim. Biophys. Acta, **107**, 141 (1965).
*6 Unless stated otherwise, the peptide and peptide derivatives described in this communication are of the L-configuration.

1) A. B. Lerner, J. D. Case, R. V. Heinzelman : J. Am. Chem. Soc., **81**, 6084 (1959).
2) A. B. Lerner, J. D. Case, Y. Takahashi : J. Biol. Chem., **235**, 1992 (1960).
3) H. Otsuka, K. Inouye : Bull. Chem. Soc. Japan, **37**, 289, 1465 (1964).
4) J. I. Harris, A. B. Lerner : Nature, **179**, 1346 (1957).

reagent, reversing the *in vitro* darkening action of α-MSH but not caffeine in contrast to melatonin which reverses the effect of both.

During the course of this investigation, we have also assayed the synthetic intermediates, N-arginyl-5-methoxytryptamine (Ⅱ) and N-(phenylalanylarginyl)-5-methoxytryptamine (Ⅲ). Analogues Ⅱ and Ⅲ, similar to melatonin, antagonize the *in vitro* action of both MSH and caffeine. The color lightening potency of all of the peptidyl tryptamines is considerably less than that of melatonin. A detailed account of these investigations are reported in this paper.

The starting material, 5-methoxytryptamine was prepared according to Szmuszkovicz, Anthony and Heinzelman.[5] It was reacted with N^{α}-benzyloxycarbonyl-N^{G}-nitroarginine[6] by the mixed anhydride procedure[6,7] to form N-(N^{α}-benzyloxycarbonyl-N^{G}-nitroarginyl)-5-methoxytryptamine which was then hydrogenated over a palladium catalyst. The product, N-arginyl-5-methoxytryptamine, was purified by ion exchange chromatography on carboxymethylcellulose (CMC)[8] and finally isolated and characterized as the dicitrate salt.

The reaction of a N^{α}-benzyloxycarbonylphenylalanyl-N^{G}-nitroarginine mixed anhydride[6,9] with 5-methoxytryptamine yielded N-(N^{α}-benzyloxycarbonylphenylalanyl-N^{G}-nitroarginyl)-5-methoxytryptamine which was then hydrogenated over a palladium catalyst. The product, N-(phenylalanylarginyl)-5-methoxytryptamine (Ⅲ), was purified by chromatography on CMC.

The preparation of Ⅰ is outlined in Chart 1. N^{α}-Benzyloxycarbonylhistidine azide[10] was reacted with Ⅲ to form N-(N^{α}-benzyloxycarbonylhistidylphenylalanylarginyl)-5-methoxytryptamine, which was then hydrogenated and chromatographed on CMC.

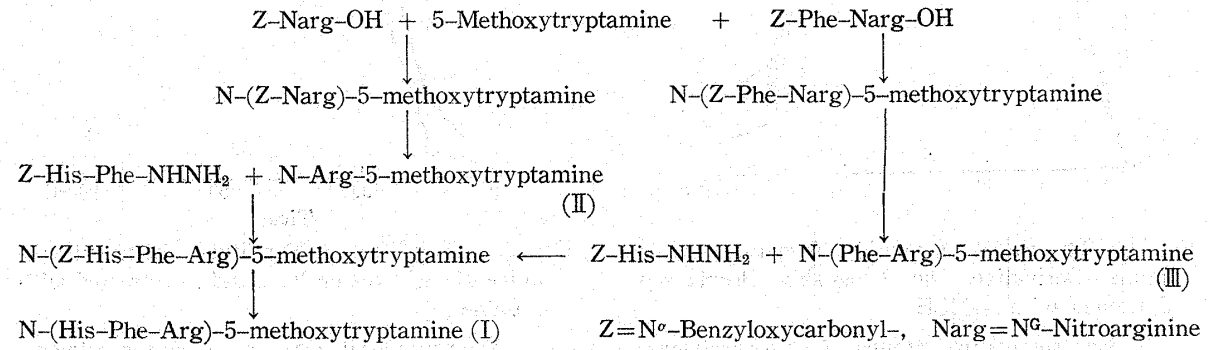

Chart 1. Synthetic Scheme of N-Peptidyl-5-methoxytryptamine Derivatives

The same compound was also prepared by hydrogenolysis of the product obtained by coupling Ⅱ with N^{α}-benzyloxycarbonylhistidylphenylalanine azide[11]. The product (Ⅰ) behaved as a single component on paper chromatography in two different solvent systems. The expected molar ratios of amino acids were obtained after acid hydrolysis (5-methoxytryptamine is destroyed by this procedure). Digestion with leucine aminopeptidase (LAP)[12] released histidine and phenylalanine in nearly equimolar amounts but the recovery of arginine was only 80 per cent of the expected value. Examination of the LAP

5) J. Szmuszkovicz, W. C. Anthony, R. V. Heinzelman: J. Org. Chem., **25**, 857 (1960).
6) K. Hofmann, W. D. Peckham, A. Rheiner: J. Am. Chem. Soc., **78**, 238 (1956).
7) J. R. Vaughan, R. L. Osato: *Ibid.*, **74**, 676 (1952).
8) E. A. Peterson, H. A. Sober: *Ibid.*, **78**, 751 (1956).
9) K. Hofmann, S. Lande: *Ibid.*, **83**, 2286 (1961).
10) R. W. Holly, E. Sondheimer: *Ibid.*, **76**, 1326 (1954).
11) K. Hofmann, H. Kappeler, A. E. Furlenmeier, M. E. Woolner, E. T. Schwartz, T. A. Thompson: *Ibid.*, **79**, 1941 (1957).
12) Partially purified (through second ammonium sulfate fractionation) LAP was prepared according to the procedure of D. H. Spackman, E. L. Smith, D. M. Brown: J. Biol. Chem., **212**, 255 (1955).

digest by paper chromatography revealed the presence of N-arginyl-5-methoxytryptamine in addition to free histidine, phenylalanine, arginine and 5-methoxytryptamine. Compound (I) was prepared by methods similar to those employed in the synthesis of analogous peptides of established optical purity.[9] We attribute the diminished susceptibility to hydrolysis of N-arginyl-5-methoxytryptamine to the slow rate of the enzymatic reaction and not to partial racemization of the arginyl moiety.

The bioassay method employed in these experiments is that described by Lerner and Wright.[13,14] Skin from *Rana pipiens* and *Rana nigromaculata* were used and comparable results were obtained in both cases. Skin was dakened with standard α-MSH for about 1 hour in a bath containing Ringer's solution. Synthetic N-peptidyl-5-methoxytryptamine was then added. In every case lightening in color of frog-skin was observed within 30 minutes as is shown in Fig. 1. The color lightening response was

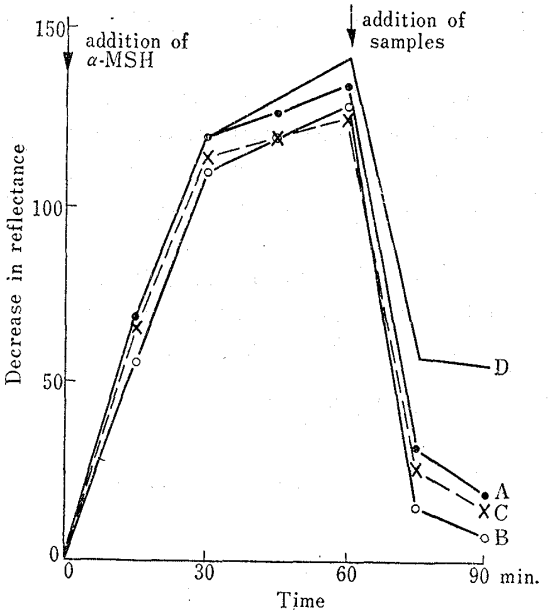

Fig. 1. The Effect of Peptidyl-5-methoxytryptamine Derivatives on Frog-skin Previously Darkened with α-MSH

Four comparable groups of skin-specimens from *Rana pipiens* were darkened with 10 MSH units of standard α-MSH. Changes in reflectance were checked at intervals of 15 minutes. The graph shows the average of the change in reflectance within each group plotted against time. After 75 minutes, 0.2 ml. of a solution of I (2.53 mg./ml), II (2.0 mg./ml.) and III (2.4 mg./ml.) were added to group A, B and C respectively. To group D, 0.2 ml. of melatonin (1×10^{-6} mg./ml.) was added. The total bath volume was 20 ml.

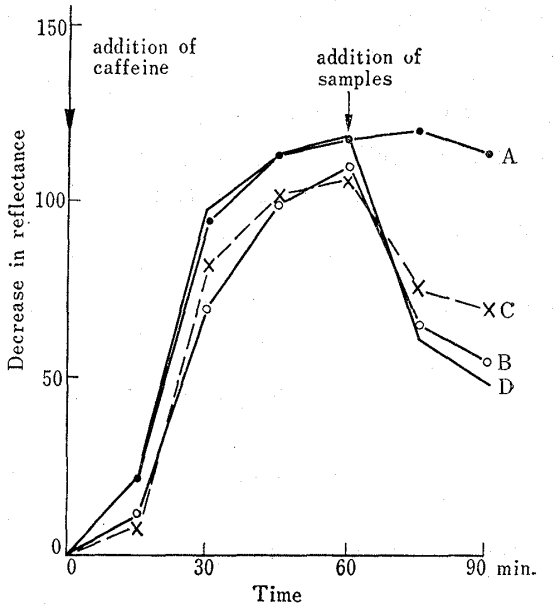

Fig. 2. The Effect of Peptidyl-5-methoxytryptamine Derivatives on Frog-skin Pretreated with Caffeine

Four comparable groups of skin-specimens from *Rana pipiens* were darkened with a Ringer's solution containing caffeine (1 mg./ml.). Changes in reflectance were measured at intervals of 15 minutes. At 60 minutes, 0.2 ml. of a solution of I (3 mg./ml.), II (2 mg./ml.) and III (2 mg./ml.) was added to group A, B and C respectively. To group D, 0.2 ml. of a melatonin solution (1×10^{-6} mg./ml.) was added.

similar to that caused by melatonin although the potency of these compounds is only about one millionth that of the natural product. Difficulty was encountered in preparing highly diluted standard solutions of melatonin with constant activity. Therefore, to determine the relative potancy of the synthetic compounds more accurately, we measured the relative amount of the compound required for a 50 per cent increase of light reflectance of skin predarkened with standard α-MSH. From these experiments, it was estimated that compound (I) was slightly more active than II and III, the

13) A. B. Lerner, M. R. Wright: "Method of Biochemical Analysis," Ed. by D. Glick, 8, 295 (1960), Interscience Publishers, Inc., New York.
14) M. R. Wright, A. B. Lerner: Endocrinology, **66**, 599 (1960).

latter two being nearly equal in activity. We wish to mention also that I reversed the *in vitro* darkening action of the one of active fragments of α-MSH, histidylphenylalanylarginyltryptophylglycine[9,15,16],*7 with the potency of 3×10^4 MSH U/g., in a concentration ratio of 1/1.*8

The behavior of frog-skin darkened for 1 hour in a bath containing caffeine is shown in Fig. 2. The addition of II or III caused lightening of frog-skin, but compound (I) exhibited no lightening activity. In further experiments, a slight increase in light reflectance was noted in a few instances when larger doses of I were employed. In such caffeine darkened skin, however, the 50 per cent level of inhibition was not attained even at concentrations of I that completely reversed MSH darkened skin. Thus I appears to be a poor inhibitor of caffeine compared to II and III.

The above experimental results indicated that the color lightening action of melatonin is maintained even after substitution of the N-acetyl moiety with strongly basic derivatives, such as arginyl, phenylalanylarginyl or histidylphenylalanylarginyl peptides although the potencies of such products are considerably lower than that of the native one.

On comparison of the structure of I and the MSH active tetrapeptide, histidylphenylalanylarginyltryptophan, it can be seen that the difference between the methoxytryptamyl and tryptophyl moieties are responsible for the contrasting physiological properties of these compounds. This relationship is further indication of the requirement for tryptophan residue in MSH-active peptides and lends weight to the view that closely related chemical derivatives of peptide hormones can act as hormone inhibitors. The effect on biological activity of increasing the size of the peptide in N-polypeptidyl-5-methoxytryptamine derivatives is under investigation as is the action of compounds (I, II and III) on chromatophores other than frog-skin melanocytes.

Experimental

General methods employed in these experiments are essentially the same as described in the Part IV[17] of this series. The following abbreviations were used for amino acids: His=histidine, Phe=phenylalanine and Arg=arginine. Rf^1 values refer to the system of Partridge[18] and Rf^2 values refer to the system of 2-butanol-ammonia[19] and expressed as a multiple of the distance traveled by phenylalanine under identical conditions.

5-Methoxytryptamine——This was prepared according to the procedure described by Szmuszkovicz, *et al.*[5] m.p. 119~120°(lit.[5] m.p. 121.5~122.5°). *Anal.* Calcd. for $C_{11}H_{14}ON_2$: C, 69.4; H, 7.4; N, 14.7. Found: C, 69.3; H, 7.2; N, 14.5.

N-(N^{ε}-Benzyloxycarbonyl-N^{G}-nitroarginyl)-5-methoxytryptamine——A mixed anhydride was prepared from N^{ε}-benzyloxycarbonyl-N^{G}-nitroarginine (1.30 g.) in ice-cold tetrahydrofuran (30 ml.) with triethylamine (0.52 ml.) and ethyl chloroformate (0.35 ml.) and added to an ice-cold solution of 5-methoxytryptamine (0.70 g.) in tetrahydrofuran (20 ml.). After stirring in an ice-bath for 3 hr., solvent was removed *in vacuo* and the residue was dissolved in AcOEt. The AcOEt extract was washed successively with 5% NH_4OH, 10% citric acid and H_2O and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue which became crystalline on standing in the cold was collected and washed with cold AcOEt; yield 1.45 g. (75%), m.p. 114~116°, $[\alpha]_D^{20}$ −11.9°(c=1.3, MeOH). *Anal.* Calcd. for $C_{25}H_{31}O_6N_7$: C, 57.1; H, 6.0; N, 18.7. Found: C, 56.9; H, 6.2; N, 18.7.

*7 This pentapeptide was prepared according to the reference 9.

*8 The potency of I could be expressed as 3×10^4-MSH U/g. considering potency of MSH active peptides which is reversed by inhibitors in a concentration ratio of 1/1.

15) K. Hofmann, M. E. Woolner, H. Yajima, G. Spühler, T. A. Thompson, E. T. Schwartz: J. Am. Chem. Soc., **80**, 6458 (1958).
16) R. Schwyzer, C. H. Li: Nature, **182**, 1669 (1958).
17) H. Yajima, K. Kubo: This Bulletin, **13**, 759 (1965).
18) S. M. Partridge: Biochem. J., **42**, 238 (1948).
19) J. F. Roland, Jr., A. M. Gross: Anal. Chem., **26**, 502 (1954).

N-Arginyl-5-methoxytryptamine——N-(N$^{\alpha}$-Benzyloxycarbonyl-N^{G}-nitroarginyl)-5-methoxytryptamine (0.70 g.) in MeOH (12 ml.) containing 30% aqueous AcOH (1.5 ml.) was hydrogenated over a Pd catalyst. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was dissolved in H_2O (30 ml.) and the solution was applied to a CMC column (3×13 cm.) which was successively eluted with the following pyridine acetate buffers at pH 5.0: 0.025*M* (2600 ml.), 0.05*M* (1300 ml.) and 0.1*M* (500 ml.). Aliquots of 17 ml. were collected at a flow rate of 5 to 6 ml. per min. Measurement of absorbancy at 280 mμ served to locate the desired product in the 0.025*M* eluate. The desired aliquots were pooled and solvent was removed *in vacuo*. The residue was lyophilized to give a hygroscopic powder; yield 0.39 g. (64%), Rf^1 0.53, Rf^2 1.01 (single ninhydrin, Sakaguchi and Ehrlich positive spot). Some tailing occurred in the column chromatography and the additional product was obtained from the front portions of the 0.05*M* eluate.

The acetate salt of N-arginyl-5-methoxytryptamine (40 mg.) was dissolved in MeOH (1.5 ml.) and the solution of citric acid monohydrate (42 mg.) in MeOH (1 ml.) was added. On addition of ether, a precipitate formed which was collected by centrifugation and washed with fresh ether; yield 50 mg. (78%), m.p. 115~121°, $[\alpha]_D^{20}$ −16.1° (c=0.3, H_2O). *Anal.* Calcd. for $C_{17}H_{26}O_2N_6 \cdot 2C_6H_8O_7 \cdot H_2O$: C, 46.6; H, 5.9; N, 11.2. Found: C, 46.8; H, 6.3; N, 11.4.

N-(N$^{\alpha}$-Benzyloxycarbonylphenylalanyl-N^{G}-nitroarginyl)-5-methoxytryptamine——A mixed anhydride was prepared from N$^{\alpha}$-benzyloxycarbonylphenylalanyl-N^{G}-nitroarginine[9] (1.45 g.) in ice-cold tetrahydrofuran (20 ml.) with triethylamine (0.4 ml.) and ethyl chloroformate (0.28 ml.) and added to an ice-cold solution of 5-methoxytryptamine (0.55 g.) in tetrahydrofuran (15 ml.). After stirring in an ice-bath for 3 hr., the solvent was evaporated, the residue was dissolved in AcOEt. The organic phase was washed successively with 5% NH_4OH, 10% citric acid and H_2O and dried over Na_2SO_4. The solvent was evaporated *in vacuo* and the residue triturated with ether. The solid product was precipitated twice from AcOEt by addition of ether; yield 1.68 g. (86%), m.p. 125°, $[\alpha]_D^{20}$ +7.3° (c=1.1, MeOH). *Anal.* Calcd. for $C_{34}H_{40}O_7N_8$: C, 60.8; H, 6.0; N, 16.7. Found: C, 60.6; H, 6.3; N, 16.5.

N-(Phenylalanylarginyl)-5-methoxytryptamine Diacetate——N-(N$^{\alpha}$-Benzyloxycarbonylphenylalanyl-N^{G}-nitroarginyl)-5-methoxytryptamine (0.67 g.) in MeOH (20 ml.) containing 20% aqueous AcOH (1.23 ml.) was hydrogenated over a Pd catalyst. After filtration and evaporation of solvent *in vacuo*, the residue was dissolved in H_2O (100 ml.). The solution was applied to a CMC column (3×18 cm.) which was eluted with the following pyridine acetate buffers at pH 5.0: 0.01*M* (390 ml.), 0.02*M* (425 ml.), 0.05*M* (850 ml.), 0.1*M* (2700 ml.), 0.2*M* (1150 ml.) and 0.5*M* (1600 ml.). Aliquots of 17 ml. were collected and measurement of absorbancy at 280 mμ served to locate the desired compound in the 0.1*M* eluate (tube No. 192~300). The contents of these tubes were pooled, concentrated *in vacuo* and the residue lyophilized; yield 0.32 g. (51%), $[\alpha]_D^{25}$ +3.9° (c=0.8, H_2O); Rf^1 0.58, Rf^2 1.5 (single ninhydrin, Sakaguchi and Ehrlich positive spot). *Anal.* Calcd. for $C_{26}H_{35}O_3N_7 \cdot 2CH_3COOH \cdot H_2O$: C, 57.0; H, 7.2; N, 15.5. Found: C, 56.6; H, 7.4; N, 15.6.

N-(N$^{\alpha}$-Benzyloxycarbonylhistidylphenylalanylarginyl)-5-methoxytryptamine——An ice-cold AcOEt solution (approximately 20 ml.) of N$^{\epsilon}$-benzyloxycarbonylhistidine azide[10] (prepared from 0.91 g. of the corresponding hydrazide) was added to a chilled solution of N-(phenylalanylarginyl)-5-methoxytryptamine diacetate (0.93 g.) and triethylamine (0.2 ml.) in dimethylformamide (20 ml.). The mixture was stirred at 4° for 24 hr. After evaporation of the solvent *in vacuo*, the residue was dissolved in H_2O-saturated *n*-BuOH and washed with three portions of *n*-BuOH-saturated H_2O. The solvent was removed *in vacuo* and the residue was triturated with AcOEt, which was then removed by decantation. The residue was dissolved in MeOH and the product was precipitated by addition of AcOEt. This procedure was repeated twice to give a powder; yield 0.74 g. (63%), $[\alpha]_D^{28}$ −6.0° (c=1.0, MeOH); Rf^1 0.81, Rf^2 1.62 (single ninhydrin negative, Pauly, Sakaguchi and Ehrlich positive spot). *Anal.* Calcd. for $C_{40}H_{48}O_6N_{10} \cdot CH_3COOH \cdot H_2O$: C, 59.8; H, 6.5; N, 16.6. Found: C, 59.9; H, 6.4; N, 16.9.

N-(Histidylphenylalanylarginyl)-5-methoxytryptamine——a) Prepared by acylation of N-arginyl-5-methoxytryptamine. The entire operation was performed in a cold room at 4°. N$^{\alpha}$-Benzyloxycarbonylhistidylphenylalanine hydrazide[11] (0.23 g.) was dissolved in 1*N* HCl (4 ml.) and in an ice-bath, a cold solution of $NaNO_2$ (0.04 g.) in H_2O (1 ml.) was added. After 5 minutes, the solution was made alkaline with a solution of 50% K_2CO_3. The precipitated azide was collected by filtration and washed with cold H_2O. This solid azide was added with stirring to a solution of N-arginyl-5-methoxytryptamine diacetate (0.11 g.) and triethylamine (0.04 ml.) in dimethylformamide (7 ml.). After 24 hr., additional azide (prepared from 0.23 g. of the hydrazide) was added and the reaction was continued for an additional 48 hr. At this stage, an examination of the reaction mixture by paper chromatography revealed the disappearance of the ninhydrin positive spot corresponding to arginyl-5-methoxytryptamine. The solvent was removed *in vacuo* and the residue was dissolved in a mixture of MeOH (30 ml.) and 50% aqueous AcOH (4 ml.) and subjected to hydrogenolysis over a Pd catalyst. After filtration, the solvent was evaporated *in vacuo* and the residue was lyophilized. The product was dissolved in H_2O (15 ml.) and applied to a column of CMC (1×15 cm.). The desired compound was eluted with some tailing by 0.25*M* pyridine acetate buffer at pH 5.0. After evaporation and lyophilization, a hygroscopic powder was obtained; yield 0.13 g. (72%), $[\alpha]_D^{24}$ −8.0° (c=0.5, H_2O); Rf^1 0.49, Rf^2 1.7 (single ninhydrin, Pauly, Sakaguchi and Ehrlich positive spot); amino acid ratios in an acid hydrolysate: $His_{1.14}Phe_{1.00}Arg_{0.97}$ (average recovery 89%); amino acid ratios in a LAP digest: $His_{1.11}Phe_{1.00}Arg_{0.80}$; examination of the hydrolysate by

paper chromatography in the system of Partridge revealed the presence of a faint spot of N-arginyl-5-methoxytryptamine (Rf^1 0.53, positive to ninhydrin, Sakaguchi and Ehrlich reagents) as well as the spots corresponding to free histidine, phenylalanine, arginine and 5-methoxytryptamine. *Anal.* Calcd. for $C_{32}H_{42}O_4N_{10}\cdot 2CH_3COOH\cdot 3H_2O$: C, 53.7; H, 7.0; N, 17.4. Found : C, 53.8; H, 7.7; N, 17.5.

b) Prepared by hydrogenolysis of the N^{α}-benzyloxycarbonyl derivative. N-(N^{α}-Benzyloxycarbonylhistidyl-phenylalanylarginyl)-5-methoxytryptamine (0.74 g.) was dissolved in a mixture of MeOH (20 ml.), H_2O (1 ml.) and AcOH (0.3 ml.) and hydrogenated over a Pd catalyst for 14 hr. The catalyst was removed by filtration, the filtrate was condensed *in vacuo* and the residue was applied to a column of CMC (3×20 cm.). The desired compound was purified as described in (a); yield 0.67 g. (97%).

Bioassay

Skin from male *Rana pipiens* or *Rana nigromaculata* was removed and stretched on plastic rings and soaked for 1 hr. in dishes containing 30 ml. of Ringer's solution before use. Four skin-specimens were used in each experiment. Darkening or lightening in color of isolated pieces of frog-skin was measured with a photoelectric photometer as described by Lerner and Wright[13,14] and each value was expressed as an average response of four specimens.

Fig. 1 and 2 illustrate the results obtained with skin-specimens from *Rana pipiens*. Table I lists the results obtained with skin-specimens from *Rana nigromaculata*. In the latter experiments α-MSH or caffeine was allowed to act for 40 minutes, after which the tryptamine derivatives were added and change in reflectance were measured after an additional 40 minutes. The amount used in these experiments is expressed as μg. per ml. in the final bath-volume (25 ml.). The required amount for 50% inhibition was obtained graphically from changes in reflectance caused by three different concentrations of the derivatives under study. In this table, increase of light reflectance (lightening in color) is expressed with a minus sign, while decrease (darkening) is shown with a plus sign.

Table I. Relative Potency of N-Peptidyl-5-methoxytryptamine Derivatives on Skin of *Rana nigromaculata*

Sample	Darkened by α-MSH (0.8×10^{-4} μg./ml., total 40 MSH U)			Darkened by caffeine (1 mg./ml.)	
	Amount used (μg./ml.)	Change in Reflectance (%)	Required for 50% inhibition (μg./ml.)	Amount used (μg./ml.)	Change in Reflectance (%)
II	5	− 26			
	10	− 40	12	20	−12
	20	− 83			
III	5	− 7			
	10	− 40	12	20	−11
	20	− 81			
I	5	− 48			
	10	− 68	5	20	+49
	20	−100			(−11)

The lightening activity of these compounds on α-MSH darkened skin of *Rana nigromaculata* was similar to that observed in *Rana pipiens*. When frog-skin of *Rana nigromaculata* was darkened with a solution of histidylphenylalanylarginyltryptophylglycine[9] (50 μg./ml., the sample possesses the activity of 3×10^4 MSH U/g.), a complete inhibition was obtained by I in the concentration of 50 μg./ml. As shown in Table I, there was some instance that caffeine-darkened skin was lightened by compound (I). But even in such case, change in light reflectance was only in the level of 11% at the concentration of 20 μg./ml. that completely reversed MSH darkened skin.

The authors express their gratitude to Prof. S. Uyeo for his encouragement during the course of this investigation. The skillful technical assistance of Miss K. Takigawa and Mr. Stanko Kulovich is gratefully acknowledged.

Summary

The N-acetyl group of melatonin was replaced with a histidylphenylalanylarginyl moiety. The product, N-(histidylphenylalanylarginyl)-5-methoxytryptamine, reversed the darkening action of α-melanocyte-stimulating hormone (MSH) on frog-skin *in vitro* in contrast to the closely related tetrapeptide, histidylphenylalanylarginyltryptophan which exhibits intrinsic MSH activity. The synthetic intermediates, N-arginyl-5-methoxytryptamine and N-(phenylalanylarginyl)-5-methoxytryptamine also reversed the action of α-MSH. The color lightening potency of these compounds was about one millionth that of melatonin.

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120. Hiroshi Hikino, Keitaro Aota, and Tsunematsu Takemoto : Structure and Absolute Configuration of Cyperotundone.*1

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The tuber of nutgrass (*Cyperus rotundus* LINNÉ (Cyperaceae)), widespread throughout the tropical and temperate zone, has long been used in folk drug and native perfumes. In Japan, it is called "Kō-bushi" and utilized as a Chinese medicine for women's diseases. Although the composition of the essential oil has been studied by a number of workers, it is only known in part.[1] Two oils of Japanese nutgrass were examined by Kimura, *et al.*[2] who described the presence of a hydrocarbon fraction and an alcohol fraction which were termed cyperene and cyperol, respectively. However, no ketonic component was found in this report,[2] though the foreign oils have been shown to contain a large amount (33~54%) of ketones.[3] In order to ascertain the composition of the oil of Japanese origin, we have re-investigated it and isolated besides α-cyperone as one of the main constituents, a sesquiterpenoid ketone of molecular formula $C_{15}H_{22}O$ for which the name cyperotundone is proposed. In a preliminary communication,[4] we have reported the structure and absolute stereochemistry of cyperotundone as shown in formula I. The present paper provides the evidence in full detail.

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*1 This paper constitutes Part Ⅳ in the series on Sesquiterpenoids. Preceding paper, Part Ⅲ, H. Hikino, Y. Takeshita, Y. Hikino, T. Takemoto : This Bulletin, **14**, 735 (1966).

*2 Kita-4-bancho, Sendai (ヒキノヒロシ, 青田恵太郎, 竹本常松).

1) For historical aspects of the research, see E. Gildemeister, F. Hoffmann : "Die Ätherischen Öle," Vol. Ⅳ, 425 (1956). Akademie Verlag, Berlin.

2) Y. Kimura, M. Otani : Yakugaku Zasshi, **48**, 971 (1928).

3) A. E. Bradfield, B. H. Hegde, B. S. Rao, J. L. Simonsen, A. E. Gillam : J. Chem. Soc., **1936**, 667.

4) H. Hikino, K. Aota, T. Takemoto : This Bulletin, **13**, 628 (1965).