MODIFICATION OF THE DISULFIDE BRIDGE IN CYCLIC MELANOTROPINS*

Michal LEBL[a]**, Wayne L. CODY[a], Brian C. WILKES[a], Victor J. HRUBY[a]***, Anna M. de L. CASTRUCCI[b] and Mac E. HADLEY[b]

[a] *University of Arizona, Department of Chemistry, Tucson, AZ 85721, U.S.A. and*
[b] *University of Arizona, Departments of Anatomy and Molecular and Cellular Biology, Tucson, AZ 85721, U.S.A.*

Received May 8th, 1984

We have prepared a cyclic analog containing a thiomethylene bridge in place of the disulfide bridge in order to further analyze the importance of a cyclic structure for the biological activity of α-MSH (α-melanotropin, α-melanocyte stimulating hormone) and to determine if the biological activity of [Cys^4,Cys^{10}]-α-MSH is related to disulfide interchange. The carba analog was based on the highly potent Ac-[Cys^4,Cys^{10}]-α-MSH_{4-13}-NH_2 fragment analog in which cysteine in residue 4 was replaced by 3-mercaptopropanoic acid (Mpa). This carba analog was slightly less active than the disulfide bridged compound in both *in vitro* bioassay systems tested (frog and lizard skin systems). Oxidation of the carba analog to the sulfoxide led to a moderate loss of activity in both assays. We also reduced the 4—13 disulfide-containing compound [Mpa^4, Cys^{10}]-α-MSH_{4-13}-NH_2 and examined the reduced analog for melanotropic activity. It was found that opening of the disulfide led to a large loss of potency. The bis-sulfhydryl compound may have been non-specifically bound to, and/or internalized inside the tissue, but did not show any inhibitory activity.

Cyclic analogs of α-MSH† that have high biological activities have recently been designed, synthesized and studied for their biological properties in several receptor systems. These compounds allowed us to analyse the importance of the peptide chain length[2-4], stereochemistry of individual amino acid residues within the cyclic structure[5-7], and the size of the disulfide ring[5,6]. In these studies, one possible explanation for the high potency of these disulfide-containing peptides is that they participate in a disulfide exchange mechanism during their interaction with the

* Part VIII in the series Cyclic Melanotropins, Part VII see ref.[5]. Part CLXXXIX in the series Amino Acids and Peptides; Part CLXXXVIII: This Journal *49*, 2562 1984.

** Permanent address: Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 166 10, Czechoslovakia.

*** Person to whom reprint requests should be addressed.

† All the chiral amino acids mentioned in this work are of L-series. The nomenclature and symbols of the amino acids and peptides follow the published recommendations[1].

receptor. This possibility was considered in the proposed mechanism of action of neurohypophyseal hormones[8], but was ruled out by the syntheses and pharmacological studies of carba-analogs of these hormones[9]. We thus decided to use this modification in an effort to better understand the mechanism of action of the cyclic melanotropin analogs.

It was known from previous studies[10] that α-MSH sensitive receptors could be effectively blocked by compounds containing a free sulfhydryl group. The reduced form of the cyclic [Cys^4,Cys^{10}]-α-MSH analogs contains two free sulfhydryl groups and might turn out to be either a specific antagonist of α-MSH, or an analog with prolonged agonist activity due to strong binding affinity to the receptor. Therefore we also have investigated the biological activities of the reduced form of the potent disulfide group containing analog *II*.

Syntheses of carba-analogs can be accomplished by a variety of methods, but the most convenient involves cyclization *via* an amide bond. This procedure has been used in the synthesis of many biologically active peptides including: oxytocin[9,11-14], vasopressin[15-18], vasotocin[19], somatostatin[20-22], calcitonin[23-25], and the A-chain of insulin[26]. The cyclization was usually carried out using a non-sterically hindered and, if possible, racemization-resistant amino acid. In the case of α-MSH, we have shown[5] that the acetylamino group in position 4 in compound *I* is not required for the full biological activity of Ac-[Cys^4,Cys^{10}]-α-MSH$_{4-13}$-NH$_2$ (*i.e.*, substitution of 3-mercaptopropanoic acid for cysteine-4 in analog *II* results in no loss in potency). This prompted us to synthesize an α-MSH analog *III* with S-(2-carboxyethyl)-homocysteine in position 10 and to cyclize it *via* the ω-carboxyl group of this residue. We synthesized the analog *III* by solid phase methodology, though we were aware that the cyclization might be accompanied by a large degree of polymerization. Using a *p*-methylbenzhydrylamine resin of low substitution (0·022 mmol/g), the synthesis was complicated by the need for long reaction times and by difficulties in monitoring the progress of coupling individual amino acids and the cyclization step with Kaiser's test[27]. When using a higher substituted resin (0·36 mmol/g) the cyclization was accompanied by a large degree of polymerization. Individual amino acids were coupled by the dicyclohexylcarbodiimide (DCC)/N-hydroxybenzotriazole (HOBt) method using Boc protected amino acids for the first four residues. After coupling the modified homocysteine residue, the synthesis was continued leaving the α-carboxylic group of S-(2-carboxyethyl)homocysteine free, and utilizing symmetrical anhydrides formed immediately prior to reaction for coupling the remaining amino-acid residues. In order to be sure that dicyclohexylcarbodiimide was not transferred to the reaction mixture we used larger than normal excesses of the Boc-amino acids. Cyclization was achieved on the resin in the presence of dicyclohexylcarbodiimide and N-hydroxybenzotriazole. The cyclic peptide thus obtained was cleaved from the resin and all the protecting groups were removed in liquid hydrogen fluoride containing 10% anisole and 5% 1,2-ethanedithiol[28]. The content

of polymers in the crude peptide was estimated by gel filtration. Purification was accomplished by ion exchange chromatography and gel filtration. The homogeneity of the product was determined by a variety of analytical techniques.

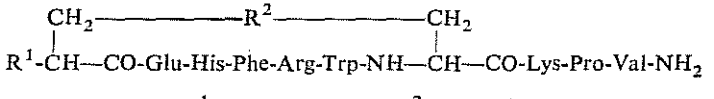
It has shown that carba-analogs of peptides can be oxidized to their sulfoxide form[29]. In the case of oxytocin analogs this usually led to a loss of biological activity[29]. In order to examine the effect of such an oxidation on cyclic α-MSH analogs, we have prepared the sulfoxide of compound *III* by oxidation with potassium periodate. The reaction was monitored by high performance liquid chromatography (HPLC) and the resulting product *IV* was desalted and purified on a reversed phase HPLC column. Proof of the sulfoxide structure was by fast atom bombardment (FAB) mass spectroscopy; MH^+, 1 284·8.

The carba analog *III* has slightly lower potency than the corresponding compound *II* which contains the disulfide bond in both biological assays employed (Fig. 1 and 2). This could be due to the very pronounced sensitivity of this class of compounds to the size of the intramolecular ring[5]. The sulfoxide *IV* was slightly less active in both assays employed (Fig. 1 and 2).

The disulfide bridge of [Mpa^4,Cys^{10}]-α-MSH_{4-13}-NH_2 (*II*) was reduced by sodium in liquid ammonia. This method was used instead of reduction by sulfhydryl groups containing compounds even though it was known that it might, possibly, damage the peptide, because we did not want any other sulfhydryl groups present.

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2

α-MSH



I, $R^1 = CH_3CONH$, $R^2 = S—S$
II, $R^1 = H$, $R^2 = S—S$
III, $R^1 = H$, $R^2 = S—CH_2$
IV, $R^1 = H$, $R^2 = S(O)—CH_2$
V, $R^1 = H$, $R^2 = SH\ HS$

The reduced analog [$Mpa(H)^4$,$Cys(H)^{10}$]-α-MSH_{4-13}-NH_2 (*V*) was dried *in vacuo* and dissolved just prior to biological testing in degassed Ringer solution. The testing was performed simultaneously on the frog and lizard skin assay systems. In both cases reduction of the disulfide led to a very large decrease in potency (in the lizard skin assay about 1 000 fold and in frog skin assay about 10 000 fold loss in activity). We did not observe any prolongation of activity for this reduced peptide, that is, the biological response was readily reversed by removing the reduced peptide from the bathing medium. In addition when tested at concentrations which displayed no intrinsic biological activity, no inhibition of α-MSH action was observed. In order to prove

that we were really dealing with the reduced compound *V* containing free SH groups, we reformed the disulfide *via* oxidation. By bubbling air through the stock solution of the reduced peptide *V* we were able to restore 30% of the original activity of [Mpa^4,Cys^{10}]-α-MSH$_{4-13}$-NH$_2$. Interestingly, bubbling air through a solution of the reduced peptide in the presence of the tissue led to no restoration of the lost activity. It is possible that the peptide in its free sulfhydryl form incorporates itself very rapidly and nonspecifically into the tissue at the concentration used in the assay (10^{-8} mol l^{-1}), such that the concentration of active substance in solution is essentially zero. The observed low activity of the bis-sulfhydryl compound could be due to a small contamination by its oxidized disulfide-containing form, but we have no evidence for this.

The above results clearly show that the high biological activity of cyclic α-MSH analogs is not due to disulfide interchange with the receptor, and that the cyclic structure is important mainly for maintaining the proper conformation which favors

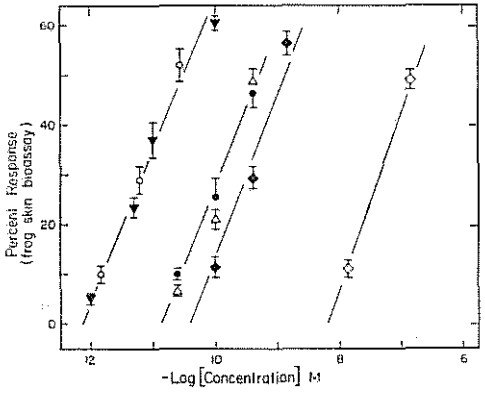


FIG. 1

Relative *in vitro* dose response curves of some α-MSH analogs determined in the frog (*Rana pipiens*) skin bioassay. Each value represents the mean, ± S.E., response (darkening) of the skins (N = 6 or more for each value in all experiments) to the melanotropins at the concentrations indicated (concentrations are given in mol l^{-1}). ● α-MSH; ○ *I*; ▼ *II*; △ *III*; ◆ *IV*; ◇ *V*

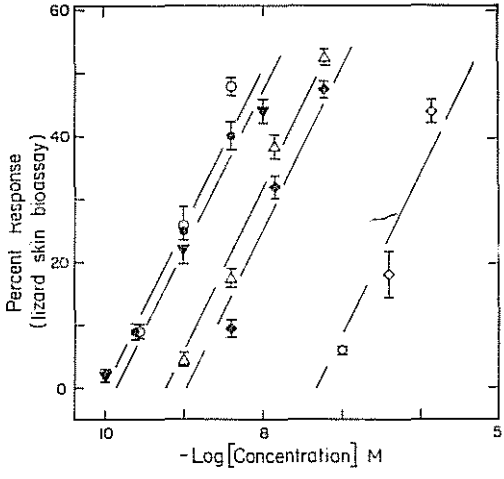


Fig. 2

Relative *in vitro* dose response curves of some α-MSH analogs determined in the lizard (*Anolis carolinensis*) skin bioassay. Conditions and symbols are the same as in Fig. 1

interaction with the receptor. The small effect of oxidation of the sulfur atom to the sulfoxide group on the biological activity of the carba analog is additional evidence in favor of the above statements. In addition, the results of these studies can be interpreted as indicating that the disulfide bridge of the cyclic α-MSH analogs is not involved in interaction with the receptor.

EXPERIMENTAL

General Methods

Capillary melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Silufol plates (Kavalier, Czechoslovakia) using the following solvent systems: *A*) 1-butanol–acetic acid–water (4 : 1 : 5, upper phase only); *B*) 1-butanol–acetic acid–pyridine–water (15 : 3 : 10 : 12); *C*) 1-butanol–pyridine–acetic acid–water (5 : 5 : 1 : 4); *D*) 2-propanol–25% aqueous ammonia–water (3 : 1 : 1). Detection was by iodine vapors and ninhydrin. ^{1}H NMR spectra were recorded on a Perkin–Elmer EM-360L spectrophotometer. Fast atom bombardment mass spectra were obtained on a Varian 311A spectrometer equipped with an Ion Tech Ltd. source with xenon as the bombarding gas. Electrophoresis was performed on Whatman 1 chromatography paper using an instrument by Gelman and 0·1 mol l^{-1} pyridinium acetate pH 5·3 at a potential drop of 20 V/cm. Detection utilized ninhydrin and the chlorination method[30]. Amino-acid analyses were obtained with a Beckman 120C amino-acid analyzer following hydrolysis for 22 h at 110°C with 4 mol l^{-1} methanesulfonic acid containing 0·2% 3-(2-aminoethyl)indole[31]. No corrections were made for destruction of amino acids during hydrolysis. High pressure liquid chromatography (HPLC) was performed on a Spectra-Physics SP-8700 instrument equipped with an SP-8400 detector, using a Vydac C18 column (25 × 0·46 cm) for analysis and a Waters RCM column (15 × 0·8 cm) for preparative work. We used 0·1% trifluoroacetic acid as the buffer and acetonitrile as the organic modifier.

N^{α}-Boc-protected amino acids and amino acid derivatives were prepared using published procedures. Before use, all amino acid derivatives were subjected to the ninhydrin test[27] for purity testing. Solvents used for chromatography were purified as previously reported[32]. The *p*-methylbenzhydrylamine resin (0·022 and 0·36 mmol/g, 1% divinylbenzene cross-linked polystyrene) was prepared by previously reported methods[33].

[Deamino-10-carba,4—10 cyclo]-α-MSH_{4-13}-NH_2 ([Hcy$(C_2H_4CO)^{10\to4}$]-α-MSH_{4-13}-NH_2) (*III*)

A) *p*-Methylbenzhydrylamine resin (4 g) was substituted with Boc-valine (0·022 mmol/g). The Boc protecting group was removed with 45% trifluoroacetic acid containing 2% anisole in dichloromethane in two treatments (30 ml for 2 and 20 min each). Subsequently, Boc-Pro, Boc-Lys(2,4-Cl_2Z) and Boc-Hcy(C_2H_4COO-t-Bu) were coupled by standard coupling methods[33] using dicyclohexylcarbodiimide (DCC) (11 fold excess) and N-hydroxybenzotriazole (HOBt) (8 fold excess). After removing the protecting groups from the coupled homocysteine derivative, subsequent couplings were performed using symmetrical anhydrides formed by the reacting appropriate amino acid derivative (12 fold excess) with DCC (5 fold excess) in dichloromethane or dimethylformamide at 0°C for 3 h. Because of difficulties in following the course of coupling by ninhydrin, all couplings were 22 h long. After the coupling of all amino acids (in order: Boc-Trp(For), Boc-Arg(Tos), Boc-Phe, Boc-His(Tos), Boc-Glu(OBzl)) the protecting

group from the amino terminal was removed and cyclization was performed in the presence of DCC (20 fold excess) and HOBt (20 fold excess). After 40 h the resin gave a negative Kaiser's test[27]. The peptide resin was washed successively with dichloromethane (3 × 10 ml), ethanol (3 × 10 ml), and dichloromethane (4 × 10 ml), dried *in vacuo* (4·1 g) and the peptide was cleaved from half of the resin (2·05 g) in liquid hydrogen fluoride (25 ml), with anisole (3 ml) and 1,2--ethanedithiol (1·5 ml) at 0°C. After 60 min the liquid hydrogen fluoride was removed *in vacuo* and the resin was washed with ethyl acetate (3 × 30 ml). The peptide was extracted with 30% acetic acid (3 × 60 ml), 10% acetic acid (3 × 60 ml), and water (3 × 50 ml). Lyophilization of the extracts gave 38 mg (66%) of a powder, which was suspended in 5 ml of 0·01 mol l^{-1} ammonium acetate (pH 4·5). The mixture was centrifuged and the supernatant was applied to a carboxymethylcellulose cation exchange column (2 × 20 cm). The column was successively eluted with 0·01 mol l^{-1} ammonium acetate, pH 4·5 and 0·1 mol l^{-1} ammonium acetate, pH 6·8 (250 ml each). The appropriate peaks were collected (280 nm detection), lyophilized and tested for α-MSH activity in the lizard skin. Significant activity was found only in one peak, which was further purified by reversed phase HPLC on an Altech C-18 column (25 × 0·46 cm) in a gradient of acetonitrile in 0·1% trifluoroacetic acid. A pure compound (1·3 mg, 1·1%) was obtained (TLC and HPLC).

B) A second synthesis was performed on a *p*-methylbenzhydrylamine resin (2 g) with an amine content of 0·36 mmol/g. For the coupling of the first four amino acids (Boc-Val, Boc-Pro, Boc-Lys(2,4-Cl_2Z), and Boc-Hcy(C_2H_4COO-t-Bu)) we used a four fold excess of the protected amino acids and DCC and a 3·5 fold excess of HOBt. For further couplings symmetrical anhydrides were used, formed from the protected amino acid (in order: Boc-Trp(For), Boc-Arg(Tos), Boc-Phe, Boc-His(Tos), and Boc-Glu(OBzl)) using an 8 fold excess of an amino acid and a 3·3 fold excess of DCC. The coupling were monitored by Kaiser's test, and the cyclization, complete in 24 h, was performed as outlined in *A*). Half of the resin was treated with liquid hydrogen fluoride as described in *A*), and 495 mg of crude peptide was obtained after lyophilization. The amount of high molecular weight products was determined by gel filtration on 5 mg of the crude peptide on a Bio-gel P-4 column (100 × 1·0 cm) in 3 mol l^{-1} acetic acid. The lyophilizate (490 mg) was suspended in 40 ml of 0·01 mol l^{-1} ammonium acetate pH 4·5 buffer, filtered through a Millipore filter (0·2 μm) and introduced onto a carboxymethylcellulose column (25 × 2·5 cm). It was eluted with a discontinuous gradient of 0·01 mol l^{-1} ammonium acetate (pH 4·5, 300 ml), 0·1 mol l^{-1} ammonium acetate (pH 6·8, 300 ml), and 10% acetic acid (300 ml). The fraction eluted at the same volume as [Mpa^4,Cys^{10}]-α-MSH_{4-13}-NH_2 was collected, lyophilized and further purified by gel filtration on Bio-Gel P-4 (100 × 1·0 cm) in 1 mol l^{-1} acetic acid; 13·2 mg (2·4%) of the purified peptide (HPLC and TLC) was obtained. The product, $k' = 2·5$ (Vydac C-18, acetonitrile–0·1% trifluoroacetic acid, 1 : 4) was oxidized by KIO_4, and this resulted in a new peak on HPLC ($k' = 1·42$) which was isolated. R_F: 0·05 (A), 0·58 (B), 0·56 (C), 0·59 (D), $[\alpha]_D$ −55·7° ($c = 0·2$, 10% acetic acid); $[\alpha]_{546}$ −69·9° ($c = 0·2$, 10% acetic acid). Amino-acid analysis: Trp 0·97, Lys 1·07, His 0·98, Arg 0·98, Glu 1·01, Pro 1·02, Val 1·08, Hcy(C_2H_4COOH) 1·03, Phe 0·94. For $C_{60}H_{85}N_{17}O_{12}S.4\,C_2H_4O_2.5\,H_2O$ (1 599); calculated: 51·08% C, 6·99% H, 14·89% N; found: 51·20% C, 6·41% H, 14·68% N. Mass spectrum: MH^+ 1 268·5.

Sulfoxide of [Hcy(C_2H_4CO)$^{10\to4}$]-α-MSH_{4-13}-MH_2 (*IV*)

The carba-analog *III* (4·5 mg) was dissolved in water (200 μl) and a saturated solution of KIO_4 in water (100 μl) was added. Progress of the reaction was followed by HPLC (Vydac C-18; 25 × × 0·46 cm; acetonitrile–0·1% trifluoroacetic acid, 1 : 4). After disappearance of starting compound *III*, the solution was injected on a Waters C-18 Radial Pak column (10 × 0·8 cm) and peptide was eluted by a gradient of acetonitrile in 0·1% trifluoroacetic acid (5 to 25% in 20 min; flow

rate 4 ml/min). After lyophilization, 1·3 mg of compound *IV* was obtained. Mass spectrum: MH^+ 1 284·8.

Experiments with the Reduced form of [Mpa^4,Cys^{10}]-α-MSH_{4-13}-NH_2

[Mpa^4,Cys^{10}]-α-MSH_{4-13}-NH_2 (*II*) (3 mg) was dissolved in liquid ammonia (6 ml) and reduced with metallic sodium (until blue color persisted for 30 s). The solution was decolorized with ammonium chloride and lyophilized. The residue was dissolved in degassed Ringer's solution (as a control, degassed Ringer's solution and α-MSH was tested in the bioassay systems with no loss of biological activity) to a final concentration of 10^{-4} mol l^{-1}. Further solution, 10^{-6} to 10^{-9} mol l^{-1} were prepared by serial dilution of the 10^{-6} mol l^{-1} stock solution. The solutions were used immediately in the bioassays and the results are summarized in Figs 1 and 2. In order to verify that the solutions actually contained the reduced peptide we tried to reform the disulfide by bubbling air through the solution. By bubbling the stock solution (10^{-4} mol l^{-1} we were able to restore 30% of the original activity of [Mpa^4,Cys^{10}]-α-MSH_{4-13}-MH_2. Upon aeration of a solution that was in contact with the lizard skin no restoration of α-MSH activity (in both cases the final concentration was 10^{-8} mol l^{-1}) was observed.

S-(2-Tert-butyloxycarbonylethyl)homocysteine

Homocysteine (2·0 g) was dissolved in liquid ammonia (100 ml) and reduced with metallic sodium until a blue color persisted for 5 min. The solution was decolorized with ammonium chloride and tert-butyl 3-chloropropanoate (2·8 g) was added. The solution was evaporated over 1 h by a stream of nitrogen and dried *in vacuo*. The residue was dissolved in water (60 ml) and extracted with ethyl acetate (2 × 30 ml). The aqueous layer was cooled to 0°C and placed on a Dowex 50 column (5 × 5 cm, H^+-cycle). The Dowex 50 was washed until neutral and the product was eluted with 5% ammonia. The solution was evaporated to give 3·85 g (97%) of a powder. R_F: (values in parenthesis are for the dicarboxylic acid formed by treatment with trifluoroacetic acid) A: 0·28 (0·17), B: 0·68 (0·50), C: 0·56 (0·28), D: 0·68 (0·50). For further analysis the sample was recrystallized from water. $[\alpha]_D$ −0·85° (c = 2, 1 mol l^{-1} acetic acid). For $C_{11}H_{21}NO_4S$ (263·4) was calculated: 50·17% C, 8·04% H, 5·32% N; found: 49·92% C, 8·27% H, 5·14% N.

N^{α}-Tert-butyloxycarbonyl-S-(2-tert-butyloxycarbonylethyl)homocysteine Dicyclohexylammonium Salt

S-(2-Tert-butyloxycarbonylethyl)homocysteine (3·8 g) was dissolved in water (130 ml) and the pH was adjusted to 10 with 4 mol l^{-1} NaOH. To the solution 50 ml of n-butanol was added, followed by 6·2 g of Boc-dicarbonate and the mixture was allowed to stir for 1 h at room temperature maintaining the pH at 10. The solution was diluted with water (120 ml) and extracted with hexane (3 × 30 ml). The aqueous layer was cooled to 0°C, acidified with 6 mol l^{-1} HCl to a pH 2, extracted with ethyl acetate (3 × 60 ml), washed with brine (50 ml), dried with Na_2SO_4, and concentrated. The oily product was dissolved in ethyl acetate, 2·83 ml of dicyclohexylamine was added and the solution was diluted with light petroleum (300 ml) and allowed to stand at 0°C. The crystalline product was filtered and dried *in vacuo* to give 6·09 g (77%). M.p. 129—130°C, R_F 0·81 (A), 0·74 (B), 0·69 (C), 0·73 (D). $[\alpha]_D$ +15·6° (c = 1·8, ethanol). ^1H NMR (free amino acid derivative): (δ, C^2HCl_3): 3·79 (t, J = 6·3 Hz, α-CH, 1-H), 2·89 (t, J = 7·4 Hz, CH_2—CO, 2 H), 2·74 (t, J = 7·6 Hz, CH_2—S, 2 H), 2·66 (t, J = 7·4 Hz, S—CH_2—CH_2—CO, 2 H), 2·09 (m, β-CH_2, 2 H), 1·60 and 1·58 (s, $C(CH_3)_3$, 9 H). For $C_{28}H_{52}N_2O_6S$ (544·8) was calculated: 61·73% C, 9·62% H, 5·14% N; found: 61·66% C, 9·73% H, 5·03% N.

Frog and Lizard Skin Bioassays

The biological activities of α-MSH and the analogs were determined by their ability to stimulate melanosome dispersion *in vitro* in the frog and lizard skin bioassay systems as previously reported[33-35]. All the solutions were prepared *via* serial dilutions from a stock solution (10^{-4} mol . l^{-1}). The frogs (*Rana pipiens*) used in these studies were obtained from Kons Scientific (Germantown, WI), and the lizards (*Anolis carolinensis*) were from the Snake Farm (La Place, LA).

Our thanks are due to Dr. J. T. Pelton for mass spectroscopic measurements. This research was supported in part by grants from the U.S. Public Health Service (AM 17420) and by grants from the National Science Foundation. M. Lebl was an exchange scientist of the Czechoslovak and U.S. Academy of Sciences who partially supported his stay.

REFERENCES

1. *Biochemical Nomenclature and Related Documents.* International Union of Biochemistry, London 1978.
2. Sawyer T. K., Hruby V. J., Darman P. S., Hadley M. E.: Proc. Nat. Acad. Sci. U.S.A. *79*, 1751 (1982).
3. Knittel J. J., Sawyer T. K., Hruby V. J., Hadley M. E.: J. Med. Chem. *28*, 125 (1983).
4. Cody W. L., Wilkes B. C., Muska B. J., Hruby V. J., de L. Castrucci A. M., Hadley M. E.: J. Med. Chem. *27*, 1186 (1984).
5. Lebl M., Cody W. L., Wilkes B. C., Hruby V.J., de L. Castrucci A. M., Hadley M. E.: Int. J. Peptide Protein Res. *24*, 472 (1984).
6. Cody W. L., Mahoney M., Knittel J. J., Hruby V. J., Hadley M. E.: J. Med. Chem., in press.
7. Hruby V. J., Wilkes B. C., Cody W. L., Sawyer T. K., Hadley M. E.: Pept. Protein Rev. *3*, 1 (1984).
8. Schwartz I. L., Rasmussen H., Livingston L. M., Marc-Aurele J. in the book: *Oxytocin, Vasopressin and their Structural Analogues, Proc. 2nd Int. Pharm. Meeting* (J. Rudinger, Ed.), p. 125. Pergamon Press, London 1964.
9. Rudinger J., Jošt K.: Experientia *20*, 570 (1964).
10. Goldman J. M., Hadley M. E.: J. Pharm. Exp. Therap. *182*, 93 (1972).
11. Jošt K.: This Journal *36*, 218 (1971).
12. Jošt K., Šorm F.: This Journal *36*, 297 (1971).
13. Kobayashi A., Hase S., Kiyoi R., Sakakibara S.: Bull. Chem. Soc. Jap. *42*, 4391 (1969).
14. Keller O., Rudinger J.: Helv. Chim. Acta *57*, 1253 (1974).
15. Sakakibara S., Hase S.: Bull Chem. Soc. Jap. *41*, 2816 (1968).
16. Hase S., Morikawa T., Sakakibara S.: Experientia *25*, 1239 (1969).
17. Procházka Z., Barth T., Cort J. H., Jošt K., Šorm F.: This Journal *43*, 655 (1978).
18. Jošt K., Procházka Z., Cort J. H., Barth T., Škopková J., Prusík Z., Šorm F.: This Journal *39*, 2835 (1974).
19. Hase S., Sakakibara S., Wahrenburg M., Kirchberger M., Schwarz I. L., Walter R.: J. Amer. Chem. Soc. *94*, 3590 (1972).
20. Sarantakis D., Teichman J., Lieu E. L., Fenichel R. L.: Biochem. Biophys. Res. Commun. *73*, 336 (1976).
21. Veber D. F., Strachan R. G., Bergstrand S. J., Holly F. W., Homnick C. F., Hirschman R., Torchiana M., Saperstein R.: J. Amer. Chem. Soc. *98*, 2367 (1976).
22. Emura J., Morikawa T., Takaya T., Sakakibara S. in the book: *Proc. 14th Symp. on Peptide Chem.* (T. Nakajima, Ed.), p. 36. Protein Research Foundation, Osaka 1977.

23. Morikawa T., Munekata E., Sakakibara S., Noda T., Otami M.: Experientia *32*, 1104 (1976).
24. Sakakibara S. in the book: *Peptides. Proc. 5th Amer. Pept. Symp.* (M. Goodman, J. Meienhofer, Eds) p. 436. Wiley, New York 1977.
25. Procházka Z., Jošt K.: This Journal *45*, 1305 (1980).
26. Jošt K., Rudinger J., Klostermeyer H., Zahn H.: Z. Naturforsch. *23b*, 1059 (1968).
27. Kaiser E., Colescott R. L., Bossinger C. D., Cook P. I.: Anal. Biochem. *34*, 595 (1970).
28. Matseuda G. R.: Int. J. Peptide Protein Res. *20*, 26 (1982).
29. Lebl M., Barth T., Jošt K.: This Journal *43*, 1538 (1978).
30. Reindel F., Hoppe W.: Chem. Ber. *87*, 1103 (1954).
31. Simpson R. J., Beuberger M. R., Lin T. V.: J. Biol. Chem. *251*, 1936 (1976).
32. Hruby V. J., Groginsky J.: J. Chromatogr. *63*, 432 (1971).
33. Sawyer T. K., Hruby V. J., Wilkes B. C., Draelos M. T., Hadley M. E., Bergsneider J.: J. Med. Chem. *25*, 1022 (1982).
34. Hruby V. J., Sawyer T. K., Yang Y. C. S., Bregman M. D., Hadley M. E., Heward C. B.: J. Med. Chem. *23*, 1432 (1980).
35. Huntington T., Hadley M. E.: Endocrinology *66*, 599 (1970).
36. Shizume K., Lerner A. B., Fitzpatrick T. B.: Endocrinology *54*, 533 (1954).

Translated by the author (M. L.)