Studies on Peptides. XLVII. Synthesis of Dogfish Melanocyte-Stimulating Hormone (MSH)

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Synthesis of dogfish MSH P11, H–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Met–NH₂, was described, in which N–β,β,β-trichloroethylcarboxyllysine and β,β,β-trichloroethylcarboxyldrazine, bearing the protecting group removable by Zn₂⁺, were applied.

The amino acid sequence of melanocyte-stimulating hormone (MSH) from dogfish (Squalus acanthias) was elucidated by Lowry and Chadwick in 1970. It was found that the sequence of the hormone, Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Met, is resemble to mammalian α-MSH. About half of its molecules have the carboxyl group at the C-terminus free (P₁) and about half are amidated (P₉). They also mentioned that about a fifth have an extra tyrosine residue on the N-terminus. This hormone, as claimed by these authors, is the first peptide hormone from non-mammalian vertebrates as far as MSH is concerned. Among these heterogeneous melanotropic principles of dogfish, we wish to record the synthesis

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\begin{align*}
\text{OBzI} & \quad \text{Z–Ser–Met–Glu–NHNH–Troc} \\
\text{OBzI} & \quad \text{Z–Ser–Met–Glu–NHNH₂} + \text{H–His–Phe–Arg–Trp–Gly–OH} & \text{Troc} \\
\text{I} & \quad \text{Z–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–OH} & \text{Z(OMe)–Lys–Pro–Met–NH₂} & \text{TFA} & \text{II} \\
\text{III} & \quad \text{Troc} \\
\text{OBzI} & \quad \text{Z–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Met–NH₂} & \text{DCC} + \text{HOBT} \\
\text{OBzI} & \quad \text{Z–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Met–NH₂} & \text{HF} & \text{Troc} \\
\text{H–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Met–NH₂} & \text{Zn–AcOH} \\
\end{align*}
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Fig. 1. Synthetic Route to Dogfish MSH P₁₁

2) Amino acid, peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by IUPAC–IUB Commission of Biochemistry Nomenclature: Biochemistry, 5, 2485 (1966); ibid., 6, 362 (1967); ibid., 11, 1726 (1972). Z=benzoylcarbonyl, Z(OMe) = p-methoxybenzoylcarbonyl, OBzI = benzyl ester, Troc = trichloroethylcarbonyl, ONP = p-nitrophenyl ester, OTCP = 2,4,5-trichlorophenyl ester.
3) Location: a) Sakyoku-ku, Kyoto; b) 2-Sagisu, Fukushima, Osaka.
of \( P_{\text{II}} \), in which our newly introduced H-Lys(Troc)–OH\(^6\) and NH\(_2\)NH–Troc\(^7\) were applied as illustrated in Fig. 1.

Three subunits, Z–Ser–Met–Glu(BOzI)–NHNH\(_2\) (I, positions 1–3), H–His–Phe–Arg–Trp–Gly–OH (II, positions 4–8) and Z(OMe)–Lys(Troc)–Pro–Met–NHNH\(_2\) (III, positions 9–11), served to construct the entire amino acid sequence of this hormone.

First, Z–Ser–Met–Glu(BOzI)–NHNH\(_2\) (I) was synthesized, as illustrated in Fig. 2, in a stepwise manner starting from Z(OMe)–Glu(BOzI)–NHNH–Troc.\(^7\) This starting protected hydrazide derivative, reported previously as oil, could be obtained as a crystalline compound by column chromatographic purification on silica. This, after treatment with trifluoroacetic acid (TFA)\(^8\) followed by neutralization with triethylamine, was condensed with Z(OMe)–Met–OH by the mixed anhydride procedure.\(^9\)

The resulting oily product, Z(OMe)–Met–Glu(BOzI)–NHNH–Troc, after treatment with TFA and subsequent neutralization, was allowed to react with Z(OMe)–Ser–OPCP to give Z(OMe)–Ser–Met–Glu(BOzI)–NHNH–Troc. Attempt to crystallize this compound has been unsuccessful. However upon treatment with zinc in acetic acid,\(^10\) the Troc group was cleaved and Z–Ser–Met–Glu(BOzI)–NHNH\(_2\) was isolated as a crystalline compound in analytically pure form, though the yield was somewhat moderate. Its homogeneity was assessed by the positive hydrazine test\(^11\) on thin-layer chromatography. Next, as illustrated in Fig. 1, the modified azide procedure\(^12\) was applied to unit Z–Ser–Met–Glu(BOzI)–NHNH\(_2\) with H–His–Phe–Arg–Trp–Gly–OH prepared according to Hofmann and Lande\(^13\) and the resulting protected octapeptide, Z–Ser–Met–Glu(BOzI)–His–Phe–Arg–Trp–Gly–OH, was obtained in analytically pure form after recrystallization from dimethylformamide (DMF) and ethyl acetate.

In order to confirm the rationality of the azide procedure of such a peptide containing the benzyl ester, an unequivocal synthesis of the above protected octapeptide was undertaken according to the scheme illustrated in Fig. 3. Z(OMe)–Glu(BOzI)–ONP was allowed to

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\begin{align*}
\text{Z–Ser–Met–NHNH}_2 & \quad \text{azide} \\
\text{Z(OMe)–Glu(BOzI)–ONP} & \quad 1) \text{ONP} \\
\text{H–His–Phe–Arg–Trp–Gly–OH} & \quad 2) \text{TFA} \\
\text{Z–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–OH} & \quad \text{OBzI}
\end{align*}
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Fig. 3. Alternate Synthesis of the Protected Octapeptide, Z–Ser–Met–Glu(BOzI)–His–Phe–Arg–Trp–Gly–OH

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react with H–His–Phe–Arg–Trp–Gly–OH (II) to give Z(OMe)–Glu(OBzI)–His–Phe–Arg–Trp–Gly–OH, which after treatment with TFA followed by neutralization, was coupled with Z-Ser–Met–NHNH₂ by the modified azide procedure. Identity of the protected octapeptides obtained by the two alternate routes was established by comparison of their melting points, RF values, specific rotation values and infrared (IR) spectra.

To prepare the protected tripeptide amide, Z(OMe)–Lys(Troc)–Pro–Met–NHNH₂ (III), Z(OMe)–Lys(Troc)–OH was first converted to the corresponding 2,4,5-trichlorophenyl ester in the usual manner. This crystalline active ester was allowed to react with H–Pro–OH in the presence of triethylamine to afford Z(OMe)–Lys(Troc)–Pro–OH, which was then coupled with H–Met–NHNH₂ by the dicyclohexylcarbodiimide (DCC) plus N-hydroxybenztriazole (HOBT) procedure as illustrated in Fig. 4. The resulting protected tripeptide amide (III) was isolated in pure form after recrystallization from ethanol. For the final coupling reaction, the above protected tripeptide amide (III) was treated with TFA as usual. As established previously, under this condition, the Troc group remained intact. The product, after conversion to the corresponding hydrochloride followed by neutralization with triethylamine, was condensed with the protected octapeptide obtained above by the DCC plus HOBT procedure. The resulting protected undecapeptide amide, Z-Ser–Met–Glu(OBzI)–His–Phe–Arg–Trp–Gly–Lys(Troc)–Pro–Met–NHNH₂, was treated, without further purification, with hydrogen fluoride according to Sakakibara, et al. As scavengers, H–Met–OH and H–Trp–OH were added during this treatment, under which the Z and benzyl ester groups could be selectively removed. The resulting partially protected undecapeptide amide, H–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys(Troc)–Pro–Met–NHNH₂, was purified by column chromatography on carboxymethyl (CM)-cellulose using pyridine acetate buffers.

In order to remove the Troc group from the above partially protected undecapeptide amide, this was then treated with zinc in acetic acid at 50° for 30 minutes and the product was isolated after purification by column chromatography on CM-cellulose. The desired undecapeptide amide was emerged from the column by pH 6.9, 0.05M ammonium acetate buffer. The synthetic dogfish MSH F₄ thus isolated was homogeneous on thin-layer chromatography. An acid hydrolysate contained constituent amino acids in ratios predicted by theory except for Trp which was destroyed during the hydrolysis. However the average recovery of each amino acid was somewhat low. When tested by dithizone, the synthetic peptide gave deep green color, indicating the presence of zinc ion in the molecule. This zinc seems to be not a contaminant, but rather bind with the peptide, since rechromatography of this sample on CM-cellulose or Sephadex G-10 was not effective to diminish this typical green color by this reagent. From the carbon content of the elemental analysis, presence of four zinc atoms could tentatively be assumed. By analogy of the zinc preparation of the synthetic ACTH tetracosapeptide, it can be concluded that this particular amino acid sequence possesses an ability to form a very tight complex with zinc, though the actual mode of binding is not known.

Synthetic peptides were submitted to the in vivo bioassay according to Nakamura, et al.,\textsuperscript{20} using african frog, *xenopus laevis*. The relative potency of synthetic dogfish MSH P\textsubscript{II} was 1/25 of \(z\)-MSH (1 \(\times 10^3\) in vitro MSH U/g).\textsuperscript{21} This value is closely in agreement with the reported value of the natural source\textsuperscript{22} and its Troc derivative was 1/14 of the above synthetic dogfish MSH. It seems noteworthy that the presence of zinc does not interfere the MSH activity of synthetic peptides.

**Experimental**

General experimental methods employed are essentially the same as described in the Part XXII\textsuperscript{23} of this series. Thin-layer chromatography was performed on silica gel (Kieselgel G. Merck). \(R_f\) values refer to the following solvent systems: \(R_f\) CHCl\textsubscript{3}-MeOH-H\textsubscript{2}O (8:3:1), \(R_f\) CHCl\textsubscript{3}-MeOH-AcOH (9:1:0.5), \(R_f\) n-BuOH-AcOH-pyridine-H\textsubscript{2}O (4:1:1:2), \(R_f\) CHCl\textsubscript{3}-MeOH-pyridine-H\textsubscript{2}O (8:3:2:1).

\(\text{Z(OMe)-Glu[OBzl]-NNH}-\text{Troc}\) — Z(OMe)-Glu[OBzl]-OH (10.0 g) was condensed with NH\textsubscript{2}NH\textsubscript{2}HCl (5.2 g) by the mixed anhydride procedure as described previously.\textsuperscript{7} The crude product was applied to a column of silica (300-400 cm), which was eluted with CHCl\textsubscript{3} and the eluates were examined by thin-layer chromatography. The fractions which contained the desired compound (\(R_f\) 0.78) were combined. Condensation of the solvent afforded the solid which was recrystallized from CHCl\textsubscript{3} yield 6.52 g (44\%). mp 85-88\(\circ\). [\(\alpha\)]\textsubscript{D} \textsuperscript{2} = -11.2\(\circ\) (c=0.8, DMF). \textit{Anal.} Caled. for C\textsubscript{35}H\textsubscript{39}O\textsubscript{2}N\textsubscript{2}Cl\textsubscript{2}: C, 48.78; H, 4.44; N, 7.11. Found: C, 48.63; H, 4.52; N, 7.00.

\(\text{Z(OMe)-Met-Glu[OBzl]-NNH}-\text{Troc}\) — Z(OMe)-Met-Glu[OBzl]-NNH-Troc (8.86 g) was treated with TFA (11 ml) in the presence of anisole (3.2 ml) at 0\(\circ\) for 45 min. Dry ether was added and the resulting oily precipitate, after drying over KOH pellets in vacuo overnight, was dissolved in tetrahydrofuran (THF) (20 ml). To this ice-cold solution, Et\textsubscript{3}N (2.8 ml) and a mixed anhydride (prepared from 4.7 g of Z(OMe)-Met-\(\text{OCH}_{3}\) with 2.1 ml of Et\textsubscript{3}N and 1.4 ml of ethyl chlorofomate) in dry THF (50 ml), were added and the mixture was stirred at room temperature for 2 hr. The solvent was evaporated and the residue was dissolved in AcOEt, which was washed with 10\% citric acid, 5% NaHCO\textsubscript{3} and NaCl-H\textsubscript{2}O, dried over Na\textsubscript{2}SO\textsubscript{4} and then evaporated to give an oily product. \(R_f\) 0.82. Attempt to solidify this compound has been unsuccessful, yield 7.2 g (67\%).

\(\text{Z-Ser-Met-Glu[OBzl]-NNH}-\text{Troc}\) — Z-Ser-Met-Glu[OBzl]-NNH-Troc (7.20 g) was treated with TFA (7.4 ml) in the presence of anisole (3.2 ml) as stated above. The oily precipitate formed by addition of dry ether was washed over KOH pellets in vacuo overnight and then dissolved in DMF (30 ml). To this solution, Et\textsubscript{3}N (2.8 ml) and Z-Ser-OPCP (4.90 g) were added and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the residue was dissolved in AcOEt, which was washed with 10\% citric acid, 5\% NaHCO\textsubscript{3} and H\textsubscript{2}O-NaCl, dried over Na\textsubscript{2}SO\textsubscript{4} and then evaporated to give an oily residue, \(R_f\) 0.86; yield 4.31 g (55\%).

\(\text{Z-Ser-Met-Glu[OBzl]-NNH}_{\text{II}}\) — Zinc dust (1 g) was added to a solution of Z-Ser-Met-Glu[OBzl]-NNH-Troc (1.0 g) in AcOH (10 ml) and the solution was stirred at room temperature for 12 hr. After filtration, the filtrate was concentrated and the residue was basified with Na\textsubscript{2}CO\textsubscript{3}. The resulting precipitate was extracted with AcOEt, which after filtration and washing with H\textsubscript{2}O-NaCl, was dried over Na\textsubscript{2}SO\textsubscript{4} and then evaporated. The solid residue was recrystallized from AcOEt; yield 0.42 g (54\%). mp 153-155\(\circ\). \(R_f\) 0.65. \textit{Anal.} Caled. for C\textsubscript{55}H\textsubscript{51}O\textsubscript{8}N\textsubscript{3}-1/2H\textsubscript{2}O: C, 54.89; H, 6.25; N, 11.43. Found: C, 54.96; H, 6.20; N, 11.28.

\(\text{Z(OMe)-Glu[OBzl]-His-Phe-Arg-Trp-Gly-OH}\) — Z(OMe)-Glu[OBzl]-ONP (2.20 g) was added to a solution of H-His-Phe-Arg-Trp-Gly-OH (1.67 g) in DMF (10 ml) containing Et\textsubscript{3}N (1.5 ml) and the mixture was stirred at room temperature for 18 hr. The solution, after neutralization with AcOH, was condensed in vacuo and the residue was triturated with AcOEt and H\textsubscript{2}O. The resulting powder was recrystallized from DMF and MeOH; yield 2.34 g (94\%). mp 179-181\(\circ\). [\(\alpha\)]\textsubscript{D} = -14.8\(\circ\) (c=0.6, DMF). \(R_f\) 0.79. \textit{Anal.} Caled. for C\textsubscript{54}H\textsubscript{52}O\textsubscript{10}N\textsubscript{5}·CH\textsubscript{3}COOH·2H\textsubscript{2}O: C, 57.95; H, 6.14; N, 14.23. Found: C, 58.00; H, 6.11; N, 14.43.

\(\text{Z-Ser-Met-Glu[OBzl]-His-Phe-Arg-Trp-Gly-OH}\) — To a solution of Z-Ser-Met-Glu[OBzl]-NNH\textsubscript{II} (0.33 g) in DMF (1 ml), 2.3\(\times\) HCl-DMF (0.48 ml) and isoaamyl nitrite (0.07 ml) were added under cooling with ice-NaCl. After 5 min, when the hydrazine test\textsuperscript{24} of the reaction mixture became negative, the solution was neutralized with Et\textsubscript{3}N (0.21 ml). To this solution, H-His-Phe-Arg-Trp-Gly-OH\textsuperscript{25} in DMF

(1.5 ml) containing Et₂N (0.14 ml) was added and the mixture was stirred at 4° for 24 hr. After evaporation of the solvent, AcOEt was added to the residue. The resulting solid was washed with H₂O and then recrystallized from DMF and MeOH; yield 0.46 g (70%). mp 223–224°. [α]₂⁰D = −24.4° (c = 0.1, DMF). RF₉ 0.46, RF₂ 0.72. Amino acid ratios in an acid hydrolysate: Ser₉,SεMet₁₈,Glu₁₀,His₈,₉₉,Phe₉₉,Ar₉₅,Gly₁₉⁰ (average recovery 97%). Anal. Calcd. for C₉₈H₁₇₁₅N₁₇S₁₅-C₇H₈COOH: C, 57.64; H, 6.05; N, 14.71. Found: C, 57.87; H, 5.86; N, 14.66.

b) Z(OMe)–Glu(OBzl)–His–Phe–Arg–Trp–Gly–OH (0.83 g) was treated with TFA (2 ml) in the presence of anisole (0.6 ml) at 0° for 60 min, when dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets in vacuo and then dissolved in DMF (3 ml). To this solution, Et₂N (0.2 ml) and the azide (prepared as stated above from 0.55 g of Z–Ser–Met–NHN₃H₂ with 0.7 ml of 4 N HCl–DMF, 0.2 ml of isomyl nitrite and 0.7 ml of Et₂N) in DMF (2.5 ml) and the mixture was stirred at 4° for 18 hr. Purification and recrystallization of the product were performed as stated in (a); yield 0.85 g (89%). mp 224–225°. [α]₂⁰D = −22.2° (c = 0.2, DMF). RF₉ 0.73. IR spectra of the compounds obtained in (a) and (b) were identical. Amino acid ratios in an acid hydrolysate: Ser₂,SεMet₁₈,Glu₁₀,His₁₉,₉₉,Phe₉₉,Ar₉₅,Gly₁₉⁰ (average recovery 94%).

Z(OMe)–Lys(Troc)–OTCP—DCC (20.8 g) was added to a solution of Z(OMe)–Lys(Troc)–OH (47.0 g) and 2,4,5-trichlorophenol (18.2 g) in AcOEt (200 ml) and the mixture was stirred at room temperature for 18 hr. The solution was filtered and the filtrate was condensed in vacuo. The residue was triturated with petroleum ether and recrystallized from AcOEt and petroleum ether; yield 54.0 g (88%). mp 60–61°. [α]₂⁰D = −4.0° (c = 0.9, DMF). RF₉ 0.95. Anal. Calcd. for C₉₈H₁₇₁₅N₁₇S₁₅-C₇H₈COOH: C, 43.38; H, 3.64; N, 4.21. Found: C, 43.59; H, 3.63; N, 4.25.

Z(OMe)–Lys(Troc)–Pro–OH—Z(OMe)–Lys(Troc)–OTCP (33.26 g) in THF (125 ml) was added to a solution of H–Pro–OH (6.91 g) in H₂O (100 ml) containing Et₂N (15.4 ml) and the mixture was stirred at room temperature for 18 hr. After evaporation of the solvent, the residue was dissolved in 5% NH₂OH (300 ml), which was washed with ether and then acidified with citric acid. The resulting precipitate was extracted with AcOEt, which was washed with H₂O–NaCl dried over Na₂SO₄ and then evaporated. The residue was triturated with petroleum ether and recrystallized from AcOEt and petroleum ether; yield 25.04 g (86%). mp 37–40°. [α]₂⁰D = −21.9° (c = 0.6, DMF). RF₉ 0.50. Anal. Calcd. for C₉₈H₁₇₁₅N₁₇S₁₅-C₇H₈COOH: C, 47.59; H, 5.19; N, 7.21. Found: C, 47.54; H, 5.25; N, 6.93.

Z(OMe)–Lys(Troc)–Pro–Met–NH₂—Z(OMe)–Met–NH₂ (0.68 g) was treated with TFA (3.7 ml) in the presence of anisole (0.5 ml) at 0° for 30 min, when dry ether was added. An oily precipitate was washed with ether, dried over KOH pellets in vacuo and then dissolved in 3.15 N HCl–dioxane (2 ml). This solution was lyophilized and the residue, after drying over KOH pellets in vacuo, was dissolved in DMF (5 ml). To this solution, Et₂N (0.9 ml), Z(OMe)–Lys(Troc)–Pro–OH (1.46 g), HOBT (0.52 g) and DCC (0.62 g) were added and the mixture was stirred at room temperature overnight. The solution was filtered and the solvent was evaporated in vacuo. The residue was dissolved in AcOEt, which was washed with 10% citric acid, 3% NH₂OH, H₂O–NaCl, dried over Na₂SO₄ and then evaporated and the resulting solid was recrystallized from EtOH; yield 1.35 g (87%). mp 145–144°. [α]₂⁰D = 18.4° (c = 0.4, DMF). RF₉ 0.80, RF₂ 0.50. Anal. Calcd. for C₉₈H₁₇₁₅N₁₇S₁₅-C₇H₈COOH: C, 47.16; H, 5.65; N, 9.82. Found: C, 47.45; H, 5.85; N, 9.88.

H–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys(Troc)–Pro–Met–NH₂—In the usual manner, Z(OMe)–Lys(Troc)–Pro–Met–NH₂ (0.11 g) was treated with TFA (0.5 ml) in the presence of anisole (0.1 ml) at room temperature for 30 min. The precipitate formed by addition of petroleum ether, was dissolved in 1 N HCl (0.15 ml) and the solution was lyophilized. The residue was dissolved in DMF (2 ml), to which, Et₂N (0.02 ml), Z–Ser–Met–Glu(OBzl)–His–Phe–Arg–Trp–Gly–OH (0.14 g), DCC (0.1 g) and HOBT (0.20 g) were added and the mixture was stirred at room temperature for 18 hr. The solution, after filtration, was condensed in vacuo and the residue was treated with AcOEt to give the solid; yield 0.18 g, RF₉ 0.84, RF₂ 0.37 (main spot). This was then treated with HF (approximately 2 ml) in the presence of anisole (0.1 ml), H–Met–OH (15 mg) and H–Trp–OH (20 mg) in an ice-bath for 30 min. The excess HF was evaporated in vacuo and the residue, after drying over KOH pellets in vacuo overnight, was dissolved in H₂O. The aqueous solution was washed with ether and then treated with Amberlite CG-4B (acetate cycle, approximately 0.5 g). The resin was removed by filtration and the filtrate was applied to a column of CM-cellulose (1.5 x 16 cm), which was eluted with following pH 5.9 pyridine acetate buffers: 0.01M (250 ml), 0.025M (250 ml), 0.05M (250 ml) and 0.1M (1400 ml). Individual fractions, 5 ml each, were collected and absorbancy at 250 µ was determined. The fractions corresponding to single peak present in 0.1M buffer (tube No. 190–240), were collected and the solvent was evaporated. Lyophilization of the residue gave fluffy white powder; yield 80 mg (45%). [α]₂⁰D = 31.2° (c = 0.1, DMF). RF₉ 0.60, RF₂ 0.33. Amino acid ratios in an acid hydrolysate: Ser₈,SεMet₁₈,Glu₁₀,His₈,₉₉,Phe₉₉,Ar₉₅,Gly₁₉⁰ (average recovery 80%). Anal. Calcd. for C₉₈H₁₇₁₅N₁₇S₁₅-C₇H₈COOH: (dried at 100° for 4 hr): C, 49.45; H, 6.05; N, 15.65. Found: C, 49.20; H, 6.30; N, 15.50.

H–Ser–Met–Glu–His–Phe–Arg–Trp–Gly–Lys–Pro–Met–NH₂ (Dogfish MSH P₂)—The above partially protected undecapeptide amide (100 mg) was dissolved in AcOH (2 ml), to which Zn dust (60 mg) was added and the solution, under N₂, was stirred at 50° for 30 min. The solution was filtered, the filtrate was condensed and the residue was lyophilized. The crude product was then applied to a column of Sephadex G-10
(2.6 × 50 cm) for desalting. The column was eluted with 10% AcOH and the absorbancy at 280 nm was determined in each fraction (3 ml). Fractions corresponding to the front peak (tube No. 34—41) were combined and the solvent was removed by lyophilization. This product was then applied to a column of CM-cellulose (2.6 × 5 cm), which was eluted with the following ammonium acetate buffers (pH 6.9): 0.005 M (500 ml), 0.01 M (500 ml), 0.02 M (500 ml) and 0.04 M (2000 ml). Individual fractions (10 ml each) were collected and the absorbancy at 280 nm was determined. Fractions of the main peak present in 0.04 M eluates (tube No. 175—205) were pooled and the solvent was removed by lyophilization to give fluffy white powder; yield 39 mg (42%). [α]D 25° = 22.5° (c = 0.1, 3% AcOH), Rf 0.40. The dithionite test gave green color. Rechromatography on Sephadex G-10 or CM-cellulose was not effective to remove the zinc complex. Amino acid ratios in an acid hydrolysate: Ser1.80, Met1.67, Glu1.60, His0.69, Phe1.00, Arg0.60, Gly1.00, Lys1.65, Pro0.80 (average recovery 63%). Anal. Calcd. for C19H26O11N10S6·4Zn(CH2COO)2 (dried at 100° for 5 hr): C, 44.36; H, 5.32; N, 12.45. Found: C, 44.64; H, 5.73; N, 12.48.

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