Table I. Absorption Ratio of Amino Acids

Amino acid	Ninhydrin color		Ultraviolet absorption	
	640/570 nm	440/570 nm	260/280 nm	250/280 nm
3,4-DOPA	0.389	0.243	0.246	0.095
m-Tyrosine	0.405	0.235	0.674	0.263
p-Tyrosine	0.356	0.205	0.535	0.174
Phenylalanine	0.404	0.208	0.266	0.278
o-Tyrosine	0.352	0.196	0.646	0.196

Therefore, it should be possible to identify these amino acids by checking not only the retention value but also the ratio of ultraviolet absorption. For the sample contaminated with ninhydrin-positive aliphatic compounds which have a similar retention time as those of hydroxylated phenylalanines, ultraviolet absorption measurement has been found to be very useful.

Analysis of hydroxylated metabolites of phenylalanine *in vivo* using the above-mentioned procedure will be reported elsewhere.

[Chem. Pharm. Bull. 24(10)2558—2563(1976)]

UDC 547.466.1.057 : 577.15.07

Studies on Peptides. LXI.[1,2] Alternate Synthesis of Human Corticotropin (ACTH)

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(Received January 26, 1976)

Succeeding to the syntheses of porcine and bovine corticotropin (ACTHs), human ACTH was alternatively synthesized by the conventional method. The synthetic peptide exhibited the *in vivo* steroidogenetic activity of 153.1 IU/mg.

In 1971, Riniker, *et al.*[4] revised the structure of human corticotropin (ACTH) proposed early by Lee, *et al.*[5] and the N-terminal portion (position 1 to 20) was confirmatively reexamined by Bennett, *et al.*[6] Leading from this firmly established formula of human ACTH (I), its total synthesis was accomplished by Sieber, *et al.*[7] using protecting groups removable by trifluoroacetic acid (TFA). Succeeding to this preliminary communication, alternate

1) Part LX: K. Koyama, H. Kawatani, H. Yajima, M. Fujino, and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **24**, 2106 (1976).
2) Amino acids, peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature: *Biochem.*, **5**, 2485 (1966), *ibid.*, **6**, 362 (1967), *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Tos=*p*-toluenesulfonyl, OBzl=benzyl ester.
3) Location: *Sakyo-ku, Kyoto, 606, Japan.*
4) B. Riniker, P. Sieber, and W. Rittel, *Nature New Biol.*, **235**, 114 (1972).
5) T.H. Lee, A.B. Lerner, and V.B. Janush, *J. Am. Chem. Soc.*, **81**, 6084 (1959); *idem, J. Biol. Chem.*, **236**, 2970 (1961).
6) H.P.J. Bennett, P.J. Lowry, and C. McMartin, *Biochem. J.*, **131**, 11 (1973).
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syntheses of human ACTH were announced by Kisfaludy, *et al.*[8] and Nishimura, *et al.*[9] More recently, its solid phase synthesis was carried out by Yamashiro and Li.[10]

Recently we synthesized two nonatriacontapeptides corresponding to the entire amino acid sequence of porcine[11] and bovine ACTHs[1] and similar procedures were next extended to the synthesis of human ACTH. Since the method we employed here is different from those of the above authors, we wish to record our results in this paper. Our synthetic route to human ACTH involves the condensation of four peptide fragments as shown in Fig. 1.

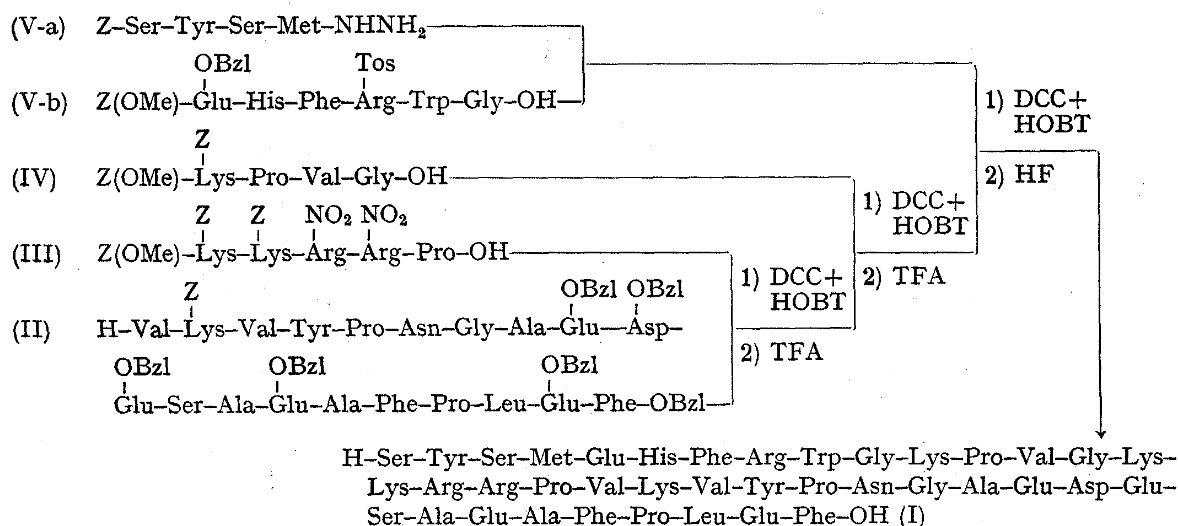

Fig. 1. Synthetic Route to Human ACTH

The partially protected eicosapeptide ester (II), H-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, an intermediate for our synthesis of human type corticotropin-like intermediate lobe peptide (CLIP),[12] served as an amino component to our present synthesis. This scheme is similar to those applied to the syntheses of porcine and bovine ACTHs in respect of taking the same fragments; Z(OMe)-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-OH (III, position 15-19) and Z(OMe)-Lys(Z)-Pro-Val-Gly-OH (IV, position 11—14). The N-terminal decapeptide unit (position 1—10) was introduced by two alternate routes; the one by the successive condensation of two available sequences; Z-Ser-Tyr-Ser-Met-$NHNH_2$ (V-a, position 1—4) and Z(OMe)-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-OH (V-b, position 5—10) as did in the synthesis of porcine ACTH and the other by the direct coupling of the decapeptide unit which was prepared after uniting two units, (V-a) and (V-b). The latter is the same scheme employed for the synthesis of bovine ACTH.

Each fragment condensation was performed by dicyclohexylcarbodiimide (DCC) in the presence of N-hydroxybenzotriazole (HOBT)[13] using a slight excess of the carboxyl component. The Z(OMe) group of intermediates was removed by TFA[14] using anisole as a scavenger and each resulting TFA salt was converted to the corresponding hydrochloride, which was subsequently neutralized with triethylamine prior to each condensation reaction.

8) L. Kisfaludy, M. Law, T. Szirtes, I. Schon, M. Sarkozi, S. Bajusz, A. Turan, A. Juhasz, R. Bake, L. Graf, and K. Medziharadszky, "Chemistry and Biology of Peptides," ed. by J. Meienhofer, Ann Arbor Pub., Michigan, Ohio, U.S. 1972, p. 299.
9) O. Nishimura, C. Hatanaka, and M. Fujino, *Chem. Pharm. Bull.* (Tokyo), **23**, 1212 (1975).
10) D. Yamashiro and C.H. Li, *J. Am. Chem. Soc.*, **95**, 1310 (1973).
11) H. Yajima, K. Kaname, Y. Kiso, A. Tanaka, and M. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **24**, 492 (1976).
12) H. Kawatani, F. Tamura, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **22**, 1879 (1974).
13) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
14) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).

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Thus, starting with the partially protected eicosapeptide ester (II), the protected nonacosapeptide ester, Z(OMe)–Lys(Z)–Pro–Val–Gly–Lys(Z)–Lys(Z)–Arg(NO_2)–Arg(NO_2)–Pro–Val–Lys(Z)–Val–Tyr–Pro–Asn–Gly–Ala–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Ala–Glu-(OBzl)–Ala–Phe–Pro–Leu–Glu(OBzl)–Phe–OBzl, was obtained after successive condensation of two fragments, (III) and (IV). Next chain elongation of this nonacosapeptide ester was performed by two alternate ways as mentioned above; the one by the DCC plus HOBT condensation of the hexapeptide unit (V-b) followed by the azide coupling of the tetrapeptide unit (V-a) and the other, by the direct condensation of the protected decapeptide, Z–Ser–Tyr–Ser–Met–Glu(OBzl)–His–Phe–Arg(Tos)–Trp–Gly–OH (V) by DCC plus HOBT. This decapeptide unit is the same unit used previously by Li, *et al.*[15] for the synthesis of a biologically active heptadecapeptide related to ACTH and differs from the decapeptide unit used for our synthesis of bovine ACTH[1] in respect that the Glu(OBzl) residue in stead of Glu(OBu^t) was employed. After the DCC plus HOBT condensation reaction, the crude products, the protected pentatriacontapeptide ester, Z(OMe)–Glu(OBzl)–His–Phe–Arg(Tos)–Trp–Gly–Lys(Z)–Pro–Val–Gly–Lys(Z)–Lys(Z)–Arg(NO_2)–Arg(NO_2)–Pro–Val–Lys(Z)–Val–Tyr–Pro–Asn–Gly–Ala–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Ala–Glu(OBzl)–Ala–Phe–Pro–Leu–Glu(OBzl)–Phe–OBzl, as well as the fully protected nonatriacontapeptide ester, Z–Ser–Tyr–Ser–Met–Glu(OBzl)–His–Phe–Arg-(Tos)–Trp–Gly–Lys(Z)–Pro–Val–Gly–Lys(Z)–Lys(Z)–Arg(NO_2)–Arg(NO_2)–Pro–Val–Lys(Z)–Val–Tyr–Pro–Asn–Gly–Ala–Glu(OBzl)–Asp(OBzl)–Glu(OBzl)–Ser–Ala–Glu(OBzl)–Ala–Phe–Pro–Leu–Glu(OBzl)–Phe–OBzl, were heated in methanol containing acetic acid to remove the dicyclohexylamidino moiety presumably attached at the N^{im} of the His residue.[16] As reported previously,[1] column chromatography on silica in the solvent system of chloroform, methanol and water (8:3:1) was effective to purify all protected intermediates and the final protected nonatriacontapeptide ester as well. Purity of these protected products was confirmed by thin-layer chromatography, acid hydrolysis and elemental analysis.

Deblocking of all protecting groups employed, Z, Tos, NO_2 and Bzl groups, from the fully protected nonatriacontapeptide ester was performed by hydrogen fluoride[17] in the presence of 2% ethanedithiol. Purification of the deblocked product was performed as described in the synthesis of porcine ACTH.[11] First, the resulting HF salt was converted to the corresponding acetate by treatment with Amberlite IR-4B. Next, the crude acetate was heated in methanol containing acetic acid to secure the removal of the dicyclohexylamidino moiety from the His residue. Met sulfoxide partially formed was reduced during this treatment by addition of dithiothreitol.[10] The product thus treated was subjected to column chromatography on Sephadex G-25 to remove scavengers used and then purified by column chromatography on CM-Sephadex. The desired peptide was eluted from the column by gradient elution with 0.3M, pH 6.9 ammonium acetate buffer. Desalting was then performed by Amberlite XAD-II and the product was finally lyophilized as a fluffy white powder.

Purity of synthetic human ACTH was confirmed by thin-layer chromatography in two different solvent systems and by disc electrophoresis at two different pH values. The hydrolysate with 3N *p*-toluenesulfonic acid[18] contained the constituent amino acids, including Trp, in ratios predicted by theory. The presence of one mole of Asn was confirmed by enzymatic (AP–M) digestion[19] as did in the synthesis of porcine and bovine ACTHs.[1,11] This synthetic peptide exhibited the *in vivo* steroidogenetic activity of 153.1 IU/mg, when compared with the activity of our synthetic porcine ACTH (148.2 IU/mg). With this synthesis, we have

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16) H. Rink and B. Riniker, *Helv. Chim. Acta*, **57**, 831 (1974).
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19) G. Pfleiderer and P.G. Celliers, *Biochem. Z.*, **339**, 186 (1963); H. Watanabe, H. Ogawa, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **23**, 375 (1975).

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accomplished the syntheses of three nonatriacontapeptides which correspond to the entire amino acid sequences of porcine, bovine (ovine) and human ACTH, so far known in mammarian sources.

Experimental

Thin-layer chromatography was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8: 3: 1), Rf_2 *n*-BuOH–pyridine–AcOH–H_2O (4: 1: 1: 2). Rf_3 *n*-BuOH–pyridine–AcOH–H_2O (30: 20: 6: 24).

Z(OMe)-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, [Z(OMe)-(human ACTH 15–39)-OBzl)]——Z(OMe)-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(O-Bzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl[11] (6.10 g) was treated with TFA (7 ml) in the presence of anisole (3 ml) in an ice-bath for 45 min. Dry ether was added and the resulting powder was collected by filtration and dissolved in 1 N HCl–dioxane (2 ml). The resulting hydrochloride was precipitated by addition of dry ether, collected by filtration and again dissolved in a small amount of dioxane, to which Et_3N (0.28 ml) was added. Dry ether was added and the free base thus precipitated was dissolved in DMF (40 ml). To this solution, Z(OMe)-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-OH[11] (3.62 g), DCC (1.24 g) and HOBT (0.81 g) were added and the solution, after stirring at room temperature for 48 hr, was filtered. The filtrate was condensed *in vacuo* and the residue was treated with ether. The resulting powder was dissolved in a small amount of the lower phase of $CHCl_3$–MeOH–H_2O (8: 3: 1, v/v) and the solution was applied to a column of silica (3×25 cm), which was eluted with the same solvent system. The eluates containing the substance of Rf_1 0.47 were combined and the solvent was evaporated. The residue was precipitated twice from DMF with ether; yield 8.08 g (97%), mp 141—145°, $[\alpha]_D^{28}$ −27.1° (*c*=0.9, DMF). Rf_1 0.47. Amino acid ratios in an acid hydrolysate: Lys 3.21, Pro 3.05, Val 1.86, Tyr 0.44, Asp 2.26, Glu 4.26, Gly 1.00, Ala 3.07, Ser 0.97, Phe 2.18, Leu 1.18 (average recovery 91%). *Anal.* Calcd. for $C_{205}H_{261}O_{52}N_{37}\cdot 5H_2O$: C, 59.11; H, 6.56; N, 12.44. Found: C, 59.10; H, 6.37; N, 12.75.

Z(OMe)-Lys(Z)-Pro-Val-Gly-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, [Z(OMe)-(human ACTH 11–39)-OBzl]——The above protected pentacosapeptide ester, Z(OMe)-(human ACTH 15—39)-OBzl, (11.0 g) was treated with TFA (13 ml) in the presence of anisole (5 ml) in an ice-bath for 45 min. The TFA salt precipitated by addition of dry ether was dissolved in a small amount of DMF, to which 1 N HCl–DMF (2.7 ml) was added. The hydrochloride was precipitated by addition of dry ether and dissolved again in a small amount of DMF. Et_3N (0.41 ml) was added and dry ether was added. The resulting solid precipitate was collected by filtration and then dissolved in DMF (100 ml). To this solution, Z(OMe)-Lys(Z)-Pro-Val-Gly-OH[11] (2.57 g), DCC (1.2 g) and HOBT (0.81 g) were successively combined. The mixture was stirred at room temperature for 48 hr and the solution, after filtration, was condensed *in vacuo*. The residue was treated with 5% citric acid and AcOEt and the resulting powder was dissolved in a small amount of the lower phase of $CHCl_3$–MeOH–H_2O (8: 3: 1) and the solution was applied to a column of silica (3×15 cm), which was eluted with the same solvent system as stated above. The eluates containing the substance of Rf_1 0.36 were combined and the solvent was evaporated. The residue was treated with H_2O and the resulting powder was precipitated twice from DMF with AcOEt; yield 10.04 g (81%), mp 144—150°, $[\alpha]_D^{28}$ −26.2° (*c*=1.0, DMF). Rf_1 0.36. Amino acid ratios in 3 N Tos-OH hydrolysate: Lys 3.81, Pro 3.95, Val 2.69, Gly 2.00, Tyr 0.65, Asp 2.33, Ala 3.35, Glu 4.35, Ser 1.08, Phe 2.36, Leu 1.12 (average recovery 92%). *Anal.* Calcd. for $C_{231}H_{298}O_{58}N_{42}\cdot 3H_2O$: C, 59.73; H, 6.60; N, 12.67. Found: C, 59.69; H, 6.46; N, 12.18.

Z(OMe)-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-Lys(Z)-Pro-Val-Gly-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, [Z(OMe)-(human ACTH 5–39)-OBzl]——Z(OMe)-(human ACTH 11—39)-OBzl (2.30 g) was treated with TFA (3 ml) in the presence of anisole (1.5 ml) and the resulting TFA salt was converted, through the corresponding hydrochloride, to the free base by treatment with 1 N HCl–DMF (0.5 ml) followed by neutralization with Et_3N (0.1 ml) as stated above. To a solution containing this free base in DMF (20 ml), Z(OMe)-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-OH[11,20] (0.93 g), DCC (0.20 g) and HOBT (0.13 g) were added and the mixture was stirred at room temperature for 48 hr. The solution was filtered, the filtrate was condensed and the residue was treated with H_2O and AcOEt. The resulting powder was dissolved in a mixture of DMF–MeOH–2 N AcOH (5: 4: 1, v/v, 25 ml). This solution, after heating at 65° for 5 hr, was condensed *in vacuo* and the residue was treated with AcOEt. The resulting powder was purified by column chromatography on silica (3×35 cm) using the solvent system of $CHCl_3$–MeOH–H_2O (8: 3: 1) as stated above. Isolation of the desired compound was similarly performed; yield 2.45 g (90%), mp 155—160°, $[\alpha]_D^{25}$ −30.8° (*c*=0.6, DMF). Rf_1 0.51. Amino acid ratios in 3 N Tos–OH hydrolysate: Glu 5.42, His 1.05, Phe 3.02, Arg

20) H. Watanabe, H. Ogawa, and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), 23, 375 (1975).

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not determined. Trp 0.92, Gly 3.00, Lys 4.36, Pro 3.82, Val 3.09, Tyr 0.90, Asp 2.31, Ala 3.38, Ser 0.89, Leu 0.96, (average recovery 85%). *Anal.* Calcd. for $C_{273}H_{348}O_{67}N_{52}S\cdot 5H_2O$: C, 59.05; H, 6.50; N, 13.12. Found: C, 59.12; H, 6.54; N, 13.24.

Z-Ser-Tyr-Ser-Met-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-OH——The title compound was prepared according to Li, *et al.*[15] mp 211—215°, $[\alpha]_D^{25}$ −20.8° (c=0.5, DMF). (lit.[15] mp 214—216°, $[\alpha]_D^{25}$ −22.3° in DMF). *Anal.* Calcd. for $C_{81}H_{96}O_{20}N_{16}S_2\cdot 2.5H_2O$: C, 56.46; H, 5.91; N, 13.00. Found: C, 56.07; H, 6.33; N, 13.45.

Z-Ser-Tyr-Ser-Met-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-Lys(Z)-Pro-Val-Gly-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-Pro-Asn-Gly-Ala-Glu(OBzl)-Asp(OBzl)-Glu(OBzl)-Ser-Ala-Glu(OBzl)-Ala-Phe-Pro-Leu-Glu(OBzl)-Phe-OBzl, [Z-(human ACTH 1—39)-OBzl]——a) By condensation of Z-(1—10)-OH and H-(human 11—39)-OBzl. Z(OMe)-(human 11—39)-OBzl (1.73 g) was treated with TFA (3 ml) in the presence of anisole (0.8 ml) and the resulting TFA salt was converted through the corresponding hydrochloride to the free base by treatment with 2.93 N HCl–DMF (0.1 ml) followed by neutralization with Et_3N (0.3 ml). To the solution containing this free base in DMF (17 ml), Z-Ser-Tyr-Ser-Met-Glu(OBzl)-His-Phe-Arg(Tos)-Trp-Gly-OH[1] (1.0 g), DCC (0.25 g) and HOBT (0.16 g) were combined and the mixture was stirred at room temperature for 48 hr. The solution, after filtration, was condensed *in vacuo* and the residue was treated with H_2O. The resulting powder was dissolved in a mixture of DMF–MeOH–2 N AcOH (5: 4: 1, v/v, 20 ml). The solution, after heating at 65° for 4 hr, was condensed *in vacuo* and the residue was treated with AcOEt. The resulting powder was purified by column chromatography on silica (3×15 cm) using the solvent system of $CHCl_3$–MeOH–H_2O (8: 3: 1) and the desired compound was isolated as stated above; yield 1.40 g (60%), mp 173—180°, $[\alpha]_D^{27}$ −24.6° (c=0.9, DMF). Rf_1 0.53. Amino acid ratios in an acid hydrolysate: Ser 2.72, Tyr 1.63, Met 0.79, Glu 5.39, His 1.09, Phe 3.00, Arg 2.17, Gly 2.82, Lys 4.05, Pro 4.08, Val 3.16, Asp 2.09, Ala 3.36, Leu 1.00 (average recovery 89%). *Anal.* Calcd. for $C_{303}H_{384}O_{74}N_{58}S_2\cdot 10H_2O$: C, 58.07; H, 6.50; N, 12.96. Found: C, 58.03; H, 6.24; N, 13.32.

b) By condensation of Z-(1—4)-$NHNH_2$ and H-(human 5—39)-OBzl. To a solution of Z-Ser-Tyr-Ser-Met-$NHNH_2$[11] (1.39 g) in DMF (15 ml), 1 N HCl–DMF (0.6 ml) and isoamyl nitrite (0.29 ml) were added under cooling with ice-NaCl. Stirring was continued for 5 min, when the hydrazine test[21] became negative. This solution was then neutralized with Et_3N (0.3 ml) and combined with a solution of H-(human 5—39)-OBzl (prepared from 6.06 g of the Z(OMe)-derivative by treatment with 9 ml of TFA in the presence of 3 ml of anisole containing 0.01 ml of mercaptoethanol at 0° for 45 min) in DMF (30 ml). The mixture was stirred at 4° for 48 hr and the solvent was evaporated. The residue was treated with ether. The resulting powder was washed with H_2O and then purified by column chromatography on silica as described above; yield 5.88 g (87%), mp 173—179°, $[\alpha]_D^{27}$ −24.7° (c=0.9, DMF). Rf_1 0.56. Amino acid ratios in an acid hydrolysate: Ser 2.79, Tyr 1.60, Met 1.00, Glu 5.14, His 0.66, Phe 2.97, Arg 2.74, Gly 2.84, Lys 3.97, Pro 3.68, Val 3.31, Asp 1.90, Ala 2.73, Leu 1.00 (average recovery 90%). *Anal.* Found: C, 58.07; H, 6.50; N, 12.79.

H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH, Human ACTH (I)——Z-(human ACTH 1—39)-OBzl (572 mg) was treated with HF (approximately 3 ml) at 0° for 60 min in the presence of anisole (1 ml) containing 2% ethanedithiol. The excess HF was removed by evaporation *in vacuo* and the residue was placed in an evacuated desiccator for 1 hr and then dissolved in H_2O (30 ml). This solution, after washing with ether, was treated with Amberlite IR-4B (type 1, acetate form, approximately 1 g) for 30 min. The solution was filtered and the filtrate was lyophilized to give a fine powder, which was dissolved in a mixture of MeOH (20 ml) and 5% AcOH (30 ml). The solution, after addition of dithiothreitol (100 mg) was refluxed for 5 hr and then filtered. The solvent was evaporated and the residue was dissolved in 5% AcOH. This solution was applied to a column of Sephadex G-25 (2.8×80 cm), which was eluted with 5% acetic acid. Individual fractions (3 ml each) were examined by ultraviolet (UV) absorbancy at 280 mμ. Fractions corresponding to the front peak (tube No. 57—70) were collected and the solvent was removed by lyophilization to give a fluffy powder; yield 461 mg (88%). This sample (130 mg) was then dissolved in H_2O (20 ml) and the solution was applied to a column of CM-Sephadex (1×3 cm), which was eluted with 0.3 M ammonium acetate buffer (pH 6.9) through a mixing flask containing H_2O (175 ml). Individual fractions (16 ml each) were examined by UV absorbancy at 280 mμ. Chromatographic pattern revealed the presence of a very minor peak (tube 6—10) and a major symmetrical peak (tube No. 12—25). Fractions corresponding to the latter main peak were collected and the solution was applied to a column of Amberlite XAD-II (3×3.5 cm) for desalting. After the column was washed with H_2O (300 ml), the desired compound was eluted with 75% MeOH containing 3% AcOH and finally lyophilized to give a fluffy powder; yield 74 mg (yield in the purification step 57%). $[\alpha]_D^{20}$ −76.5° (c=0.6, 1% AcOH). (lit.[9] $[\alpha]_D^{24}$ −87.6° in 1% AcOH). Rf_3 0.53 (lit.[9] 0.51). Rf_2 0.50. Under identical conditions, Rf_3 of porcine and bovine ACTH were both 0.51 (at 18°) respectively. Disc electrophoretic mobility on 15% polyacrylamide gel (0.5×6.0 cm, 5 mA/tube) at pH 8.3 (0.38 M glycine–Tris buffer) was 1.9 cm after 55 min from the origin to the anode and at pH 4.0 (0.3 M glycine–AcOH buffer) was 2.6 cm after 130 min to the cathode. Amino acid ratios in 3 N Tos-OH hydrolysate: Ser 2.79, Tyr 2.03, Met 1.00, Glu 5.19, His 0.66, Phe 3.00, Arg 2.74, Trp 0.88, Gly 3.01, Lys 3.97, Pro 3.68, Val 3.31, Asp

21) H.E. Ertel and L. Horner, *J. Chromatog.*, 7, 268 (1962).

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1.90, Ala 2.73, Leu 0.78 (average recovery 89%). Amino acid ratios in Ap-M digest (theory is given in parenthesis): Ser+Asn 3.37 (4 calcd. as Ser), Tyr 2.32 (2), Met 1.01 (1), Glu 5.44 (5), His 0.76 (1), Phe 3.00 (3), Arg 3.17 (3), Trp 0.91 (1), Gly 2.98 (3), Lys 4.06 (4), Pro 4.04 (4), Val 3.30 (3), Asp 0.65 (1), Ala 2.98 (3), Leu 1.06 (1) (average recovery 77%). *Anal.* Calcd. for $C_{207}H_{308}O_{58}N_{56}$–$8CH_3COOH \cdot 14H_2O$: C, 50.78; H, 7.03; N, 14.87. Found: C, 50.75; H, 6.61; N, 14.73.

Acknowledgement The authors express their sincere appreciations to Drs. A. Tanaka and M. Nakamura of Central Research Laboratory, Shionogi Pharm. Co. for these biological assays.

[Chem. Pharm. Bull. 24(10)2563—2566(1976)]

UDC 547.94.057 : 541.541.63.04

Studies on the Syntheses of Analgesics. XLI.[1] Optical Resolution of (±)-N-Cyclopropylmethyl-3-hydroxy-9-azamorphinan (Studies on the Syntheses of Heterocyclic Compounds. Part DCLXIX[2])

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(Received January 27, 1976)

Optical resolution of (+)-*N*-cyclopropylmethyl-3-hydroxy-9-azamorphinan (I) was successfully effected with (+)-*O,O*-dibenzoyltartaric acid or (2*R*: 3*R*)-2′-nitrotartranilic acid as the resolution agent. The optically active compounds thus obtained were tested for their analgesic activity and antagonistic effect of morphine analgesia.

Previously, we reported the several synthetic methods of the 9-azamorphinan ring system[4] and found this type of compounds to have an analgesic activity. Especially, the title compound *N*-cyclopropylmethyl-3-hydroxy-9-azamorphinan (I) was found to be about twice as potent as pentazocine in the analgesic activity, but to show no side effects such as addiction.[4d] Therefore, we have examined the pharmacological activity of the optically active compound of I and attempted to accomplish the optical resolution of I. Here we wish to report the successful resolution of I and also the analgesic activity of the optically active compounds.

Firstly, we investigated the optical resolution of compounds (I—III) in several solvents by using (+)-*O,O*-dibenzoyltartaric acid,[5] which was widely used as a resolution agent in morphinan system compounds, and (2*R*: 3*R*)-2′-nitrotartranilic acid,[6] which was developed as an effective resolution agent by Montzka, Pindell and Matiskella.[6] Both of them effected successfully the resolution of I in 2-propanol and in 90% ethanol while cooling in refrigerator to give the crystalline salt of optically active isomer. Furthermore, (+)-binaphthylphosphoric

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