Meeting of Kanto Branch, Pharmaceutical Society of Japan, Tokyo, Nov. 1978.
2) S. Sakai, H. Takayama, and T. Okamoto, *Yakugaku Zasshi*, **99**, 647 (1979).
3) S.W. Pelletetier and Z. Djarmati, *J. Am. Chem. Soc.*, **98**, 2626 (1976).
4) H. Beierbeck and J.K. Saunders, *Can. J. Chem.*, **54**, 2985 (1976).
5) The structure of neoline (2) was previously determined unambiguously by the correlation with delphisine (8,14-diacetylneoline) whose structure was confirmed by X-ray crystallographytic study of its hydrochloride.[8]
6) K. Wiesner, H.W. Brewer, D.L. Simmons, D.R. Babin, F. Bickelhaupt, J. Kallos, and T. Bogri, *Tetrahedron Lett.*, **1960**, 17.
7) K. Omura, A.K. Sharma, and D. Swern, *J. Org. Chem.*, **41**, 957 (1976); S.L. Huang, K. Omura, and D. Swern, *ibid.*, **41**, 3329 (1976).
8) S.W. Pelletetier, Z. Djarmati, S. Lajsic, and W.H. DeCamp, *J. Am. Chem. Soc.*, **98**, 2617 (1976).

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Synthesis of a Heptacosapeptide Amide corresponding to the Entire Amino Acid Sequence of Chicken Gastrin Releasing Peptide (GRP)[1]

The heptacosapeptide amide corresponding to the entire amino acid sequence of chicken gastrin releasing peptide was synthesized using a new amide forming reagent, 3-acyl-thiazolidine-2-thione, and a new deprotecting system, 1 M trifluoromethanesulfonic acid (TFMSA)-thioanisole in TFA.

Keywords——total synthesis of chicken gastrin releasing peptide; trifluoromethanesulfonic acid-thioanisole deprotection; a new amide forming reagent; 3-acyl-thiazolidine-2-thione; side reaction at the Trp residue; Curtius rearrangement in an azide condensation reaction

Succeeding to the structural elucidation of porcine GRP,[2] McDonald *et al.*, characterized the similar heptacosapeptide with similar bombesin-like activity from chicken intestine.[3] Within this molecule, nine amino acids differ from those of porcine GRP. However its C-terminal tridecapeptide portion is identical with that of porcine origin except for one amino acid residue at position 19 (Figure).

Following to the synthesis of porcine GRP,[4] we wish to report the synthesis of this newly found GRP from chicken origin. The method we employed for the present synthesis is essentially the same as employed for the former synthesis of porcine GRP. Amino acid derivatives bearing protecting groups removable by 1 M TFMSA-thioanisole[5] was employed; *i.e.*, Lys(Z), Arg(Mts)[6] and Ser(Bzl). The Met residue was protected as its sulfoxide.[7]

Of seven peptide fragments, three fragments, (**1**), (**2**) and (**4**), are identical with those employed for our previous synthesis of porcine GRP. The other four fragments were newly

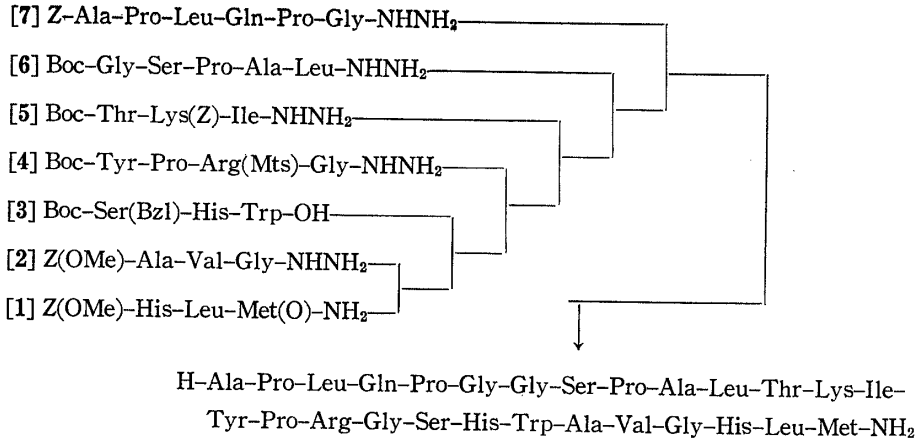

Fig. 1 Synthetic Route for the Chicken Gastrin Releasing Peptide

synthesized due to structural variation of these two peptides, for which a new coupling procedure with 3-acyl-thiazolidine-2-thione[8] was preferentially employed. Ser(Bzl) was adopted to make purification of fragment (**3**) easier, since in the trial synthesis, Boc–Ser–His–Trp–OH was found to be partially soluble in water.

Seven fragments were assembled successively by the Honzl and Rudinger's azide procedure,[9] except for fragment (**3**). This Trp-containing fragment was condensed by the DCC-HOBT procedure[10] to minimize possible racemization. After introduction of Trp-residue into the chain, the Boc group was adopted as the Nα-protecting group, since much less side reactions are involved during the TFA deprotection,[11] compared with the Z(OMe) group.[12] In addition, anisole containing EDT[13] was employed as a cation scavenger to minimize such side reaction at the Trp residue.

Most of the fragment condensation proceeded smoothly as expected, except for the azide condensation of fragment (**5**). This reaction had to be performed at more lower temperature (−10°C) than usual to minimize possible Curtius rearrangement.[14] The product obtained in a conventional manner afforded a low recovery of Ile in an acid hydrolysate.

The protected heptacosapeptide thus obtained was purified by gel-filtration on Sephadex LH-60 using DMF as an eluant and deprotected with 1M TFMSA-thioanisole in TFA in the presence of *m*-cresol at 0°C for 90 min to remove all protecting groups employed.

As performed in the previous synthesis of porcine GRP, the deprotected peptide was converted to the corresponding acetate, treated with dilute ammonia to reverse possible N→O shift[15] and incubated with dithiothreitol to reduce the Met(O). The reduced product was then purified successively by gel-filtration on Sephadex G-25, ion-exchange chromatography on CM-cellulose and partition chromatography on Sephadex G-25 with the solvent system of *n*-BuOH–EtOH–0.01M NH_4OAc= 4: 1: 5.

Purity of synthetic chicken GRP was ascertained by TLC [*Rf* 0.63 in *n*-BuOH–AcOH–Pyr–H_2O=4: 1: 1: 2], HPLC [retention time 5.80 min, on Cosmosil (5C18) (4.6×150 mm) column using the solvent system, 5 mM ammonium acetate–acetic acid buffer (pH 4.2): ethanol=70: 30], and amino acid analysis in 6N HCl hydrolysate [Thr 0.99, Ser, 1.86, Glu 0.99, Pro 4.06, Gly 3.97, Ala 2.76, Val 1.00, Met 0.75, Ile 0.87, Leu 3.00, Tyr 0.96, Lys 1.02, His 1.78, Arg 1.00 (recovery of Val 83%) (Trp content (0.80) was confirmed by acid hydrolysis with 4N MSA)].

Synthetic chicken GRP was found to possess the activity equivalent to that of synthetic porcine GRP, when assayed plasma RI-gastrin level, after *i.v.* administration into rats.

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References and Notes

1) Amino acids, peptides and their derivatives are of the L-configuration. The following abbreviations were used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Mts=mesitylene-2-sulfonyl, Bzl=benzyl, DCC=dicyclohexylcarbodiimide, HOBT=N-hydroxybenzotriazole, DMF=dimethylformamide, EDT=ethanedithiol, TFA=trifluoroacetic acid, MSA=methanesulfonic acid, HPLC=high performance liquid chromatography.
2) T.J. McDonald, H. Jörnvall, G. Nilsson, M. Vagne, M. Ghatei, S.R. Bloom, and V. Mutt, *Biochem. Biophys. Res. Comm.*, **90**, 227 (1979).
3) T.J. McDonald, H. Jörnvall, M. Ghatei, S.R. Bloom, and V. Mutt, *FEBS Letters*, **122**, 45 (1980).
4) H. Yajima, K. Akaji, N. Fujii, M. Moriga, M. Aono, and A. Takagi, *Chem. Pharm. Bull.*, **28**, 2276 (1980).
5) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J.C.S. Chem. Comm.*, **1974**, 107; Y. Kiso, S. Nakamura, K. Ito, K. Ukawa, K. Kitagawa, T. Akita, and H. Moritoki, *J.C.S. Chem. Comm.*, **1979**, 971.
6) H. Yajima, M. Takeyama, J. Kanaki, and K. Mitani, *J.C.S. Chem. Comm.*, **1978**, 482.
7) B. Iselin, *Helv. Chim. Acta*, **44**, 61 (1961).
8) H. Yajima, K. Akaji, Y. Hirota, and N. Fujii, *Chem. Pharm. Bull.*, **28**, 3140 (1980).
9) J. Honzl and J. Rudinger, *Coll. Czech. Chem. Comm.*, **26**, 2333 (1961).
10) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
11) Yu. B. Alakhov, A.A. Kiryushkin, V.M. Lipkin, and G.W.A. Milne, *J.C.S. Chem. Comm.*, **1970**, 406; E. Wünsch, E. Jaeger, L. Kisfaludy, and M. Löw, *Angew. Chem.*, **89**, 330 (1977).
12) H. Ogawa, T. Sasaki, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, **26**, 3144 (1978).
13) J.J. Sharp, A.B. Robinson, and M.D. Kamen, *J. Am. Chem. Soc.*, **95**, 6097 (1973).
14) K. Inouye, K. Watanabe, and M. Shin, *J.C.S. Perkin I*, **1977**, 1905; K. Inouye and K. Watanabe, *ibid.*, **1977**, 1911.
15) S. Sakakibara, in "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins," Vol. 1, B. Weinstein Ed., Marcel Dekker, New York, 1971, p. 51.

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