Synthesis of the Docosapeptide Corresponding to the Entire Amino-acid Sequence of Porcine Motilin

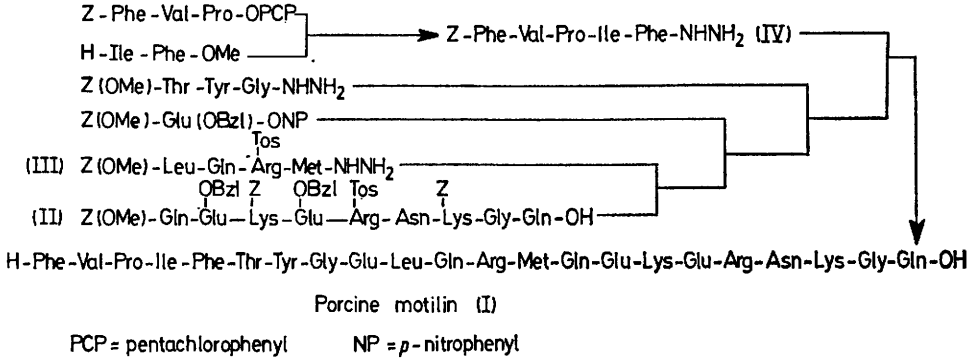
By HARUAKI YAJIMA,* YOSHIYUKI KAI, and HIROKI KAWATANI
(*Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan*)

Summary The docosapeptide corresponding to the entire amino-acid sequence of porcine motilin, a gastric motor activity-stimulating polypeptide, has been synthesised.

THE entire amino-acid sequence of porcine motilin (I), a gastric motoractivity-stimulating polypeptide, was determined by Brown *et al.*[1] in 1974, after a minor correction of their 1973 formula.[2] We have synthesized the docosapeptide corresponding to the newly revised sequence of motilin by a method (Scheme) different from that employed by Wünsch *et al.*[3] who synthesized [13-Nle, 14-Glu]-motilin in 1973.

The protected tetrapeptide hydrazide, Z(OMe)-Leu-Gln-Arg(Tos)-Met-$NHNH_2$ (III), was synthesised by the stepwise elongation method starting with Z(OMe)-Arg(Tos)-Met-OMe, which was obtained by the dicyclo-hexylcarbodiimide (DCC) condensation procedure.[10] The *p*-nitrophenyl ester and pentachlorophenyl ester procedure[11] were employed for introduction of Z(OMe)-Gln-OH and Z(OMe)-Leu-OH respectively. The resulting, Z(OMe)-Leu-Gln-Arg(Tos)-Met-OMe was treated with hydrazine to give (III).

In order to prepare the *N*-terminal pentapeptide hydrazide, Z-Phe-Val-Pro-Ile-Phe-$NHNH_2$ (IV), Z-Phe-Val-Pro-OH was treated with pentachlorophenyl trichloroacetate[12]



SCHEME

The *C*-terminal protected nonapeptide, Z(OMe)-Gln-Glu(OBzl)-Lys(Z)-Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH (II) was synthesized in a stepwise manner by the active ester procedure starting from H-Gly-Gln-OH.[4] The Z(OMe) group removable by trifluoroacetic acid (TFA)[5] was used as a protecting group. Z(OMe)-Asn-OH, Z(OMe)-Gln-OH, and Z(OMe)-Glu(OBzl)-OH were introduced to the peptide chain by the *p*-nitrophenyl ester procedure,[6] Z(OMe)-Lys(Z)-OH by the 5-chloro-8-quinolyl ester procedure,[7] and Z(OMe)-Arg(Tos)-OH by the 2,4-dinitrophenyl ester procedure.[8,9]

to give the corresponding pentachlorophenyl ester, which was allowed to react with H-Ile-Phe-OMe and the resulting protected pentapeptide ester was converted into (IV) in the usual manner.

The entire amino acid sequence of motilin was constructed as in the Scheme. Except for the incorporation of the Glu(OBzl) residue, each fragment condensation was performed by the modified azide procedure[13] to avoid the risk of racemization. Every intermediate and the fully protected docosapeptide, Z-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu(OBzl)-Leu-Gln-Arg(Tos)-Met-Gln-Glu(OBzl)-Lys(Z)-

Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln-OH, were purified by column chromatography on silica using $CHCl_3$–MeOH–H_2O (8 : 3 : 1, v/v) as solvent system and characterized by elemental analysis and acid hydrolysis.

All protecting groups from the docosapeptide were removed by HF[14] at 0° for 60 min. Anisole and Met were used as scavengers. The deblocked product was treated with Amberlite IR-4B (acetate form) and then purified by column chromatography on Sephadex G-25 followed by CM-Sephadex. The desired peptide was eluted with 1% AcOH in the former step and by gradient elution with ammonium acetate buffer (0·2 M, pH 6·9) in the latter step. NH_4OAc in the desired fractions was removed by lyophilization. The synthetic docosapeptide thus purified exhibited a single spot on t.l.c. and gave satisfactory elemental and amino acid analyses.

Biological activity was assayed by Dr. Zen Itoh, Department of Surgery, Gunma University, School of Medicine. It was found that by an intravenous infusion to dogs, our synthetic peptide exhibited characteristic contractile activity patterns of the stomach and the duodenum, which were quite similar to those of burst activity of those organs.

(*Received*, 15*th December* 1974; *Com.* 1530.)

[1] H. Schubert and J. C. Brown, *Canad. J. Biochem.*, 1974, **52**, 7.
[2] J. C. Brown, M. A. Cook, and J. R. Dryburgh, *Canad. J. Biochem.*, 1973, **51**, 533.
[3] E. Wünsch, J. C. Brown, K. H. Deimer, F. Drees, E. Jaeger, J. Musiol, R. Schart, H. Stocker, P. Thamm, and G. Wendlberger, *Z. Naturforsch.*, 1973, **28c**, 235.
[4] Y. Shimonishi, S. Sakakibara, and S. Akabori, *Bull. Chem. Soc. Japan*, 1962, **35**, 1966.
[5] F. Weygand and K. Hunger, *Chem. Ber.*, 1962, **95**, 1.
[6] M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1959, **81**, 5688.
[7] H. D. Jakubke, *Chem. Ber.*, 1966, **99**, 2944; *Z. Chem.*, 1966, **6**, 52.
[8] J. A. Farrington, P. J. Hextall, G. W. Kenner, and J. M. Turner, *J. Chem. Soc.*, 1957, 1407.
[9] M. Bodanszky and M. A. Ondetti, *Chem. and Ind.*, 1966, 26.
[10] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[11] J. Kovacs and M. Q. Ceprini, *Chem. and Ind.*, 1965, 2100.
[12] M. Fujino and C. Hatanaka, *Chem. Pharm. Bull.* (*Japan*), 1968, **10**, 929.
[13] J. Honzl and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1961, **26**, 2333.
[14] S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, 1967, **40**, 2164.