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Synthesis of Granuliberin-R, a New Frog Skin Peptide from *Rana rugosa*[1)]

Granuliberin-R, H–Phe–Gly–Phe–Leu–Pro–Ile–Tyr–Arg–Arg–Pro–Ala–Ser–NH_2, was synthesized in two alternative routes; the one, after removing three different protecting groups employed, *i.e.*, Z(OMe), *p*-methoxybenzenesulphonyl from Arg and Bzl from Ser by methanesulphonic acid and the other, Z and nitro from Arg by hydrogenolysis.

Keywords——frog skin peptide from *Rana rugosa*; mast cell degranulating activity; deprotection by mathanesulphonic acid; N^G-*p*-methoxybenzenesulphonyl-Arg; DCC plus N-hydroxybenzotriazole condensation; DCC plus N-hydroxynorbornenedicarboxinide condensation

Granuliberin-R is a peptide with the potent mast cell degranulating property, isolated from skins of *Rana rugosa* and its amino acid sequence (I) was disclosed in 1976.[2)]

The dodecapeptide amide corresponding to the entire amino acid sequence of this new frog skin peptide was synthesized using amino acid derivatives bearing protecting groups removable by methanesulphonic acid (MSA),[3)] *i.e.*, Arg(MBS)[4)] and Ser(Bzl). The TFA labile Z(OMe) group[5)] served as the temporal protection of the α-amino function of intermediates.

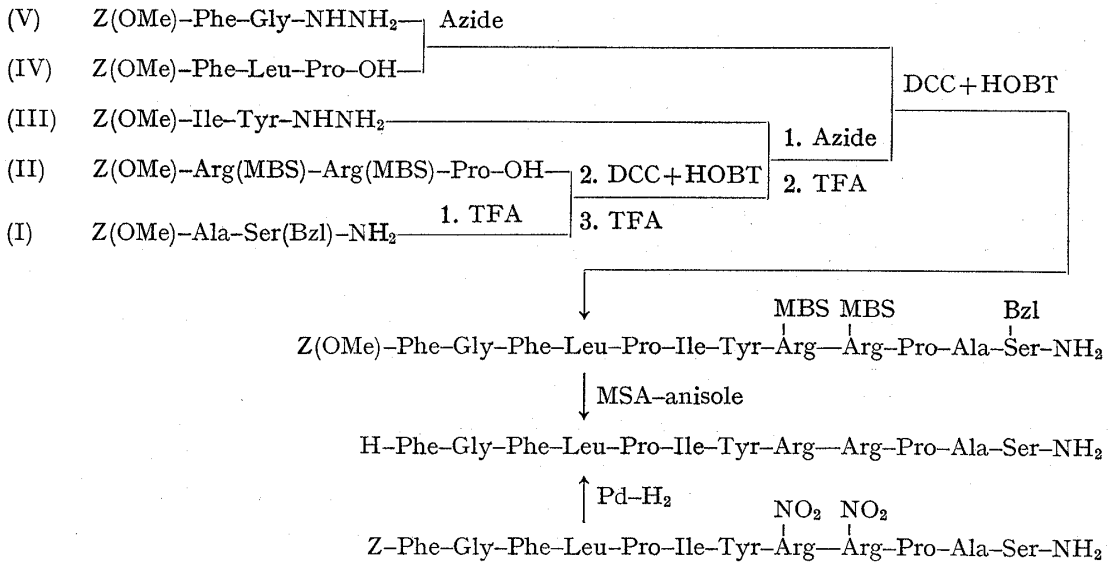

Fig. 1. Synthetic Route to Granuliberin-R

1) Amino acids, peptides and their derivatives are of the L-configuration. Following abbreviations were used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, MBS=*p*-methoxybenzenesulphonyl, DCC=dicyclohexylcarbodiimide, TFA=trifluoroacetic acid.

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Five peptide fragments prepared in a conventional manner were selected as the building blocks; *i.e.*, Z(OMe)–Ala–Ser(Bzl)–NH_2 (I, mp 182—185°, $[\alpha]_D^{25}$ +3.1° in MeOH. *Anal.* Calcd. for $C_{22}H_{29}N_3O_6$: C, 61.52; H, 6.34; N, 9.79. Found: C, 61.74; H, 6.28; N, 9.84.), Z(OMe)–Ile–Tyr–$NHNH_2$ (III, mp 221—222°, $[\alpha]_D^{25}$ −23.9° in DMF. *Anal.* Calcd. for $C_{24}H_{32}N_4O_6$: C, 61.00; H, 6.83; N, 11.86. Found: C, 61.08; H, 6.92; N, 11.63.), Z(OMe)–Phe–Gly–$NHNH_2$ (V, mp138—140°, $[\alpha]_D^{25}$ −20.7° in DMF). *Anal.* Calcd. for $C_{20}H_{24}N_4O_5$: C, 59.99; H, 6.04; N, 13.99. Found: C, 60.24; H, 5.95; N, 14.02.) and the two Pro-terminal tripeptides, Z(OMe)–Arg(MBS)–Arg-(MBS)–Pro–OH (II, mp 124—126°, $[\alpha]_D^{24}$ −15.6° in DMF. *Anal.* Calcd. for $C_{40}H_{53}N_9O_{13}S_2$: C, 51.54; H, 5.73; N, 13.53. Found: C, 51.28; H, 5.56; N, 13.36.) and Z(OMe)–Phe–Leu–Pro–OH (IV, mp 80—83°, $[\alpha]_D^{24}$ −47.1° in DMF. *Anal.* Calcd. for $C_{29}H_{37}N_3O_7$: C, 64.54; H, 6.91; N, 7.79. Found: C, 64.47; H, 6.88, N, 7.70.).

Necessary peptide fragments thus obtained were then assembled according to the scheme illustrated in Fig. 1. The Z(OMe) group was removed from intermediates by the TFA-anisole system[5] prior to each condensation reaction. The Rudinger's azide procedure[6] was employed for introduction of the two hydrazides (III) and (V) and the DCC plus N-hydroxybenzotriazole (HOBT) procedure[7] was employed for condensation of the two Pro-terminal units, (II) and the N-terminal pentapeptide, Z(OMe)–Phe–Gly–Phe–Leu–Pro–OH resulted by the above azide condensation of (IV) and (V) respectively. The resulting protected dodecapeptide amide, Z(OMe)–Phe–Gly–Phe–Leu–Pro–Ile–Tyr–Arg(MBS)–Arg(MBS)–Pro–Ala–Ser(Bzl)–NH_2 (mp 149—153°, $[\alpha]_D^{22}$ −40.0° in DMF. *Anal.* Calcd. for $C_{99}H_{129}N_{19}O_{23}S_2$. 1.5 H_2O: C, 58.16; H, 6.50; N, 13.02. Found: C, 58.12; H, 6.45; N, 13.02.) and protected intermediates were purified by batchwise washing with 5% citric acid and water followed by repeated precipitation from tetrahydrofuran or dimethylformamide with appropriate solvents, such as ether or ethyl acetate.

For deprotection, the above protected dodecapeptide amide was exposed to MSA in the presence of anisole at room temperature for 60 minutes. The deblocked product, precipitated with ether as an oil, was converted to the corresponding acetate with Amberlite CG-4B (acetate form) and purified by column chromatography on CM-cellulose. The desired product was eluted as a single peak by gradient elution with ammonium acetate buffer (0.2 M, pH 6.9) and finally desalted by column chromatography on Sephadex G-15 to give a white fluffy powder.

The synthetic dodecapeptide amide ($[\alpha]_D^{25}$ −91.5° in 1 N AcOH) exhibited a single spot on thin layer chromatography (Kieselgel G, Merck) with the identical *Rf* value (0.78 in *n*-BuOH–AcOH–pyridine–H_2O=30: 6: 20: 24 v/v) with that of the natural peptide. Complete removal of the protecting groups by MSA was proved by satisfactory recovery of Arg and Ser in a hydrolysate with aminopeptidase (AP–M)[8] (Phe 1.97, Gly 1.00, Leu 1.02, Pro 1.94, Ile 1.03, Tyr 1.00, Arg 2.03, Ala 0.98, Ser 0.93, average recovery 92%).

Alternatively, the identical dodecapeptide amide was obtained after hydrogenolysis of Z–Phe–Gly–Phe–Leu–Pro–Ile–Tyr–Arg(NO_2)–Arg(NO_2)–Pro–Ala–Ser–NH_2, which was prepared by the DCC plus N-hydroxynorbornenedicarboximide condensation[9] of three peptide subunits, the N-terminal and middle pentapeptides and H–Ala–Ser–NH_2 successively.

The synthetic peptide was consistent chromatographically with natural granuliberin-R. The degranulating activity of the synthetic peptides prepared by alternative routes in the rat peritoneal mast cells was found equivalent to that of the natural source. It seems interesting to note that the dodecapeptide lacking the C-terminal amide exhibited only the one 5 th of the parent molecule.

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