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SYNTHESIS OF GRANULIBERIN R. AN APPLICATION OF THE 'HOLD-IN-SOLUTION' METHOD

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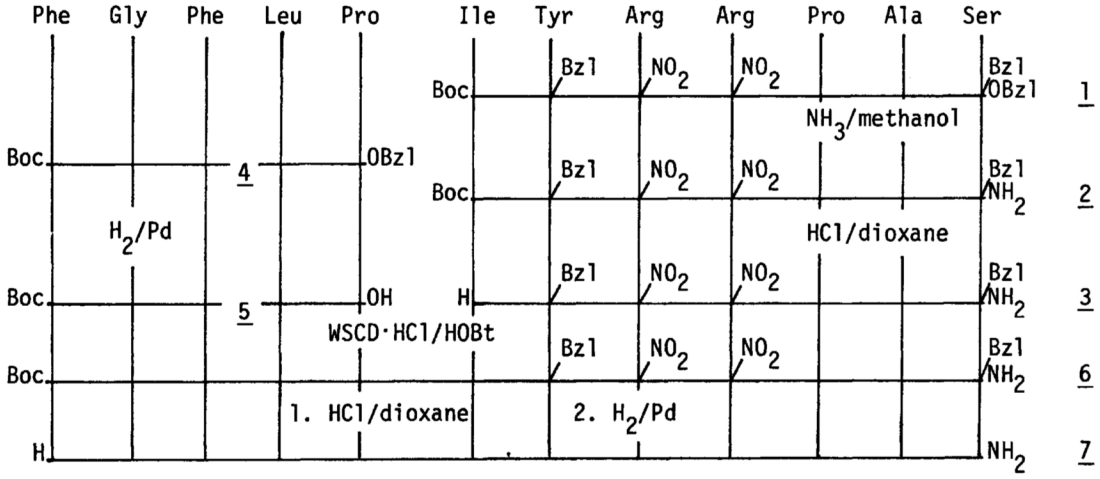
Granuliberin R, a new dodecapeptide isolated from Rana rugosa, was synthesized. The 'hold-in-solution' method was applied to the preparation of the fragment peptides.

Granuliberin R,[2] a mast cell degranulating peptide, was synthesized in order to substantiate its amino acid sequence proposed as H-Phe-Gly-Phe-Leu-Pro-Ile-Tyr-Arg-Arg-Pro-Ala-Ser-NH_2 (7).[3] The dodecapeptide 7 was constructed as illustrated in Fig. 1.

The fragment peptides, 1 and 4, were synthesized by the 'hold-in-solution' method proposed in the previous paper,[4] and were obtained in a short period of time in overall yields of 61% and 68%, respectively, based on each C-terminal residue. In the preparation of 1, gel formation owing to the poor solubility of the resulting peptide 1 in 1,2-dichloroethane (DCE) reduced the rate of the coupling of Boc-isoleucine with H-Tyr(Bzl)-Arg(NO_2)-Arg(NO_2)-Pro-Ala-Ser(Bzl)-OBzl. Repeating acylation of the amine component in N, N-dimethylformamide yielded 1 sat-

Fig. 1.



isfactorily. Hydrogenolysis of 4 followed by reprecipitation from ethyl acetate-diethyl ether gave the C-terminal free peptide 5. TLC, R_f (silicagel, chloroform-methanol, 7:3): 0.73. Amino acid ratios in the acid hydrolysate (in const. boiling HCl, 110 °C, 16 h): Pro 1.01, Gly 0.96, Leu 1.01, Phe 2.03. The benzyl ester 1 was converted by the usual method to the amide 2, which was reprecipitated from ethanol-diethyl ether. TLC, R_f (silicagel, 1-butanol-acetic acid-water, 4:1:1): 0.73 with a trace spot at 0.56. Amino acid ratios in the acid hydrolysate[5]: Ser 0.86, Pro 1.08, Ala 1.17, Ile 0.89, Arg + Orn 1.57. By acid treatment in DCE followed by addition of 2 M Na_2CO_3, 2 yielded the N-terminal free heptapeptide amide 3, which was acylated in a mixture of DCE and water with 2 eq. amounts each of 5, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD·HCl), and 1-hydroxybenzotriazole (HOBt). After completion of the reaction, the mixture was washed with 0.1 M HCl and water. Removal of the N-terminal Boc-group of 6 and catalytic hydrogenolysis of the remaining protectors yielded 7. It was purified by SP-Sephadex chromatography (with a gradient of sodium acetate buffer, 0.05 - 0.35 M, pH 6.5) and, additionally, partition chromatography (Sephadex G-25, 1-butanol-acetic acid-pyridine-water, 100:3:10:100). The highly purified product 7 was obtained in a 17% yield from 2. TLC, R_f (silicagel, 1-butanol-acetic acid-pyridine-water, 16:3:10:12): 0.65. Amino acid analyses: the acid hydrolysate; Ser 0.94, Pro 2.02, Gly 1.02, Ala 1.03, Ile 0.98, Leu 1.02, Tyr 0.97, Phe 2.00, Arg 2.03; the aminopeptidase M digest; Ser 0.87, Pro 2.14, Gly 0.93, Ala 1.02, Ile 1.02, Leu 1.07, Tyr 1.03, Phe 1.89, Arg 2.03. $[\alpha]_D^{25}$ -83.1° (c 0.56, 1 M acetic acid). Found: C, 52.03; H, 6.72; N, 15.06%. Calcd for $C_{69}H_{103}O_{14}N_{19}\cdot 3CH_3COOH\cdot 7H_2O$: C, 52.10; H, 7.52; N, 15.39%.

The synthesized peptide exhibited the same biological potency and chromatographic behavior as those of the natural peptide. Thus the structure proposed by Nakajima and Yasuhara was substantiated, and moreover the applicability of the 'hold-in-solution' method was proved in the present preparation of granuliberin R.

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References and Notes

1) To whom correspondence should be addressed.
2) T. Yasuhara and T. Nakajima, "Proceedings of the 14th Symposium on Peptide Chemistry" ed. by T. Nakajima, Protein Research Foundation, Osaka (1977), p. 159. T. Nakajima and T. Yasuhara, Chem. Pharm. Bull. (Tokyo), in press.
3) In the 97th Annual Meeting of the Pharmaceutical Society of Japan, it was presented by T. Nakajima et al. that the C-terminal residue of granuliberin R is serineamide.
4) S. Nozaki, A. Kimura, and I. Muramatsu, Chem. Lett., 1057 (1977).
5) Because of being fully protected, no tyrosine was detected. A part of N^G-nitroarginine degraded to give ornithine.

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