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### Occurrence of a New Active Peptides on Smooth Muscle and Bradykinin in the Skin of *Rana nigromaculata* Hallowell

Similar to some amphibian skin,<sup>1)</sup> methanol extracts of wet skin of *Rana nigromaculata*, a Japanese amphibian, was found to contain some active peptides which possess a potent stimulant action on isolated rat uterus.

The present communication describes the purification and identification of these active peptides. Separatory method of the peptides is shown in Chart 1.

**Characterization and Structure of the Peptide I**—The peptide gave a single dimethylaminonaphthalenesulfonyl (DNS) derivative by thin-layer chromatography on silica gel H. The peptide was stable against tryptic digestion, but cleaved with chymotrypsin to yield three fragments including free arginine. DNS-valine was obtained as the N-terminus when the DNS-peptide I was hydrolyzed with 6 N HCl at 105° for 24 hours, and following amino acids were also determined by amino acid analyzer: Thr<sub>1</sub>, Pro<sub>3</sub>, Gly<sub>1</sub>, Phe<sub>2</sub>, Arg<sub>1</sub>. From the results of Edman degradation combined with DNS method,<sup>2)</sup> which was performed to the last sequence, it was concluded that the structure of the peptide I was val<sup>1</sup>-thr<sup>6</sup>-bradykinin (Val-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg). Relative contracting activity of val<sup>1</sup>-thr<sup>6</sup>-bradykinin on isolated rat uterus was about 1/5 to that of bradykinin.

**Characterization of the Peptide II**—This peptide was identified with bradykinin by the evidence described below. DNS derivative of the peptide showed the same *R<sub>f</sub>* value as DNS bradykinin on a thin-layer of silica gel H. N-terminal amino acid was arginine. Chromatographic behaviors of the DNS-peptide II after the treatment with trypsin or chymotrypsin were identical to that of DNS-bradykinin. Amino acid composition was also identical to bradykinin.

Complete details will be published elsewhere. Total synthesis and further pharmacological investigation of val<sup>1</sup>-thr<sup>6</sup>-bradykinin are in progress.

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2) W.R. Gray and B.S. Hartley, *Biochem. J.*, **89**, 379 (1963).

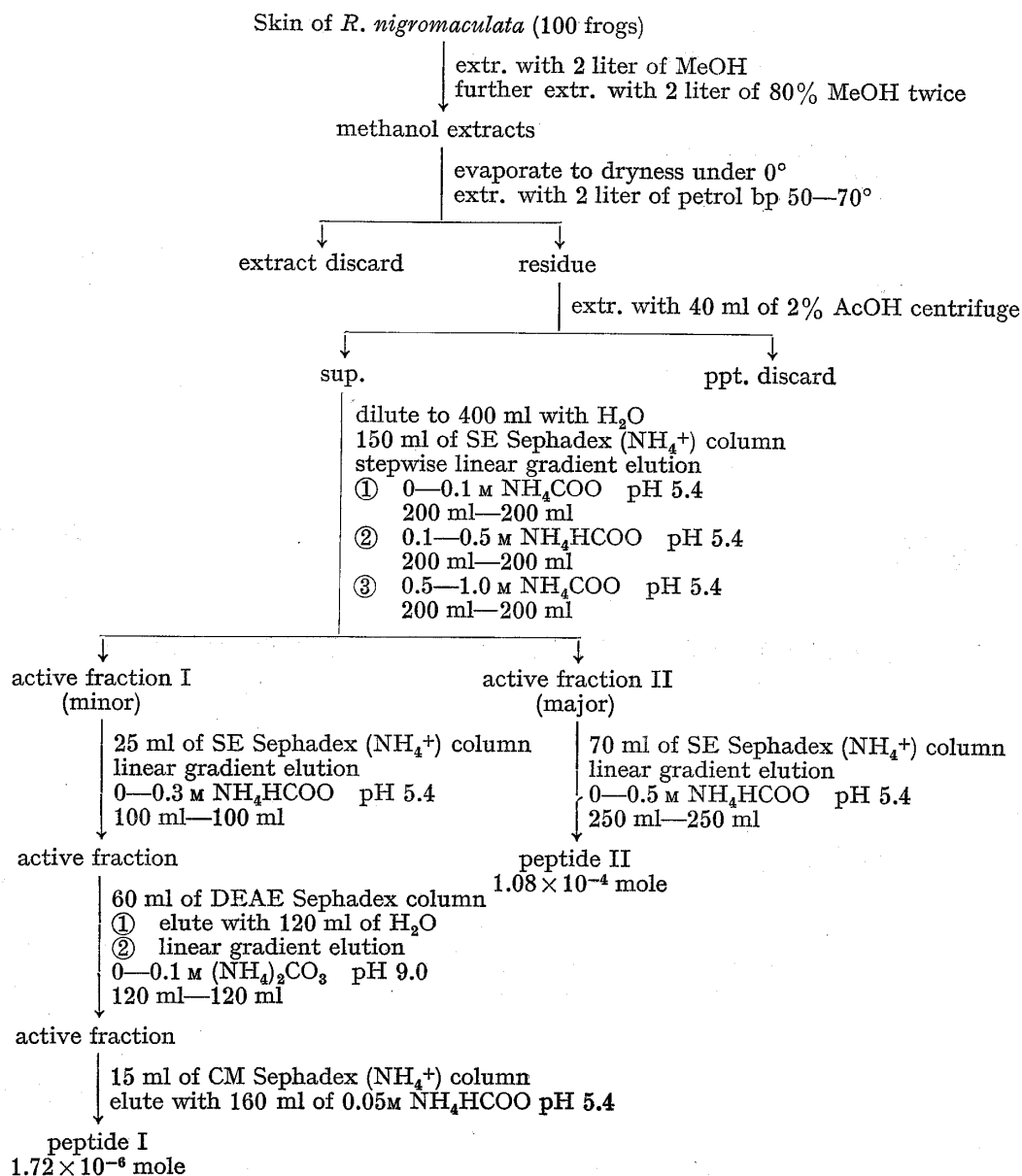


Chart 1

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