(**10**) of mp 147—148.5° (17% yield from **5**) and 2α-*p*-anisylmethyl-3α-acetoxy-4β-hydroxypyrrolidine (**1**) of mp 119.5—121°[12] (11% yield from **5**), which were separated by silica gel column chromatography. **10** was identified with the sample obtained by removal of the carbobenzyloxy group from **7a**. **1** exhibited the same infrared and nuclear magnetic resonance spectra and the same behavior on thin-layer chromatogram as the sample of natural anisomycin.[13] The synthetic *dl*-anisomycin showed half the activity against *Candida albicans*, as compared to that of the natural antibiotic.

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12) Repeated recrystallization of **1** from benzene–hexane gave polymorphic crystals which showed a higher, but not-sharp melting point.
13) The sample of anisomycin was supplied by Dr. L. Delcambe, International Center of Information on Antibiotics, Liége, Belgium, to whom we wish to express our appreciation.

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On the Third Active Peptide on Smooth Muscle in the Skin of *Rana nigromaculata*

HALLOWELL

In the previous communication, occurrence of two kinds of peptide, val[1]–thr[6]–bradykinin and bradykinin, has been demonstrated in the skin of *Rana nigromaculata*.[1]

The present communication describes the purification and amino acid sequence of the third peptide (peptide III). Separation of the peptide III from the bradykinin fraction, which corresponded to active fraction II in the previous communication,[1] was carried out as shown in chart I.

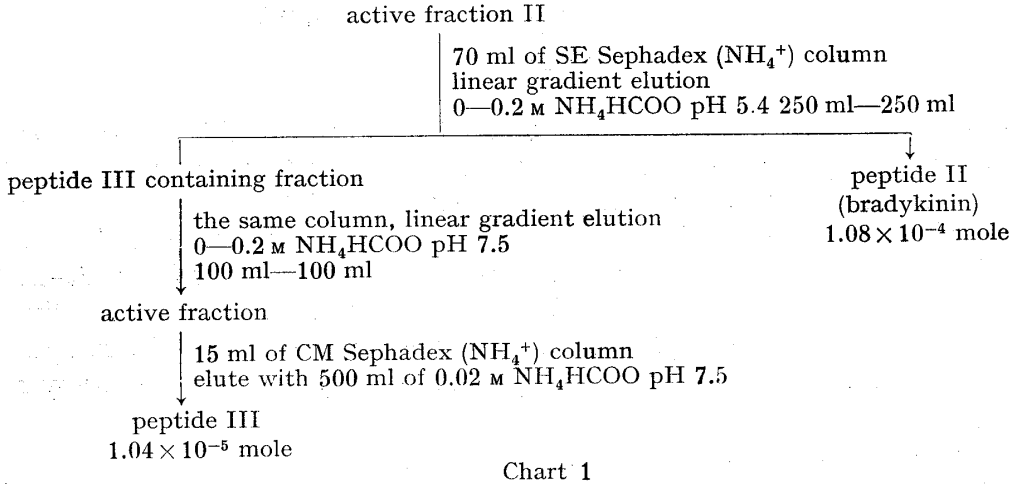


Chart 1

1) T. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **16**, 769 (1968).

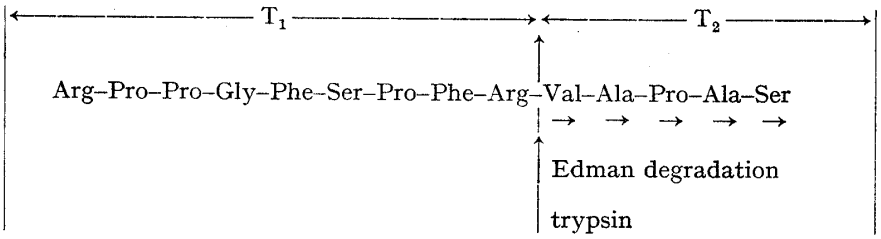
Characterization of the Peptide III

The peptide showed a single dimethylaminonaphthalenesulfonyl (DNS) derivative with thin-layer chromatography on silica gel H. The *Rf* value was slightly lower than that of DNS-bradykinin with a solvent system of isopropanol-methyl acetate-28% ammonia (7:9:4).

DNS-arginine was obtained as the N-terminus when the DNS-peptide was hydrolysed with 6N HCl at 105° C for 24 hours, and the following amino acids were also determined by an amino acid analyser: Ser_2, Pro_4, Gly_1, Ala_2, Val_1, Phe_2, Arg_1. The smooth muscle stimulant activity of the peptide was increased more than 20 times by the treatment with trypsin, but was lost by the treatment with chymotrypsin. The DNS-peptide was also split to give two fragments by trypsin. One was a fluorescent peptide (DNS-T_1), and the other was a nonfluorescent (T_2). DNS-T_1 was identical with DNS-bradykinin in the amino acid composition and chromatographic behavior.

These results indicate that the peptide III has a bradykinin sequence at the N-terminal position. An additional tryptic peptide, T_2, which has to be located in the C-terminal position, consists of the following amino acids: Ser_1, Pro_1, Ala_2, Val_1. Sequential analysis of the peptide was performed by Edman degradation combined with DNS method.[2]

The following structure was deduced as the peptide III.



Although this sequence does not appear to be related to the mammalian kininogens of Suzuki,[3a] Habermann[3b] or Pierce,[3c] it will suggest the occurrence of an another kininogen in amphibian.

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

3) *a*) T. Suzuki, S. Iwanaga, S. Nagasawa and T. Sato, "Hypotensive Peptides," ed. by E.G. Erdös, N. Back, and F. Sicuteri, Springer-Verlag, New York, Inc., 1966, p. 149; *b*) E. Habermann, *ibid.*, p. 116; *c*) J.V. Pierce and M.E. Webster, *ibid.*, p. 130.