of the determination of the C-terminal sequence of sturine B [1] enabled the complete amino-acid sequence of sturine B to be established: H-Ala-Arg_5-Ser-Ser-Arg-Pro-Glx-Arg_6-His-Gly-Arg_2-Gly-Arg_4-OH.

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SYNTHESIS OF A HEXAPEPTIDES RELATED TO ELEDOISIN

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UDC 547.964.4

In order to investigate the mechanism of the action of kinins, we have synthesized by a new method lysylphenylalanylisoleucylglycylleucylmethionine amide (I, 1-6, Table 1, Scheme) – a substance possessing a strong hypotensive and myotropic action [1]. In all cases (with the exception of E 5-6) for the formation of the peptide bond we used 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) [2] in solution in diformamide or ethanol (for H 1-6) in the presence of N-methylmorpholine. Compound E 5-6 was obtained as described by Lübke et al. [3]. After the transfer of the reaction products into ethyl acetate, the starting materials were separated by washing the solutions with sodium bicarbonate and potassium bisulfate.

The benzyloxycarbonyl and tert-butyloxycarbonyl groups were split off by treatment with solutions of hydrogen bromide and hydrogen chloride in acetic acid, respectively; the p-nitrobenzyl ester was cleaved by catalytic hydrogenolysis.

Compound E 2-4 was recrystallized from water, G 1-4 from 50% methanol, and H 1-6 from a mixture of dimethylformamide and water. The final product I 1-6 was purified by dissolution in water, filtration, and lyophilization of the filtrate; mp 241°C (decomp.) (240-243°C, decomp. [4]), $[\alpha]_D^{23}$ −16.3° (c 0.8; acetic acid) (−16.8° [4]). The results of amino-acid and elementary analyses agreed with the calculated figures. In the biological test* (contraction of the guinea-pig ileum), I 1-6 showed an activity more than twice as great as that of bradykinin, which agrees with literature information [4]. (See scheme.)

*The tests were performed by Z. P. Auna and V. E. Klusha.

TABLE 1. Electrophoretic and Chromatographic Constants of the Compounds Synthesized

Peptide	E_{His}*	Rf in the following systems†				
		1	2	3	4	5
B 3–4	0	0,45	0,95	0,98	0,85	0,96
C 3–4	0,71	0	0,10	0,50	0,50	0,50
D 2–4	0	0,40	0,95	0,98	0,90	0,85
E 2–4	0,56	0	0,35	0,90	0,75	0,80
F 1–4	0	0,18	0,95	0,90	0,85	0,96
G 1–4	0	0,02	0,90	0,97	0,86	0,88
H 1–6	0	0,02	0,90	0,90	0,86	0,88
I 1–6	0,76	0	0	0,70	0,55	0,20

*The electrophoretic mobility E_{His} was determined on type "S" [medium] paper (Leningrad Paper Mill No. 2) in 1 N acetic acid.

†For thin-layer chromatography on silica gel we used "Silufol" plates and the following solvent system: 1) chloroform–acetic acid (95 : 5); 2) chloroform–methanol–acetic acid (85 : 10 : 5); 3) n-butanol–acetic acid–pyridine–water (15 : 3 : 10 : 12); 4) n-propanol–concentrated aqueous ammonia (84 : 37); 5) n-butanol–isopropanol–water–monochloroacetic acid (65 ml : 15 ml : 20 ml : 3 g).

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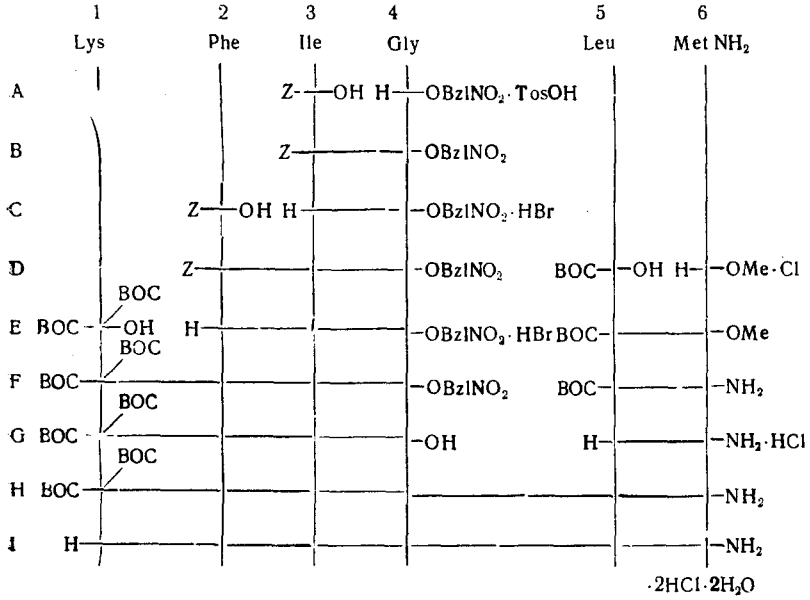
Scheme of the Synthesis



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LOCALIZATION OF THE ACTIVITY OF LEUCINE AMINOPEPTIDASE AFTER ELECTROPHORESIS IN ACRYLAMIDE GEL

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A method has been developed for localizing the activity of leucine aminopeptidase after electrophoresis in acrylamide gel with the aid of the substrate L-leucine p-nitroanilide [2].

The leucine aminopeptidase was isolated from "orizin" – a mixture of the proteins of Asp. oryzae (obtained in the Moscow enzyme factory) by precipitation with ethanol and chromatography on columns of DEAE-cellulose at pH 5.6 and 6.9 and hydroxylapatite. Disk electrophoresis was performed at pH 8.3 in tris-glycine buffer on a "Canalco-Europe" instrument (Holland), using an 11.2% acrylamide gel and 70×6 mm tubes. The duration of an experiment was 1.5 h at a current strength of 3 mA/gel and a temperature

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