Phospholipase A_2	Number of histidine residues (per monomer)		Activity, %
	Total	Modified	
Native	3.96	0	100
Modified with p-bromophenacyl bromide	3.43	0.54	44
Modified with bromomethyl adamantyl ketone	2.87	1.12	1–2

On the other hand, as has been shown previously, in a series of phospholipases A_2 from various sources, the histidine residue located in the active center of the enzyme is surrounded by hydrophobic amino acid residues which probably interferes with the access of the modifying reagent to the histidine residue [5]. In the phospholipase A_2 from *V. orientalis*, however, there are considerably more hydrophobic amino acids than in the phospholipases A_2 from other sources [2]. In view of this, the more hydrophobic molecule of bromomethyl adamantyl ketone was used to modify the enzyme under investigation. It was found that this reagent leads to 100% inactivation of the enzyme, although after a longer period (Fig. 1, curve 2). At the same time, the kinetic curve of the inactivation of the enzyme with time has a linear nature. Analysis of the amino acid compositions of the native and modified proteins (see above) show that bromomethyl adamantyl ketone is capable of modifying the functionally important histamine residues in both subunits of the dimer of phospholipase A_2 from *V. orientalis* venom. The results obtained permit the conclusion that there is a functionally important histidine residue in the active center of the molecule of *V. orientalis* venom phospholipase A_2, as well.

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SYNTHESIS OF MINILULIBERIN

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Compounds possessing the capacity for regulating ovulation processes are of interest for a number of fields of medicine and agriculture. The development of methods for obtaining such compounds is an urgent problem.

The group of compounds capable of regulating ovulation processes by stimulating the liberation of the luteinizing hormone includes miniluliberin which is structurally related to the immunostimulator tuftsin [1, 2] and consists of the tripeptide of (I) [3] (here and below, all amino acids are of the L series).

$$\overset{1}{\text{H}}\text{Thr}-\overset{2}{\text{Lys}}-\overset{3}{\text{Arg}}-\text{OH} \qquad (1)$$

We have performed the synthesis of miniluliberin (I) by schemes 1 and 2, involving the stepwise formation of the tripeptide in solution with the use as the amino components of benzyl (system 1) or p-nitrobenzyl (scheme 2) esters of the C-terminal amino acid and intermediate dipeptides, and as the activated carboxylic components the pentafluorophenyl esters [4], or mixed anhydrides of the protected lycine and threonine, respectively.

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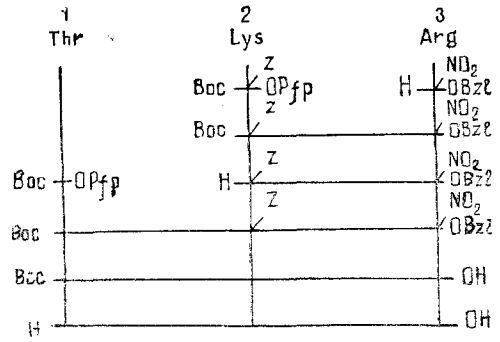
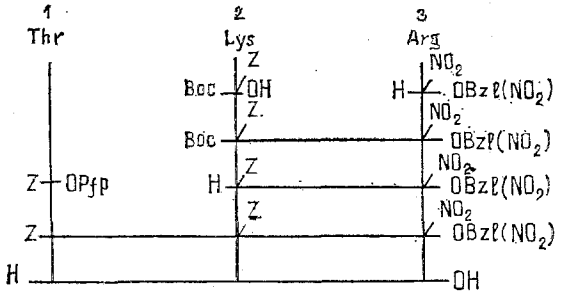


Scheme 1

In the realization of scheme 1, in addition to the tripeptide (I) we obtained its tert-butoxycarbonyl derivative (Ia), which is of independent interest.

To eliminate the tert-butoxycarbonyl protective groups, the intermediate peptides were treated with a 4.5 N solution of hydrogen chloride in dioxane. The benyloxycarbonyl and p-nitrobenzyl protective groups were eliminated by catalytic hydrogenolysis in the presence of palladium black.

The structures of all the compounds obtained follow unambiguously from the method of their synthesis, and their individuality was confirmed by chromatographic determinations and analytical results.



Scheme 2

Miniluliberin (I) — mp 120-123°C (decomp.), R_f 0.18 [acetone—25% ammonia—water (7:2:4)], 0.23 [pyridine—butan-1-ol—acetic acid—water—ethyl acetate (2:2:2:2:1)], 0.10 [isopropanol—25% ammonia—water (7:4:8)] (TLC on Silufol UV-254 plates); $[\alpha]_D^{21}$ −25° (c 1.00; methanol). Amino acid analysis: Thr 0.90 (1); Lys 1.00 (1); Arg 0.98 (1).

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