Chemistry of Natural Compounds, Vol. 29, No. 6, 1993

FOR THE 80th ANNIVERSARY OF THE BIRTH OF ACADEMICIAN A. S. SADYKOV

THE INVESTIGATION OF THE STRUCTURE AND FUNCTION OF NATURAL BIOREGULATORS (LEGACY OF A. S. SADYKOV AND PROSPECTS OF DEVELOPMENT)

Sh. I. Salikhov

In this year, the scientific community of Uzbekistan is marking the 80th anniversary of birth of the outstanding chemist Academician A. S. Sadykov.

We may consider the start of A. S. Sadykov's scientific activity to be work in the field of the chemistry of secondary metabolites — alkaloids and diterpenoids [1, 2]. These investigations, and also the original work of I. P. Tsukervanik and S. Yu. Yunusov, brought the chemistry of the natural compounds of Uzbekistan to the world level and opened up new chapters in the chemistry of heterocyclic compounds and conformational analysis and permitted the formulation of the problem of the interrelationship between the structure, functions, and biological activity of compounds.

A. S. Sadykov was a wide-ranging specialist on natural compounds. In the 50s-60s numerous alkaloids of the α,β-dipyridyl group, a broad range of bases of the isoquinoline, tropolone, and quinolizidine series, and various N-oxide forms of natural bases were isolated and their structures were established in the problem laboratory and in the department of the chemistry of natural compounds of Tashkent State University, the majority of these new compounds having unique structures [3, 4].

In contrast to what had become the traditional approach at that time, A. S. Sadykov posed three questions [5]: how do features of the structure of natural substances determine their physicochemical properties, their reactivity, and their biological activity. The solution of these problems led the school of natural-compound chemists that he had created to a higher level in this field of science.

Profound investigations with the use of mathematical modeling and the employment of a wide range of spectral methods and radiospectroscopy permitted the establishment of a definite role of the spatial forms and conformational states of polycyclic bases in the dynamics of processes of complex-formation, hydrolysis, oxidation, dehydrogenation, and isomerization.

The main stereoelectronic and energetic characteristics of the molecules of these substances were determined, and this permitted the prediction of the direction of shifts in conformational equilibria in reactions with various reagents. On the basis of these studies, purposefully directed synthesis from alkaloids and heterocycles related to them began to be developed and a number of new physiologically active substances with specific properties were obtained, while, at the same time, it was shown that neurophysiological, antitumoral, and hypotensive action and a number of other physiological effects depend substantially on the spatial structures of the molecules of natural compounds and their derivatives [6].

An object of particular interest to A. S. Sadykov throughout his life was the complex chemical study of the cotton plant, and his idea of this crop as an inexhausible source of valuable substances was confirmed by the fact that more than 100 individual compounds have been isolated from various vegetative and generative organs of the cotton plant, among them being representatives of many classes of organic chemistry [7]. Investigations of the polyphenols of the cotton plant — flavonols, anthocyans, proanthocyanidins, and gossypol pigments — proved to be the most fruitful [8].

Among the polyphenols of the cotton plant, a special position is occupied by gossypol — a terpenoid aldehydonaphthol with a specific nature of its electron distribution that is shown in the mutual influence of its numerous functional groups. A

A. S. Sadykov Institute of Bioorganic Chemistry, Uzbekistan Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 780—799, November-December, 1993.

 0009-3130/93/2906-0698$12.50

study of the conformational states of the molecules of gossypol and its derivatives under various conditions, and numerous chemical transformations in oxidation, esterification, and condensation reactions,
enabled the existence of a dynamic equilibrium between the tautomeric forms of gossypol to be established and the phenomenon of atropoisomerism to be revealed for the case of its methyl ethers [9-11].

An estimate of the descriptors of the structure of a large number of gossypol derivatives and compounds modeling it by the method of regression analysis, in combination with structural-functional analysis, permitted a number of correlations to be established between structure and physiological action. It was shown that almost all the gossypol derivatives studied are immunotropic; their immunomodulating action bears a dose-dependent nature and under otherwise identical conditions depend on the nature and structural features of an amine component [12].

The greatest activity is possessed by derivatives with *ortho*-located hydroxy groups while the type of activity (antiviral, interferon-inducing, immunomodulating) is determined by the nature of substituents in the aldehyde groups [13-15].

The high specific activity of gossypol derivatives has permitted the creation and introduction into health-care practice of the antiviral drug linament gossipola [gossypol liniment] (3%), the antiherpes drug maz' megosina [megosin salve] (3%), and the immunosuppressor batriden, which is used in liver and kidney transplants, chronic glomerulonephritis and some other autoimmune diseases [16, 17].

Gossypol is a unique substance [18-23], giving clathrates with practically all low-molecular-mass organic compounds (out of 110 substances tested, no exceptions have been detected). For the identification of the clathrates and the investigation of their desolvation, use has been made of powder diffractometry, NMR spectroscopy, derivatography, and thermomicroscopy.

Gossypol possess unusual polymorphism, consisting in the formation of eight x-radiographically identified polymorphs, six of which are obtained only on the decomposition of clathrates with the channel type of structure, belonging to various groups of isostructural complexes even in the absence of thermotropic polymorphic transformations.

To elucidate specific features of crystalline forms of gossypol and the reason for its universal ability to form clathrates, clathrates and non-solvated forms of modified gossypol have been synthesized: dianilinegossypol, diphenylethylaminegossypol, and gossypol hexamethyl ether. The crystallographic parameters of 24 clathrates and of six nonsolvated forms have been determined, and complete x-ray structural analyses have been made for three clathrates and four unsolvated forms. A number of general and particular structural rules have been been found on the basis of the voluminous material obtained, and these have been represented in the form of a complete logical map of the crystal chemistry of gossypol.

Characteristic for gossypol, as for the majority of phenolic compounds, is a capacity for interacting with the functional groups of proteins and nucleic acids and inhibiting the activity of a number of enzymes, and this, together with the possibility of suppressing undesirable free-radical processes arising in the organism in pathological states, forms the basis of the molecular mechanism of their action [24, 25]. Moreover, it has been shown that the formation of the phenolic compounds of the cotton plant is also the basis of one of the protective reactions of the plant against infection by the fungus *Verticillium dahliae* Kleb., the degree of development of the disease depending on the rate of formation of phytoalexins and other antibiotic substances in the plants [26].

It has been established by investigations of the structure-function relationships of numerous gossypol derivatives and nitrogen-containing alicyclic bases that their biological effect is based on interaction with the most important components of the cell, and, in the first place, with membrane receptor systems mediating intracellular processes.

The attention devoted to the investigation of the proteins of plasmatic membranes that has increased in the last decade is connected with the appearance of new evidence of the many-sided and important role of these proteins in the vital activity of the plant organism. Among them, are the endo- and exogeneous factors of differentiation and of intercell contacts, enzymes, receptors, lectins, stress proteins, etc.. On the other hand, the increasing demands for cellulose and, in particular, for cotton fibers is requiring the creation of higher-yielding varieties of cotton with fibers having improved technological properties, which makes the investigation of the mechanisms of the regulation of the biosynthesis of cellulose and the search for new methods for improving its quality an urgent matter. In view of this, particular significance is acquired by a study of the action of hormones, their interaction with membrane proteins, and the topology of the membrane proteins, since it is just these structures that perform the highly specific hormone receptor, intercellular, and other types of interactions of plant cells.

Interest in plant hormones is going far beyond the framework of plant physiology, since it has been established that they exhibit a stimulating action on cells, governing the synthesis of certain proteins, including the organizing polyenzyme systems for the synthesis of cellulose, lignin, polysaccharides, mucopolysaccharides, and other biomacromolecules.

In its turn, protein synthesis occupies a central position in the metabolism of the cell and forms a direct expression of the information stored in genes. The search for the mechanisms regulating the manifestation of genes and thereby mediating

the whole pattern of growth and development of the organism, in which the key role is played by hormones, has been and remains one of the tasks of bioorganic chemistry and molecular biology.

Modern ideas on the mechanism of the action of hormones are based on an analysis of the phenomena observed in vivo and in vitro in animal tissues and cells. It has been established that the influence of hormones depends on their interaction in the cell with specific protein receptors. Binding with the latter initiates the transport through the plasmatic membrane of one or more ions (Ca^+, Na^+, K^+) and activates membrane-associated adenylate cyclase, as a result of which the intracellular concentration of c-AMP rises. The main role of c-AMP in the cell is the stimulation of the phosphorylation of proteins, which is catalyzed by protein kinases. It has been postulated that the phosphorylation of the ribosomal proteins may affect the nature and amount of the proteins synthesized; i.e., the hormone, on forming a complex with the corresponding receptor, regulates the synthesis of proteins by acting directly on the genetic material.

The question arises: do phytohormones act in a similar way to animal hormones or is their function connected with some other mechanism? It is known that animals and plants have one and the same genetic code. The cells of higher plants and animals are of the eukaryotic type. Higher organisms contain one and the same set of DNA molecules, but different cells synthesize completely differently proteins; nevertheless, this generalization cannot a priori be extended to the hormonal regulation of the vital activity of the plant organism.

In plants, the fine regulation of the action of phytohormones is distinguished by exceptional selectivity and specificity. Against this background, the effect of their polyfunctionality is particularly striking. The molecules of phytohormones have small dimensions and, at first sight, it is difficult to imagine how their comparatively simple molecular structure ensures the specificity and many-sideness of their action. They can be considered as a tool for the specific induction of gene sequences present under the control of the given phytohormone.

A detailed investigation of the mechanism of the action of hormones in plant cells will enable us to obtain additional information on the process of the transmission and transformation of the hormonal signal in the living cell from evolutionary aspects. The elucidation of the structure and function of the receptor proteins and the proteins induced by the hormone–receptor complex will permit us to approach the generation of molecular-genetic markers in order to obtain transgenic varieties of cotton.

Investigation of the phytohormone receptors is made difficult by the fact that these biomolecules are present in plant tissues in extremely small amounts and also because of the experimental difficulties of their isolation and purification and the absence of clear biochemical markers; at the same time, the usual methods of analytical chemistry are unsuitable for their identification. The problem is being appreciably simplified by the use of phytohormones labeled with radioactive isotopes and of various specific sorbents, and also of monoclonal antibodies to functionally important antigens of the cytoplasmatic membranes. In view of this, radiochemical methods of synthesizing the main classes of phytohormones with a high activity, of the order of 2-10 GBq/ml, have been developed [27, 28] for identifying the hormone-binding proteins in all stages of purification and obtaining a number of affinity sorbents containing the corresponding phytohormones and their analogues as ligands.

The first of these to be isolated from cotton seedlings were highly purified auxin-binding protein (ABP), cytokinin-binding protein (CBP), ethylene-binding protein (EBP), and abscisin-binding protein (ABABP) [29, 30] and gibberellin-dependent DNA-binding protein (GA_3-DBP) from ovules [31, 32] corresponding to the criteria for evaluating phytohormone receptors. Thus, it was established that all the phytohormones studied stimulated the activity of DNA-dependent RNA-polymerase in isolated chromatin (IAA, BAP, ethylene) and the nuclei of cotton seedlings (GA_3) predominantly in complexes with hormone-binding proteins.

Up to the present time, information has appeared in the literature only on ethylene-receptor proteins of the membrane type. Our isolation of a water-soluble ethylene receptor permits the assumption that in plant hormones, just as in animal hormones, two action pathways in the cell are possible: one of them is the generation of a second mediator at the level of the plasmatic membrane, and the other is penetration into a target cell and binding with receptors in the nucleus. The specificity of the receptor for GA_3 has been confirmed in experiments in which gibberellin was replaced by auxin: here no stimulation of RNA polymerase activity was observed. In experiments with isolated bean seedling nuclei a species-specificity of the GA_3-DBP, in the presence of which the activity of RNA polymerase rose considerably, was established.

In addition to this, the influence of phytohormones, hormone-binding proteins, and their complexes on the level of synthesis of protein in in vitro systems has been studied. This showed that not only the phytohormones themselves (BAP and ethylene) and the hormone-binding proteins individually but also — to a large degree — their complexes activate the processes of protein biosynthesis in an isolated chromatin system [33, 34]. It is characteristic that inhibitors of ribosomal protein

synthesis — puromycin and cycloheximide — exerted no influence on its level. An investigation of the products of synthesis in comparison with a control showed that in samples with the CBP–BAP complex three new components appeared, with MM 14-20 kDa, and in samples with the EBP—ethylene complex two new polypeptides, with MM 48-68 kDa, respectively [35].

On induction with protoplasts from cotton seedlings for 1 hour, the hormone-receptor complex GA_3–DBP likewise stimulated the synthesis of polypeptides with MM 180, 168, 157, and 152 kDa, while GA_3 itself led to the inhibition of the synthesis of a component with MM 133 kDa; the other protein bands were identical with those of a control experiment. Because of the absence from the literature of a detailed description of the genes and their products that are under the control of gibberellin in the cotton plant, it was impossible to identify the above-described protein bands. Nevertheless, this experiment shows that the GA–DBP complex is capable of stimulating the synthesis of new polypeptides.

In view of this, attention is directed to the fact that in the model synthesis of RNA in isolated chromatin in the presence of a hormone–receptor complex the intensive inclusion of labeled nucleotides ([^{33}P]-UTP) and amino acids ([^{35}S]-methionine) into the TCA-precipitable material begins simultaneously in the first minutes of incubation. The addition of rifampicin — a specific inhibitor of the synthesis of RNA polymerase — almost completely completely suppresses the synthesis of RNA and considerably quenches the synthesis of protein, which shows a possible linkage of these processes. The results obtained are of interest for understanding the mechanism of the physiological action of phytohormones both at the membrane and genetic level.

The investigation of the molecular mechanism of hormonal regulation is indissolubly connected with the problem of introducing modern intensive and ecologically harmless technologies for the cultivation of cotton: the creation of insect-resistant varieties and of new effective biorgulators of fruit-bearing and defoliation. Work on the search for new defoliants is connected not only with an improvement of known biological tests for leaf-shedding but also with the creation of new ones more closely imitating the physiological processes taking place in the plant during leaf-shedding.

At the present time it has been established that, in the main, three phytohormones participate in the process of forming a separating layer in leaves: ethylene, auxin, and cytokinin. At the same time, it has been shown that a number of the most effective defoliants — Dropp (thidiazuron), butifos (DEF), and tsitodef — possess a pronounced cytokinin activity. It is assumed that their defoliating activity is directly connected with their cytokinin activity and is exhibited through an enhancement of the synthesis of ethylene in the leaves [36]. All this permits us to propose a new principle for screening defoliants by establishing the specific biochemical effects determining the shedding of leaves.

The mechanism of the action of defoliants at the molecular level is not so far clear, but several key points in their application exist. In the first place, it has been reliably established that defoliants, like the other growth regulators, interact with the receptor proteins of phytohormones, causing specific changes in the processes of transcription and translation [37]. In the second place, defoliants activate the ethylene-producing system [38]. In the third place, defoliants induce the biosynthesis of polysaccharide hydrolases [39]. These facts enable us to formulate the following criteria for selecting effective defoliants: 1) competition with phytohormones for specific binding sections; 2) activation of RNA polymerase and protein kinase; 3) activation of protein synthesis in in vitro systems (isolated chromatin, chloroplasts); 4) stimulation of the secretion of ethylene; and 5) increase in the activity of cellulase and of pectinase in the separating zone. Thus, if a compound under trial corresponds to all the criteria listed above one may expect a high defoliating activity.

Up to the present time, the mechanism of the physicochemical processes lying at the basis of the biosynthesis of the cotton fiber have remained unelucidated; the set of effectors for this system of reactions is unknown. From this point of view, it appeared of interest to study the influence of a protein isolated from ovules (GA_3–DBP) and its complex with GA_3 on the cellulose-synthesizing process, since the synthesis of the cotton fiber is under the control of gibberellin. In cotton protoplasts, a stimulating action of a gibberellin–protein complex on the inclusion of ^{14}C-labeled glucose in the alcohol-soluble material has been shown, the increase of inclusion being due to the synthesis of cellulose with an unbranched β-1→4 structure.

The replacement of GA_3 by auxins did not lead to a stimulation of cellulose synthesis. Actinomycin D, added to the incubation mixture in a concentration suppressing the synthesis of cellulose by 50%, completely eliminated the effect of the GA_3–protein complex. This permits the assumption that the action of the hormone–receptor complex consists in the activation of a group of gibberellin-sensitive genes having some relationship to the cellulose-synthesizing system. The stimulating influence of the GA_3–DBP complex on the synthesis of cellulose of the secondary wall of protoplasts indicates that the protein under study is a gibberellin receptor capable of participating in the process of regulating the formation of the secondary cell wall. As is known, cotton fiber is a cell the mechanisms of the formation of which are largely identical with the synthesis of the cellulose of the secondary cell membrane. In addition, two types of enzymes participate directly in the synthesis of the cotton

cellulose fiber; pyrophosphorylase, synthesizing a precursor to uridine diphosphate glucose (UDPG), and a glucan-synthesizing complex ensuring the addition of the glucose from UDPG to the growing cellulose chain.

Glucan-synthetase activity hs been studied in the fibers of several varieties of cotton and it has been shown that the accumulation of cellulose with the development of elements of the fruit takes place untypically in different varieties but its absolute amounts at the moment of maturation are practically identical [40, 41].

In cotton-growing, in order to obtain low plants convenient for mechanical harvesting, in addition to mechanical methods use is made of treatment with retardants — synthetic compounds interfering with the biosynthesis of gibberellins and thereby causing the formation of short-stemmed plants. However, we have recorded different effects of retardants — Morphonol and Pix — on the glucan-synthetase activity of the fibers of different varieties (Andizhan-9 and S-6524).

The study of the regulatory proteins of the plant includes an investigation of various biopolymeric systems located on the surface and in the cytosol of the plant cell. The participation and determining role of various glycoproteins of plant and animal cells in the processes of vital activity, including intercellular contacts, proliferation and differentiation, and systems of reception and immunity are generally known. Among them, the greatest attention has been attracted by the lectin-like proteins (LLPs) and hydroxyproline-rich proteins of the cotton plant, since the chemical aspects of the biological effect of plant glycoproteins, including highly specific protein-carbohydrate interactions, have scarcely been studied. However, it is precisely these interactions that are responsible for their participation in the regulation of cell processes at the molecular and intercellular level.

Lectin-like proteins and some of their components were first isolated from the cotton plant, these being obtained on the basis of their affinity for biosorbents, including the d-galactosyl protein (LLP-1) the Con-A–binding protein (LLP-2) and a hydroxyproline-rich glycoprotein (extensin). These proteins are of interest as stress factors participating in the protective strategy of plants and the extension of the cell wall [42,43]. The biochemical analysis of these proteins has shown the presence in them of a complex system of glycoproteins with hemagglutinating activity and different carbohydrate specificities.

In order to study the structural-functional characteristics of these complex membrane-bound glycoproteins, use has been made of an immunochemical approach involving monoclonal antibodies (mABs). This method, based on hybridoma technology, is one of the latest and most rational methods for the identification, separation, and epitopic analysis of such heterogeneous protein systems as membrane proteins.

A set of mABs to the membrane and lectin-like proteins has been obtained, and their antigenic properties have been studied. Cross-immunochemical analysis by the ELISA method has shown that some mABs isolated from producing hybridomas react both with ordinary membrane proteins and also with lectin-like proteins, which shows the common nature of their antigenic determinants and the possible localization of these proteins in membrane structures [44]. The nature of the overlapping of the immunochemical reactions of mABs with LLP-1, LLP-2, and lectins of known specificity (Con-A, RCA_{120}, peanut lectin) enables the structure of the carbohydrate domains in the molecules of the lectin-like glycoprotein to be judged. Moreover, two mABs exhibiting affinity to extensin have been detected.

Thus, the reactivities of protein antigens to a set of mABs can be used as markers for membrane epitopes associated with a specific function, and also for the purposeful fractionation of heterogeneous membrane proteins. Another direction of the use of monoclonal antibodies is the creation of new types of biologically active substances with a narrowly-directed action (drugs, immunotoxins, diagnostic agents) and also chemical agents including antibody enzymes catalyzing chemical reactions.

At the beginning of the 80s, it became clear that traditional genetic-selection methods of obtaining new varieties of agriculturally important plants, including cotton, were incapable of satisfying the ever-increasing demand of agricultural for promising varieties, which led to the use of the latest methods of genetic and cell engineering for the creation of a transgenic cotton plant possessing a complex of agriculturally valuable and ecologically useful genes.

In order to find methods for the regeneration of single cells to form fertile cotton plants, an enormous number of genotypes has been screened for callusogenicity and embryogenicity, which has revealed embryogenic industrial varieties [45]. Clones have been obtained containing key genes responsible for resistance to fungal diseases — a gene for the synthesis of chitinase, and a gene for the synthesis of 1,3-glucanases and phenylalanine ammonia lyase (PAL). The protein products of the first two genes digest the cell wall of the fungus and thereby prevent its multiplication in the tissues of the host plant, while the products of the PAL genes (there are five varieties of them) are responsible for the formation of phytoalexins from gossypol, thereby ensuring the resistance of the plants to pathogens.

Another biotechnological approach is also being developed which is based on the introduction of insectotoxic genes from *Bacillus thuringiensis* into the cotton gene. This permits the creation of varieties of transgenic cotton plants resistant to agricultural pests.

At the beginning of the 80s, in view of the strained ecological situation in the Republic of Uzbekistan, investigations into the chemistry of insect pheromones were begun on the initiative of Academician A. S. Sadykov. One of the indications of the unfavorable situation in this field is the massive development of harmful insects causing great losses to the economy, and also a decrease in the number of useful insects.

In recent times, the development of chemical agents for the fight against insects has taken place in the direction of the search for highly effective compounds acting on such processes as the transmission of nervous excitation, feeding, respiration, digestion, and the water-salt metabolism. A substantial disadvantage of such pesticides is the low selectivity of their action, which is connected with the similarity of the metabolic processes in animal organisms. At the same time, quite insufficient attention is being devoted to the search for means of acting on communication systems and information channels through which, to a considerable degree, regulatory interrelationships between the elements of natural biological systems are effected.

All this has led to the creation of a a new concept for the protection of plants which envisages the development and introduction of a system of means for directing the dynamics of the population of harmful insects that enable their damaging activity to be kept at an economically imperceptible level with the minimum adverse action on the other components of the environment. The most promising direction is the development of methods for the artificial control of the development, multiplication, and behavior of insects based on the use of small-molecule bioregulators.

In the Institute of Bioorganic Chemistry, scientific-research work is being carried out intensively on the identification and synthesis of the sex, aggregation, and trail pheromones of economically important insects of Uzbekistan in order to use them in pheromone traps with the aim of predicting the state and dynamics of the change in the populations of insects. The study of pheromones has largely promoted work on the synthesis of a considerable number of compounds having a definite stereospecific structure, the study of conformations, the creation of their functional analogues — active synthetic pheromones — and the development of means and methods for their practical utilization.

As a result of fundamental investigations using the methods of bioorganic chemistry and chemical ecology, the sex pheromones of the pests of the cotton plants — the turnip moth and the cutworm moth of the Central Asian population — have been identified [46].

Methods have been developed for the synthesis of monoenic pheromones and pheromones containing nonconjugated diene systems using alkyne intermediates and the phase-transfer variant of the Wittig-Horner reaction. The Knoevenagel reaction for the synthesis of trans-alkene compounds has been modified. Stereoselective methods have been developed for the synthesis of pheromones of insect pests of the cotton plant and some other crops and also of agaricultural stocks [47-53].

In the practical aspect, devices have been developed for the manufacture of the component parts and the industrial output of sets of pheromone traps. Even at the present time, the pheromones of the boll worm and the turnip moth are being widely used in agricultural practice.

During the last two decades, fundamental investigations have been developed in the Institute on biologically active proteins and peptides from the venoms of Central Asian animals. These investigations have been directed to the search for and the study of the structure and physiological action of neurotoxins, enzymes, and peptides from the venoms of arthropods in connection with their participation in the generation and transmission of nervous impulses — one of the most important processes of the vital activity of higher organisms. The considerable progress achieved by world science in the field of the study of the mechanism of the transmission of nervous impulses in synapses is connected with the use of neurotoxins of animal and plant origin specifically interacting with the ion channels and other molecular complexes of the synapses. Widely known among them are tetrodotoxin, saxitoxin, batrachotoxin, aconitine, and the neurotoxins of scorpions and actiniae, the use of which as "tools" has promoted the formulation of an idea of the structural organization of the sodium, potassium, calcium, and anion channels. Electro- and neurophysiological investigations with the aid of such membrane-active compounds have given an impulse to the chemical study of the receptor system of cells.

In the Institute at the present time, about ten highly specific protein neurotoxins and enzymes and also more than ten small-molecule peptides possessing an immunomodulating and antitumoral action have been isolated from the venoms of Central Asian animals and studied in all aspects; the complete primary structures of most of them have been established.

As is known, in the molecular form or as subunits, many neurotoxins with a presynaptic action possess a phosphorylase activity and are homologous with pancreatic phosphorylase which, to some degree, permits an idea to be obtained of possible active sections responsible for their catalytic and toxic properties. The toxins of the venoms of hymenoptera, which, as we have discovered, possess phosphorylase activity, have no structural homology with pancreatic phosphorylase and are therefore of considerable interest for the structural-functional relationships of this class of proteins.

```
                                                  20
F-N-P-C-P-Y-S-D-D-T-V-K-M-I-I-L-T-E-Q-N-K-K-Q-D-F-Y-T-L-
F-N-P-C-P-Y-S-D-D-T-V-K-M-I-I-L-T-E-Q-N-K-K-Q-D-F-Y-T-L-
                          40
D-T-I-G-E-H-N-Q-F-N-K-L-T-A-K-S-Q-V-V-F-I-V-W-Q-T-G-I-G-
D-T-I-G-E-H-N-Q-F-N-K-L-T-A-K-S-Q-V-V-F-I-V-W-Q-T-G-I-G-
      60                                          80
D-A-I-T-A-R-A-G-Y-I-F-L-L-D-S-G-L-A-M-L-V-D-K-Y-Q-M-A-P-
D-A-I-T-A-R-A-A----------S-G-L-A-M-L-V-D-K-Y-Q-M-A-P-
                                100
V-A-S-D-I-K-L-C-N-A-G-C-Y-C-K-P-T-Y-V-I-S-V-K-K-A-I-Q-F-
V-A-S-D-I-K-L-C-N-A-G-C-Y-C-K-P----------A-I-Q-F-
                  120                                 140
A-W-E-H-K-C-V-G-I-R-C-S-D-P-G-V-P-T-D-G-L-S-G-R-P-H-Y-G-
A-W-E-H-K-C-V-G-I-R-C-S-D-P-G-V-P-T-D-G-L-S-G-R-P-H-Y-G-
E-T-L-H-K-V-R-S-Y-N-G-K              OPT-1
E-T-L-H-K-V-R-S-Y-N-G-K              OPT-2
```

Fig. 1. Comparison of the structures of the orientotoxins ORT-1 and ORT-2.

Orientotoxin I — a toxin with a presynaptic action possessing lysophospholipase activity (ORT-1) — and orientotoxin II — a highly toxic phospholipase A_2 (ORT-2) — have been isolated from venom of the giant hornet *Vespa orientalis*. The orientotoxins obtained are electrophoretically homogeneous proteins with MM 16 and 17.5 kDa.

As structural investigations have shown, ORT-1 and ORT-2, while differing in their functional activities, are structural homologues [54]. It can be seen from Fig. 1 that the polypeptide chain of orientotoxin I differs from that of orientotoxin II by two hydrophilic clusters in positions 64-70 and 101-108. Furthermore, a comparison of the amino acid sequences of ORT-1 and ORT-2 with the sequences of known phospholipases shows that in their primary structures orientotoxins I and II differ considerably both from pancreatic lipase and from toxic phospholipases A_2 from other sources.

In spite of the structural differences, we have observed a functional similarity, namely: the dependence of the catalytic activities of the enzymes on the pH, the temperature, metal ions, and detergents. It has been shown by modification that in the catalytic part of the active center of the molecule of phospholipase A_2 from *Vespa orienalis* venom there is a functionally important histidine residue which is surrounded by a hydrophobic cluster of amino acid residues.

Thus, its structural and functional characteristics have shown that phospholipase A_2 from the venom of *Vespa orientalis* is an individual structural type of lipolytic enzymes.

Interesting results have been obtained in a study of the structure and mechanism of the action of the venom of the spider *Segestria florentina* and its neurotoxins Sf-1 and Sf-2 [55, 56].

The physiological action of neurotoxin Sf-I, as of the whole venom of the spider *S. florentina*, is shown in a change in the action potential of the surface fibers of the frog sartorius muscle and in the paralysis of cockroaches on intraperitoneal injection that is not connected with hemolytic cholinesterase and protolytic activities. Employing the usual methods of protein chemistry, the complete amino acid sequences have been established for neurotoxins Sf-1 and Sf-2 and an insectotoxin of the venom of the spider *Segestria florentina* (Figs. 2-4).

A whole series of neurotoxins with a presynaptic action has been isolated from the venom of various *Latrodectus* species of spiders, including: α- and β-latrotoxins (*L. tredecimguttatus*), α-LP- and β-LP-latrotoxins (*L. pallidus* subsp. *Pavlovski* Chorit, and α-LD- and β-LD-latrotoxins (*L. dachli* Lev.) [57].

In an investigation of the components of the kallikrein-kinin system in the venom of the spider *L. tredecimguttatus* a high kininase activity has been detected for the first time [58, 59], and a homogeneous bradykinin-inactivating ensyme has been isolated. With respect to its molecular mass (81 kDa) the enzyme isolated is closer to kininases from plant sources or microorganisms. The results of physicochemical investigations permit the conclusion that the molecule of the kininase from the karakurt [black widow] spider consists of a single polypeptide chain with a blocked N-terminus consisting of 724 amino acid residues. Of the other features of the structures of the enzymes, attention is merited by the presence of free SH groups, which permits this kinase to be assigned to the SH-dependent proteinases. In addition to the enzyme kininase, two bradykinin-potentiating peptides, with molecular masses of 10 and 25 kDa and a broad spectrum of physiological action on enzymes have been isolated for the first time from the venom of the spider *Latrodectus tredecimguttatus*.

ARG-GLU-PHE-MET-TRP-LEU-TYR-GLY-VAL-TYR-ARG-ASP-LYS-GLU-
-CYS-TYR-PRO-CYS-ARG-TRP-GLU-THR-CYS-CYS-ASP-ILE-SER-GLU-
-THR-PRO-LYS-ILE

Fig. 2. Primary structure of neurotoxin Sf-I.

ARG-GLU-GLN-MET-LEU-TRP-GLY-ARG-VAL-CYS-TYR-ILE-ARG-ASN-
-HIS-CYS-ASN-SER-ASP-GLY-CYS-SER-LYS-LEU-LYS-LYS-ALA-ALA-
-CYS-GLY-GLU-ARG-THR-PRO-ARG-VAL-CYS-ASP-ILE-ALA-THR-CYS-
-LEU

Fig. 3. Primary structure of neurotoxin Sf-II.

ARG-GLN-ASP-MET-VAL-ASP-GLU-SER-VAL-CYS-TYR-ILE-THR-ASP-
-ASN-ASN-CYS-ASN-GLY-GLY-LYS-CYS-LEU-ARG-SER-LYS-ALA-CYS-
-HIS-ALA-ASP-PRO-TRP-GLU-LEU

Fig. 4. Primary structure of insectotoxin S1T.

O1: ALA-ARG-PRO-HYS-GLY-PHE-SER-PRO-PHE-ARG-VAL-ASP
O2: ALA-ARG-PRO-PRO-GLY-PHE-SER-PRO-PHE-ARG-VAL-ASP

Fig. 5. Structures of vespakinins O1 and O2.

The use of vasoactive peptides in medicine is extremely limited; the main reasons for this are the briefness and polyfunctionality of their action in the animal organism. In order to create highly specific and long-acting peptide bioregulators, numerous structural-functional investigations have been made of natural vasoactive peptides with the aim of creating new drugs. In the Institute, in addition to high-molecular-mass proteins, low-molecular-mass peptide components, including bradykinin-like peptides with a prolonged action, have been isolated from venoms. Thus, eight peptides possessing the activity of kinins have been isolated in the homogeneous form for the first time from the venoms of the wasp *Palistis gallicus* and the hornet *Vespa orientalis*, their physicochemical properties have been studied, and their complete amino acid sequences have been established. The primary structures of two of them — vespakinins O1 and O2 are shown in Fig. 5 [60].

In an investigation of the functional properties of the peptides isolated, an agreement of the parameters of the myotropic and hypotensive activities was demonstrated, which showed the monotypic nature of the molecular mechanisms forming the basis of these physiological effects. It was established that the two peptides, not being analogues of bradykinin, have a mixed type of inhibition of the binding of the ligand with bradykinin receptors. This opens up possible routes for the search for and creation of new drugs.

It must be mentioned that the protein-peptide toxins and enzymes isolated from the venoms of Central Asian animals are finding wide use. Being distinguished by high specificity, these substances are used as fine "tools" for the study of the molecular mechanism of the transmission of nervous impulses and also as "probes" for the isolation of the components of biomembranes and the study of their structures and the principles of their functioning.

The chemical investigation of the receptor systems of animal cells is being developed intensively with the use of other types of membrane-active substances — synthetic analogues of the natural neurotransmitter acetylcholine. The normal functioning of the cholinergic system as a whole is ensured by various enzyme complexes participating in the synthesis and resynthesis and the mobilization and immobilization of the transmitter. The key role in these processes is that of cholinesterases (CEs) — enzymes controling the level of the transmitter in the synaptic gap and ensuring the restoration of the initial state of the receptor–channel complex after the action of the transmitter [61].

In view of this, under the direction of Academician A. S. Sadykov, all-sided investigations were undertaken of the active surface of CEs by the method of substrate-inhibitor analysis with the aid of specially synthesized homologous series of substrates and inhibitors — esters of carboxylic acids and of acids of phosphorus containing the residues of certain alkaloids and simpler heterocyclic compounds, which permitted the elucidation of the role of productive and unproductive sorption in

enzymatic catalysis and also the revelation of the mechanism of selective action in relation to acetylcholinesterase (ACE) and butyrylcholinesterase (BuCE) [62].

In these investigations it was established that in the mechanism of the inhibition of CEs the main role is played by hydrophobic interactions between the nonpolar groups of the molecules of organophosphorus compounds (OPCs) and the hydrophilic sections of the active surface of the enzyme located in the regions of both the ionic and the esterase centers. In view of this, other urgent matters are the supplementation of the arsenal of tools by new synthetic organophosphorus inhibitors (OPIs) in the hydrocarbon radicals of which ether/ester groupings leading to a disturbance of hydrophobicity are included and the study of the influence on acetylcholinesterase activity of the introduction of a hydrophilic grouping into the phospholipid part of the OPI molecule and also of features of the metabolism of these compounds in the organism, which may serve as a theoretical basis for the purposesful synthesis of physicologically active substances with given properties [63].

The interaction of a large set of new OPCs forming several homologous series with the CEs of insects and arthropods has shown a substantial difference between the structures of the active centers of the CEs of warm-blooded animals and insects. Investigations by the method of substrate-inhibitor analysis of the enzymes of the metabolism, such as the carboxylesterases (CBEs) and cytochrome-P-450-dependent monooxygenases of warm-blooded animals and insects have given the possibility of making a close approach to the problem of the resistance of insect pests of agricultural crops to insectoacaricides and to the creation of a series of highly specific thiosubstrates inhibiting CBEs which may find use as synergists of known insectoacaricides.

Together with investigations of natural and synthetic neuroregulators, work on the study of other types of membrane-active substances — cytotoxins — has been developed in the Institute.

Cytotoxins Vc1 and Vc5, isolated earlier from the Central Asian cobra have proved to be convenient materials for the study of the structural features of biological membranes and of the processes taking place in them. The interaction of these cytotoxins with natural and model biological membranes has been studied with the aid of physicochemical methods. The results published in [64-66] have permitted the statement that, on interaction with phospholipid liposomes and proteoliposomes from rat brain, these cytotoxins form domain structures including lipid and toxin molecules in their composition; the mobility of the fatty-acid residues of the phospholipid residues then decreases along their whole length.

A model has been proposed of the localization of the cytotoxins in the lipid complex of biomembranes according to which the cytotoxin molecules are embedded in the hydrophobic region of the liposomes and the hydrophilic parts of the cytotoxin molecule are then localized at the phase-separation boundary [66]. Furthermore, it has been shown for the first time that cytotoxins Vc1 and Vc5 induce the fusion of membranes with different chemical compositions, the probability and mechanism of a cytotoxin-induced fusion of membranes being determined both by the nature of the distribution of the charged, hydrophilic, and hydrophobic amino acids in the tertiary structures of the cytotoxins and also by the charge and shape of the phospholipid molecules. The fusogenic properties of the cytotoxins are due to their capacity for initiating polymorphic transformations of the lipid matrix of membranes [67].

In addition to those mentioned above, one more membranotropic property of cytotoxins has been found — their capacity for producing water-permeable pores forming channels through which many ions and, in particular, ferricyanide, praseodinium, manganese, etc., ions penetrate.

The study of cytotoxins Vc1 and Vc5 with the aid of physicochemical methods has enabled physiological and biochemical results on the interaction of cytotoxins with native biological membranes to be interpreted [68].

The cytotoxins also include a little-studied group of small-molecule peptides — the thionins (MM about 6 kDa). Interest in these substances is due to the broad spectrum of the toxic effects characteristic of them and their possible participation in the protective system of plants. Thus, thionins with bactericidal, cancerostatic, and anticancerogenic effects have been isolated from various plant sources. A study of the action of the thionin from the nut of *Pyrularia pubera* on model membranes has revealed its membranotropic properties, which are shown in its capacity for modifying model membranes, leading to a breakdown in their structural organization [69]. The thionin is an active fusogen and an inhibitor of the energetic processes both of whole cells (thymocytes) and of cell organelles (mitochondria).

It must be mentioned that in the process of functioning of cell membranes these low-molecular-mass proteins have no strictly defined functions; their functional properties are determined by the lipid composition of the membranes with which they are interacting. Appearing as membrane modifiers, under certain conditions they may stabilize the lamellar packing of lipids and other conditions induce the formation of nonbilayer structures. The nature of the modification, determined by the charges and structural features not only of the membrane-active polypeptides and of the lipids, corresponds to the minimum energy of the system on their interaction. The fairly diverse phospholipid compositions of biological membranes are responsible for the

possibility of the existence of a multiplicity of lipid structures on interaction with membrane-active polypeptides. This expands the functional possibilities of biological membranes and explains their high mobility.

An important direction of the investigations of modern membranology is the question of how membranes of different types, including plasmatic membranes, acquire their characteristic set of proteins determining their specific functional properties. The results of the work of a group in the Institute permit this question to be answered to some degree. It has been shown on simple model systems how important are differences in the affinity of two practically identical polypeptides for membranes with different phospholipid compositions. This permits the assumption that a biological membrane having a complex lipid composition is capable of very accurately identifying what are, at first sight, insignificant structural noncorrespondences between the proteins and the configuration of the lipid system; i.e., a biological membrane with a definite lipid composition, as it were, "recognizes" and "selects" the proteins appropriate to it. Apparently, it is just the lipid composition that determines both the set of proteins and, in combination with them, the functional properties of a membrane [70].

It is obvious that all the scientific directions considered here that have been developed by A. S. Sadykov and his colleagues, are united by the problem of realizing a biological function at the chemical level. The investigations of hormone–receptor, enzyme-substrate, and enzyme-inhibitor interactions and the biological functions of membrane-active proteins, low-molecular-mass effectors, and other groups of natural substances are based on the revelation of the physicochemical nature of the processes of recognition at the molecular level.

The establishment of many of the scientific directions listed here became possible thanks to A. S. Sadykov's understanding of the tendencies of the development of modern science, important results being achieved by a combination of chemical, biological, biophysical, and medical investigations with the demands of practice and the solution of applied problems. Constant attention to the practical use of the scientific results obtained has led to the development of new physiologically active drugs and technologies that have found use in the national economy. Among them is the creation of emulsifiers, surface-active and textile-auxiliary agents, fungicides and growth stimulators, food dyes, and drugs with a broad action spectrum.

The main alkaloid of the plant *Anabasis aphylla* — anabasine hydrochloride — has been introduced into medical practice as an antismoking drug [71]. When the structure of anabasine was changed by introducing various radicals into its molecule, its pharmacological properties also changed substantially. Anabasine derivatives obtained by adding alkyl and aryl radicals to its piperidine nitrogen retained the capacity of anabasine for exciting the central H-cholinoreceptors but to a considerably smaller degree than anabasine itself. The presence of an additional piperidine ring in the anabasine molecule (the alkaloid anabasamine) led to the appearance of central H-cholinolytic properties. The alkaloid anabasamine has also been found to have analgesic, hypothermic, and tranquilizing properties [72, 73].

A subsequent detailed investigation of anabasamine has shown its high thrombocyte-stimulating action, and a high antiinflammatory activity has been found that exceeds the action of such important nonsteroid antiinflammatory agents as indomethocin, butadion (phenylbutazonene), and voltaren (diclofenac sodium). Unlike these drugs, anabasamine possesses an antiulcer effect.

Derivatives of heterocyclic amines undergo a number of chemical transformations in the organism. In the intact organism, these proceses may be considered as a special case of the phenomenon of detoxication, leading to the formation of products readily eliminated from the organism. In more complex situations connected with pathologies, for example, not only the quantitative but also the qualitative spectrum of the metabolites changes, which has a substantial effect on the physiological activity of the drugs.

A characteristic feature of compounds of low molecular mass is their capacity for rapid transformation and elimination from the organism. Where amines are used as drugs, only a small part of the material exerts its useful action and the remainder is at least useless or, in the majority of cases, actually harmful. When amines are combined with polymers, not only quantitative but also qualitative changes are observed due to the physiochemical features of the macromolecules. This new direction in the chemistry of natural and physiologically active compounds was formulated in the seventies with the direct participation of Academician A. S. Sadykov. As a result, a whole series of polymeric derivatives of anabasine, lupinine, ephedrine, colchichine, and other alkaloids and heterocyclic amines with a broad spectrum of biological activity was obtained. The introduction of these compounds into medical practice required the solution of a whole range of problems relating to the fate of the drugs in the organism: absorption, bioaccessibility, and rate of elimination, and also the dependence of these parameters on molecular mass, molecular-mass distribution, charge, hydrophobicity, etc. [74, 75].

A large and complex program uniting chemists, biochemists, pharmacologists, doctors, and technologists was developed under the direction of A. S. Sadykov, as a result of the performance of which the antiviral preparation linament gossipola 3%

and the immunosuppressor batriden, used in kidney transplants and for the treatment of glomerulephritis and some other autoimmune diseases, were created and and introduced into industry in comparative short times [76, 77]. The successful introduction of the new drugs into medical practice promoted the development of a universal method for obtaining ^{14}C-radioactively-labeled gossypol derivatives (batriden, megasin, ragosin) which permitted the study of their biotransformation and pharmacokinetic features [78-80]. A number of compounds synthesized from gossypol are undergoing preclinical and clinical study at the present time. Recently, certain polymers — especially polyvinylpyrrolidone (PVP) and cellulose and their derivatives — have been employed ever more widely for the creation of new medicinal forms with increased bioaccessibility in which an insoluble or poorly soluble drug is present in the molecularly disperse state. The solubility of such macromolecular systems is apparently greatly enhanced through solvation.

Investigations in this field have permitted a number of new water-soluble forms of drugs possesing antihelminthic, immunosuppressor, interferon-inducing, and hemostatic activities to be obtained.

The comparative evaluation of these pharmacokinetic parameters of the initial compounds and their water-soluble complexes has shown that the polymeric matrix substantially increases the bioaccessibility of the drug and the rate of its distribution through the organs and tissues, retards its metabolism, and prolongs its therapeutic action [81]. These features are also characteristic for drugs based on gossypol. In the case of a complex of batriden with PVP the rate of absorption and the accummulation of the drug in immunocompetent organs rises considerably; at the same time the elimination of the drug from the organism takes place faster and more completely.

Thus, the investigation of the biotransformation of derivatives of heterocyclic amines and polyphenols combining in themselves so many diverse and original properties has not only expanded our understanding of common structure-function relationships in bioorganic chemistry but has also led to methods for their practical use. A domestic school of researchers has been set up which continues even today to work with modern methodology including radiochemical and stereospecific synthesis, physicochemical analysis, quantitative methods of evaluating biodestruction, and the development of new drugs with preplanned pharmacokinetic characteristics.

The vigorous development of biorganic chemistry, which is the fundamental basis for biotechnology, requires a considerable shortening of the times of introduction of the advances of science into medicine, agriculture, and industry. This is particularly urgent for independent Uzbekistan, since it is just today that biotechnology is determining the level of scientific and technical progress, while the unique scientific legacy of Academician A. S. Sadykov, which is being developed by his pupils and colleagues, is unconditionally a necessary intellectual undertaking for the future.

It appears to us that the most promising scientific developments from the fundamental and applied point of view are those in the following directions:

— a deepening of investigations in the field of the study of the structure and function of bioregulators ensuring chemical control over the interrelationships of different organs formed by the cell systems of one organism and acting on the receptors of others, revealing the structural-functional nature of this type of interactions (regulators of the ecological type);

— the development of the biotechnology of substances providing chemical control over the interaction between different cell systems of the organs of plants, animals, and Man that act on the receptor cell systems of the given organism (control of physiology); and

— the investigation of the structure, properties, and mechanisms of the action of bioregulators of intracellular origin, including not only known messengers but also those the appearance and action of which depend substantially on the time of existence of the given type of cells, the state of their macromolecular complexes, and the polyfunctional systems of a eukaryotic organism.

On the whole, considering investigations in bioorganic and biotechnological directions connected with the optimization and intensification of cotton-growing in the Republic, one may come to the conclusion that the creation of a fairly complete but limited set of specific bioregulators of the cotton plant determining the processes of growth, differentation, nutrition, the ageing of the tissues of certain organs, and the biosynthesis of the fiber protein is an urgent matter. Extremely important is the development of new approaches and the creation of effective means for combating pests that selectively decrease the populations of definite species of insects, fungi, and bacteria, including bioregulators of the activation of phytoalexins in the plant itself.

One of the successes of investigations of Academician A. S. Sadykov's school is the opening up of new sources of extremely valuable and unique biopolymers possessing biological functions and selective physiological action (enzymatic, immunological, and immunostimulating, antitumoral, hormonal, and membranotropic). The task consists in a more detailed investigation of the interconnection between structure and biological function and of features of processes of recognition at the

molecular level as applied to each type of biologically active substances, using the whole arsenal of experimental and theoretical physics, bioorganic chemistry, hybridoma technology, molecular genetics and gene-cell engineering, with the simultaneous creation of a basis for their biotechnology and a methodology of their use in medicine, industry and agriculture.

REFERENCES

1. A. S. Sadykov, Materials for a Bibliography of the Scientists of Uzbekistan [in Russian], FAN, Tashkent (1989), p. 119.
2. A. S. Sadykov, The Chemistry of *Anabasis aphylla* Alkaloids [in Russian], Izv. AN. UzSSR, Tashkent (1956), p. 224.
3. A. S. Sadykov, Kh. A. Aslanov, and Yu. K. Kushmuradov, Alkaloids of the Quinolizidine Series [in Russian], Nauka, Moscow (1970).
4. M. K. Yusunov, B. Chommadov, and Kh. A. Aslanov, Khim. Prir. Soedin., No. 3, 419-420 (1985).
5. A. S. Sadykov, The Development of Bioorganic Chemistry in Uzbekistan. IVth Franco-Soviet Symposium on the Interactions of Proteins and Nucleic Acids (September, 1977) [in Russian], Fan (Tashkent, 1978).
6. A. S. Sadykov, Problems of Regulation Synthesis and Metabolism of Natural Compounds, in: Frontiers of Bioorganic Chemistry and Molecular Biology, IUPAC, Pergamon Press, Oxford (1978), pp. 225-237.
7. A. S. Sadykov, Cotton, the Miracle Plant [in Russian], Nauka, Moscow (1985, p. 143.
8 A. Ismailov, Sh. Yu. Islambekov, and A. Karimdzhanov, Questions of Transplantology [in Russian], Tashkent (1982), pp. 82-85.
9. F. G. Kamaev, N. I. Baram, A. Ismailov, V. B. Leont'ev, and A. A. Sadykov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 5, 1000-1010 (1979).
10. N. I. Baram, L. Biktimirov, Kh. L. Ziyaev, and A. Ismailov, Khim. Prir. Soedin., No. 5, 650-654 (1988).
11. N. I. Baram, G. A. Ismailova, A. M. Biktimirov, and Kh. L. Ziyaev, Khim. Prir. Soedin.,No. 5, 647-650 (1988).
12. N. I. Baram, A. Ismailov, L. Biktimirov, R. Z. Paizieva, and Kh. L. Ziyaev, Problems and Prospects of the Development of the Chemistry of Natural and Physiologically Active Substances [in Russian], Fan, Tashkent (1989). pp. 78-100.
13. A. S. Sadykov, F. I. Ershov, A. S. Novokhatskii, Kh. A. Aslanov, and S. A. Auelbekov, Interferon Inductors [in Russian], Fan, Tashkent (1978).
14. S. A. Auelbekov, F. I. Ershov, and Kh. A. Aslanov, The Connection of Structure and Function in Low-Molecular-Mass Interferon Inductors, in: The Molecular Biology of Viruses [in Russian], Meditsina (Moscow (1985), pp. 209-220.
15. A. A. Sadykov, Kh. Aslanov, and S. A. Aulbekov, Low-Molecular-Mass Interferon Inductors. Connection of Structure and Function, in: Natural Polyphenols and their Derivatives as Antiviral Drugs and Interferon Inductors [in Russian], Fan, Tashkent (1981), pp. 3-29.
16. A. S. Sadykov, S. A. Vichkanova, A. Ismailov, L. B. Goryunova, Z. Sh. Shukurov, V. V. Peters, and L. G.Martynova, Ekspress-Informatsiya. Novye Nekarstvaennye, No. 1, [Express Information. New Drugs], No. 10, 18-22 (1983).
17. N. I. Baram, L. Biktimirova, Kh. L. Ziyaev, and A. Ismailov, Information Report [in Russian], FAN, Tashkent (1991).
18. M. Gdaniec, V. T. Ibragimov, and V. N. Dadabaev, Acta Crystallogr., **C48**, 810 (1990).
19. M. Gdaniec, V. T. Ibragimov, and S. A. Talipov, J. Inclusion Phen. Molec., Recognition, **9**, 231 (1990).
20. B. T. Ibragimov, S. A. Talipov, B. N. Dababaev, G. B. Nazarov, and T. F. Aripov, Khim. Prir. Soedin., No. 5, 675-679 (1988).
21. B. T. Ibragimov, S. A. Talipov, T. F. Aripov, and A. S. Sadykov, J. Inclusion Phen. Molec. Recognition, **8**, 323 (1990).
22. B. T. Ibragimov, M. Gdaniec, and B. N. Dadabaev, J. Inclusion Phen. Molec. Recognition, **8**, 333 (1990).
23. B. T. Ibragimov, S. A. Talipov, and M. Gdaniec, J. Inclusion Phen. Molec. Recognition, **8**, 409 (1990).
24. A. S. Sadykov, A. Ismailov, S. T. Zakhidov, and N. I. Baram, Ontogenez, No. 4, 346-357 (1985).
25. S. T. Zakhidov, S. P. Pozdnyakov, I. V. Uryvaeva, A. Ismailov, N. I. Baram, and A. S. Sadykov, Genetika, **23**, No. 5, 922-924 (1987).

26. Z. S. Abdullaev, Sh. Yu. Islambekov, A. Karimdzhanov, A. Ismailov, K. V. Vasil'eva, T. A. Gladkikh, and L. V. Metlitskii, Uzb. Biol. Zh., No. 5, 3-7 (1981).
27. Kh. K. Avlyanov, O. N. Veshkurova, A. A. Takanaev, and Sh. I. Salikhov, Khim. Prir. Soedin., No. 6, 699-702 (1992).
28. Ya. G. Ziyaev, O. N. Veshkurova, and Sh. I. Salikhov, Dokl. Akad. Nauk RUz, No. 4-5, 53-54 (1992).
29. Kh. K. Avlyanov, O. N. Veshkurova, A. A. Takanaev, and Sh. I. Salikhov, Khim. Prir. Soedin., No. 6, 702-706 (1992).
30. Sh. I. Salikhov, I. A. Markova, and A. K. Tonkikh, Fiziol. Rast., **37**, No. 4, 103-108 (1990).
31. M. Kh. Abidova, A. A. Abdukarimov, and G. T. Mavlyanov, Dokl. Akad. Nauk UzSSR, No. 1, 52-53 (1989).
32. M. Kh. Abidova, G. T. Mavlyanov, and I. N. Grigina, Dokl. Akad. Nauk UzSSR, No. 11, 52-53 (1990).
33. O. Kh. Saitmuratova, D. Tursunova, and V. B. Leont'ev, Uzb. Biol. Zh., No. 6, 55-56 (1978).
34. O. Kh. Saitmuratova, Neirokhimiya, **9**, No. 3, 350-404 (1990).
35. N. A. Evgrafova, O. N. Veshkurova, and A. A. Takanaev, Khim. Prir. Soedin., No. 4, 590-593 (1993).
36. N. F. Zubkova, É. V. Bukashkina, and O. N. Kulaeva, Agrokhimiya, No. 3, 91-97 (1987).
37. V. I. Kefeli, The Physiological Foundations of Defoliation and the Production Process [in Russian], Fan, Tashkent (1991).
38. N. F. Zubkova, É. V. Bukashkina, Kh. A. Sharepkova, and O. I. Kulaeva, Dokl. Akad. Nauk. SSSR, **272**, No. 4, 1011-1013 (1983).
39. J. C. Thomas and F. R. Katterman, Plant Physiol., **81**, 681-683 (1986).
40. É. Ch. Mustakimova, F. A. Ibragimov, V. M. L'vov, and A. A. Akhunov, The Activity of the Glucan Synthetase of Cotton Fiber in the Dynamics of the Plant, in: IIIrd All-Union Conference on the Biosynthesis of Cellulose and other Components of the Cell Wall [in Russian], Kazan' (1990), p. 52.
41. A. Akhunov, F. A. Ibragimov, and A. Ch. Mustakimova, International Conference on Biotechnology and Agriculture and Forestry. Abstracts, Dehli (1993), p. 74.
42. D. J. Meyer, C. L. Afonso, and D. W. Galbraith, J. Cell Biol., **107**, 163 (1988).
43. J. P. Stafstrom and L. A. Staenelin, Plant Physiol., **81**, 234 (1986).
44. Yu. S. Mangutova, Z. S. Khashimova, D. M. Kataleva, M. Suslo, and V. B. Leont'ev, Dokl. Akad. Nauk UzSSR, No. 3, 49 (1989).
45. A. A. Abdukarimov, M. Kh. Abidova, and S. A. Dzhataev, Problems and Prospects of the Development of the Chemistry of Natural and Physiologically Active Substances [in Russian], Fan, Tashkent (988), pp. 393-412.
46. M. M. Kasymzhanova, A. S. Ismailov, Ya. S. Zhiyaev, V. S. Abdukakharov, Ya. S. Ziyaev, and A. A. Abduvakhabov, Uzb. Khim. Zh., No. 3, 46-48 (1991).
47. G. G. Verba, V. S. Abdukakharov, and A. A. Abduvakhabov, Khim. Prir. Soedin., No. 5, 698-700 (1985).
48. G. G. Verba, A. M. Bikurova, and A. A. Abduvakhabov, Khim. Prir. Soedin., No. 1, 143-145 (1988).
49. L. M. Bikulova, G. G. Verba, F. G. Kamaev, and A. A. Abduvakhabov, Khim. Prir. Soedin., No. 5, 682-683 (1988).
50. M. M. Kasymzhanova, V. S. Abdukakharov, F. G. Kamaev, and A. A. Abduvakhabov, Khim. Prir. Soedin., No. 6, 783-784 (1988).
51. V. S. Abdukakharov, M. M. Kasymkhanova, G. S. Shakirzyanova, and A. A. Abduvakhabov, Khim. Prir. Soedin., No. 4, 568-569 (1990).
52. G. G. Verba, L. G. Bikulova, A. A. Abduvakhabov, and F. G. Kamaev, Khim. Prir. Soedin., No. 3, 444 (1991).
53. M. M. Kasymshanova, V. S. Abdukakharov, and A. A. Abduvakhabov, Khim. Prir. Soedin., No. 5, 598-588 (1992).
54. A. S. Korneev, Sh. I. Salikhov, and M. U. Tuichibaev, Bioog. Khim.,**15**, No. 1, 127-129 (1989).
55. N. Kh. Sagdiev, L. A. Valieva, A. S. Korneev, A. A. Sadykov, and Sh. I. Salikhov, Bioorg. Khim., **13**, No. 8, 1013-1018 (1987).
56. N. Zh. Sagdiev, A. A. Sadykov, P. B. Usmanov, D. Kalikulov, B. A. Tashmukhamedov, and A. S. Sadykov, Dokl. Akad. Nauk SSSR, **282**, No. 1, 463-465 (1985).
57. A. S. Sadykov, A. Akhunov, and Sh. I. Salikhov, Karakurt [Black Widow] Venom [in Russian], Fan, Tashkent (1985).
58. A. A. Akhunov, Z. Golubenko, and A. S. Sadykov, Dokl. Akad. Nauk SSSR, **257**, No. 6, 1478 (1981).
59. A. S. Sadykov, A. A. Akhunov, I. I. Chernetskaya, and Z. Golubenko, in: Abstracts of the VIIth Soviet-Indian Symposium on the Chemistry of Natural Compounds [in Russian], Tbilisi (1983), p. 90.
60. A. S. Korneev, Sh. I. Salikhov, and M. U. Tuichiev, Bioorg. Khim., **15**, 127 (1989).

61. A. S. Sadykov, E. V. Rozengart, A. A. Abduvakhabov, and Kh. A. Aslanov, Cholinesterases. Active Center and Action Mechanism [in Russian], Fan, Tashkent (1976).
62. M. I. Kabachnik, A. A. Abduvakhabov, I. I. Agabekova, A. P. Brestkin, et al., Usp. Khim., **93**, 1050 (1970).
63. A. P. Brestkin, E. V. Rozengart, and A. A. Abduvakhabov, Usp. Khim., **52**, No. 10, 1624 (1983).
64. T. F. Aripov, V. A. Salakhutdinov, Z. Salikhova, and A. S. Sadykov, Gen. Physiol. Biophys., **3**, 489 (1984).
65. T. F. Aripov, I. A. Rozenshtein, B. A. Salakhutdinov, and A. S. Sadykov, Biofizika, **31**, 716 (1986).
66. T. F. Aripov, I. A. Rozenshtein, and E. E. Gussakovskii, Khim. Prir. Soedin., No. 2, 226 (1986).
67. B. A. Salakhutdinov, T. F. Aripov, A. S. Sadykov, K. A. Aslanov, and M. U. Babaev, Khim. Prir. Soedin., **7**, No. 8, 1233 (1981)
68. T. F. Aripov, I. A. Rozenshtein, V. A. Salakhutdinov, Gen. Physiol. Biophys., **6**, No. 4, 343 (1987).
69. S. O. Kolusheva, B. A. Salakhutdinov, T. F. Aripov, and L. P. Vernon, Khim. Prir. Soedin., No. 4, 594-597 (1993).
70. M. J. Dufton and R. S. Hader, J. Mol. Biol., **115**, 177 (1977).
71. S. Kh. Nasirov and I. S. Khazbievich, Pharmacology of the *Anabasis aphylla* L. Alkaloids and Clinical Use of Anabasine Hydrochloride [in Russian], FAN, Tashkent (1982).
72. I. S. Khazbievich, On the Pharmacology of the Akaloid Anabasamine, in: The Pharmacology of Plant Substances [in Russian], Fan, Tashkent (1983).
73. Kh. S. Mukhamedzhanova, U. A. Sadykova, and A. Kh. Aminov, The Antiulcer Action of the Alkaloid Anabasamine, in: The Organization and Economics of the Pharmacy, Technology, and Pharmacology of Some Physicologically Active Substances [in Russian], Tashkent (1986).
74. F. R. Khalikova, The Influence of the Alkaloid Anabasine and its Polymer (PAMAK) and Copolymers on the Respiration and ECS of Animals, in: The Pharmacology of Plant Substances [in Russian], FAN, Tashkent (1973).
75. É. K. Kashkova, The Pharmacology of Polymeric Derivatives of Lupinine [in Russian], Dissertation ... Candidate of Biological Sciences (1975).
76. A. S. Sadykov, U. A. Aripov, A. I. Ismailov, et al., USSR Inventors' Certificate No. 459230; Byul. Izobret. No. 5 (1975), p. 35.
77. A. S. Sadykov, U. A. Aripov et al., USSR Inventors' Certificate No. 545634; Byul. Izobret., No. 5 (1977), p. 81.
78. U. A. Aripov, A. D. Sakhibov, B. Z. Kasymov, Kh. L. Ziyaev, L. Biktimirov, N. I. Baram, and A. Ismailov, Khim.-Farm. Zh., No. 3, 269 (1983).
79. U. A. Aripov, A. D. Sakhibov, B. Z. Kasymov, Kh. L. Ziyaev, L. Biktimirov, N. I. Baram, and A. Ismanlov, Khim.-Farm. Zh., No. 5, 908 (1983).
80. F. I. Ershov, V. I. Sumin, A. M. Saintkulov, N. I. Baram, É. B. Tazulakova, and A. Ismailov, Antibiotiki i Biotekhnol. Prom., **47**, No. 1, 26 (1992).
81. V. I. Sumin, N. I. Baram, A. I. Ismailov, and B. Z. Kasymov, Questions of Transplantology and Immunocorrective Therapy: Collection of Scientific Papers [in Russian], TashGosMI, Tashkent (1986).