BIOLOGICAL ACTIVITY OF GOSSYPOL AND ITS DERIVATIVES

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This review of the literature and of the authors' own investigations is devoted to the biological activity of gossypol and its derivatives. Certain results of the structural—functional analysis of gossypol ethers and the products of its condensation with amines and CH-acids are discussed. The results of pharmacokinetic investigations of radioactively labeled samples of gossypol and its physiologically active derivatives are given.

In spite of the fact that gossypol (Gp) — the specific pigment of the cotton plant — has been known since the end of the last century [1], it has remained the object of great attention by chemists, physiologists, biochemists, biophysicists, and physicians up to the present time.

The presence of two aromatic naphthalene fragments and of six hydroxy groups, two of which are in *ortho* positions relative to carboxy groups, and capacity for tautomeric transformations — all this is responsible for features of the chemical properties and biological activity of Gp. It is just the polyfunctionality of the Gp molecule that ensures wide possibilities of its modification and explains the broad spectrum of its physiological activity.

The history of the study of gossypol is connected with attempts to get rid of it as a toxic substance interfering with the use of cottonseed meal as a high-calorie fodder. It is only since 1963, when the antitumoral activity of Gp was first shown [2] that our relationship to it has changed and it has begun to be considered as a potential drug. A high virucidal activity of Gp was shown later [3] and it was established that its antiviral action is exhibited in relation to a whole series of arbo- and myxo viruses and herpes virus [4-7]; in low concentrations it almost completely inhibits many RNA and DNA viruses [8], which has permitted the use of Gp for the treatment of some diseases of viral etiology [9]. Great interest has been caused by a report of the capacity of Gp for inhibiting the human immunodeficiency virus *in vitro* [10, 11].

At the present time, antimalarial [12] and antibacterial activity, tested on more than 60 strains of Gram-positive and Gram-negative bacteria [13], ulcer-healing [14, 15], and hypoglycemic [16] action have been shown for Gp. Publications of recent years have stated that Gp possesses the properties of a neurotoxin [17], and a rodenticide [18] and may exert an antithyroidal function on young female rats [19].

Investigations of the antitumoral action of Gp have received further developments [20-25], and a comparatively high activity of the (—)-isomer has been reported [26].

Reports on the study of the contraceptive activity of Gp have appeared extremely widely within the framework of the program of the World Health Organization [27-31], and it has been proposed as a safe male antifertility agent [32-34]. As in the investigation of its antitumoral action, a whole series of experiments on various animals have revealed a high contraceptive activity of (—)-Gp as compared with (+)-Gp and (±)-Gp [35]. Thus, different effects of the Gp enantiomers have been observed in their action on lactate dehydrogenase-X of the rat testis [36]; at the same doses (—)-Gp was twice as effective as (±)-Gp; (+)-Gp was totally inactive [37]. (—)-Gp lowered the level of ATP in tubular fragments of hamster spermatids and inhibited isoenzyme C_4 of lactate dehydrogenase, in contrast to (+)-Gp, which was ineffective here [38, 39]. The characteristics of the action of (—)-Gp are connected, in particular, with a lower degree of its binding with tissue proteins. The latter circumstance also explains the considerably shorter period of half-elimination of (—)-Gp from the organism as compared with (+)-Gp [40].

Gp has proved to be the first immunosuppressor of plant origin [41] and its investigation in this connection has led to the creation of a preparation used in kidney transplantation and in the treatment of such autoimmune diseases as chronic glomerulonephritis and allergodermatosis [42].

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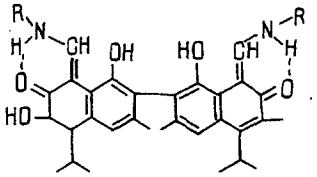
Possible explanations of the broad spectrum of physiological action of Gp are its capacity for inducing the formation of interferon in *in vitro* and *in vivo* systems [43-45], and also its inhibiting action on numerous enzyme systems. It is known that Gp exerts an influence on transport ATPases [46-50], suppresses the activity of sperm-specific lactate dehydrogenase-X [38-40, 51-54], inactivates malate dehydrogenase and glutathione transferase [55], succinate dehydrogenase, cytochrome oxidase, and succinate oxidase [56], catechol *o*-methyltransferase [57], oxidoreductase and NADP-dependent glutamate dehydrogenase [58], DNA polymerase [59], adenylate cyclase [60], and cyclic adenosine 3,5-monophosphate diphosphoesterase [61], is a powerful inhibitor of NAD^+-dependent dehydrogenase, which is responsible for the inactivation of the prostaglandins [62], and exhibits a suppressive action on the synthesis of progesterone [63].

One of the factors responsible for the biological activity of Gp must be considered if its capacity for generating superoxide anion-radicals [64, 65] and affecting the level of free-radical processes in the membrane and the cell [66]. In addition to this, Gp is a membrane-active substance and induces permeability predominantly for hydrogen ions [67, 68]. However, in this process the membranes become appreciably permeable for other uni- and bivalent cations, as well, in accordance with the sequence $H^+:Ca^{2+}:K^+:Na^+:Mg^{2+}:Ba^{2+}$ = 1.0:0.49:0.33:0.22:0.11:0.10 [68]. This increase in proton permeability may be responsible for the inhibiting action of Gp on some membrane transport systems [69]. A study of the influence of various tautomeric forms of Gp on membranes has shown that these effects are connected with its enolic form [70]. All that has been said above shows the exceptional nature of the Gp molecule not only from the point of view of its chemical structure — it is unique among the group of natural polyphenols — but also in connection with the extremely broad spectrum of its biological action.

GOSSYPOL DERIVATIVES AND THEIR BIOLOGICAL ACTION

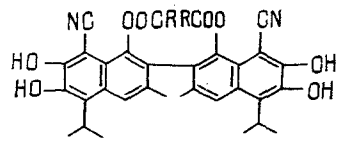
Among existing methods for finding and constructing physiologically active substances (screening, the modification of known drugs, the modification of natural compounds, fundamental research), one of the most fruitful has proved to be the modification of natural compounds, which enables a colossal variety of substances with better properties than those of the initial substrate to be obtained [71, 72]. Investigations on the chemical modification of Gp, the molecule of which presents broad possibilities for this, may serve as a clear confirmation of what has been said.

Gp imines, in the molecules of which an additional center has appeared which ensures interaction with the cell macromolecules through hydrogen bonds, hydrophobic interaction, the formation of complexes, etc., is of interest in this connection. At the present time, about 80 Schiff bases of Gp with the general formula



have been synthesized [73-82], and individual representatives of them have been shown to possess antiviral [80, 83] interferon-inducing [84-86], immunomodulating [81, 87], ulcer-healing, and other forms of physiological activity [88-90].

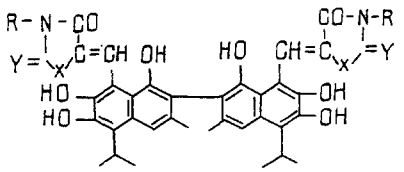
In recent years, a number of reports have appeared on the synthesis of imines and bis-Schiff bases of Gp with chlorine- and fluorine-containing amines [91-93]. peri-Acylated nitriles of Gp with the general formula [94-96]



possess antiviral and antimalarial activity, and for these a high affinity for the binding sites of bilirubin from human serum albumin and values of K_α ~ 30 times higher than for Gp have been shown [97]. From these compounds a drug has been developed that is effective in use against herpes simplex virus (type II) [98].

In spite of the fact that products of the condensation of Gp with CH-acids may be no less interesting for obtaining biologically active substances, comparatively few compounds of this type have been described [99, 100]. Only recently has a

publication appeared on Gp derivatives for the production of which use was made of thiazolid-4-ones as compounds containing active methylene groups [88]. Among this group of substances with the general formula



X=O; Y=S; R=H;
Y=O; R=aryl
X=NH; Y=S; NH; R=H;
X=Y=S; R= alkyl, aryl;
X=S; Y=NH; R=H;
Y=O; R=H, aryl

substances with a high antitumoral activity have also been found. When barbituric and thiobarbituric acids were used as the CH-acids, substances were obtained which possessed a pronounced immunosuppressive action [42].

Among Gp derivatives substituted in the hydroxy groups (ethers and esters) [101], no interesting physiologically active substances whatever have been found [88]. A comparative consideration of the results of a study of the antitumoral [83, 88] immunosuppressive [87, 88], and contraceptive [102] activities, and also of the inhibiting action on spermatozoal fructolysis and on the anionic transport of erythrocytes, of methyl (di-, tetra-, hexa-) ethers and their various derivatives [104] has shown that the substitution of the hydroxy groups leads to an appreciable fall in activity or even to its complete disappearance. It is interesting to note that while the dimethyl ether of Gp exhibits a weak immunosuppressive action, its condensation products with the general formula

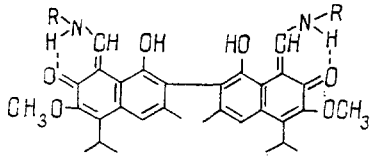


exhibit a weak immunostimulating effect [105].

A structural—functional analysis of numerous Gp derivatives has permitted several conclusions to be drawn about the dependence of their biological activities on their structures. Thus, a study of ethers and esters has shown the necessity for the presence of unsubstituted hydroxy groups in the molecule for the manifestation of activity [83, 87, 88, 102-104]. A definite contribution to the formation of activity is made by aldehyde groups, since their absence — for example, in the case of apogossypol — is accompanied by a fall in activity [87, 106]. As can be seen from a study of their antiviral, interferon-inducing, immunomodulating, and antitumoral action, the main factor determining the type of physiological activity of Gp derivatives is the nature of the substituents in their aldehyde groups [87, 88]. Thus, the product of the condensation of Gp with barbituric acid is an active immunosuppressor while the use of thiobarbituric acid as substituent in the aldehyde groups leads to a substance that is completely inactive in this respect [87].

It has been shown for a large number of Gp imines that they are all immunotropic, and the magnitude and type of their immunomodulating activity (inhibition or stimulation) depend on the nature and position of the substituents in the amine component. For example, the use of *o*-aminobenzoic acid as the amine component leads to a compound possessing immunostimulating action, the addition of *m*-aminobenzoic acid to Gp leads to the appearance of a weak immunosuppressive effect, and the product of the interaction of *p*-amino benzoic acid with Gp is inactive. A similar correlation has been traced in the products of the condensation of Gp with the isomeric aminophenols and sulfanilamides [87].

In the case of the Gp imines, it is very easy to trace the dependence of the immune response on the dose and the methods of administering the substance (before or after the action of an antigen), which is generally characteristic for immunomodulators. The different directions of the immunomodulating action of Gp derivatives has also been confirmed by an investigation of their inhibiting action on K^+, Na^+-ATPase, and lactate dehydrogenase [46, 107, 108]. Investigations of the actions of Gp derivatives on the structure of mitochondria have revealed that their effect is milder than that of Gp, and the ionic permeability induced by various derivatives has shown a selectivity differing from that of Gp [68]. The dependence of the permeability of the membrane on the concentration and the pH of the medium found in these investigations has shown the promising nature of the use of various gossypol derivatives with the aim of acting selectively on the permeability of biomembranes for particular ions. The selective action on biomembranes and the inhibition of enzyme systems and bioenergetic processes in the cell, together with the established fact of the modification of the lymphocyte membranes of the spleen with the aid of a number of imines and thiazolid-4-one derivatives of Gp [108], may be considered as one of the possible mechanisms of their action as immunosuppressors.

An important point revealed in the study of the biological activity of Gp derivatives is their lower toxicity, as compared with Gp itself, in *in vitro* and *in vivo* systems. In order to study the toxic effect of Gp derivatives, a system of oxidative phosphorylation localized in the membrane of liver mitochondria was used, the link between the toxic effect of Gp and its influence on the energy-conversion system in the mitochondria serving as a prerequisite for this purpose [109, 110]. Substitution of the functional groups, both the hydroxy and the aldehyde groups, is always accompanied by a fall in toxicity [87, 88, 111]. The most toxic among the Gp derivatives studied proved to be derivatives of gossypolone — the quinone of gossypol [112, 113] — which is in harmony with the known fact of the realization of the toxic action of natural phenolic compounds on the animal organism through the phenol—semiquinol—quinone system [114, 115].

Apparently, the mechanism of the inhibiting effect of Gp derivatives, as of Gp itself, on the activity of enzymes and enzyme systems is based on their capacity for interacting with the amino groups of proteins and enzymes and producing complexes with the metal ions forming components of enzymes, and also their influence on the level of free-radical processes in membranes and cells. What is very important, in the majority of cases, their inhibiting action has a competitive, reversible, nature.

METHODS OF DETERMINING GOSSYPOL AND ITS DERIVATIVES

The increasing interest of recent years in gossypol and its physiologically active derivatives has made it necessary to generalize information on methods for their quantitative determination. Several reviews devoted to this question are known [116-119], and therefore we give information published in the last 10-15 years.

It can be seen from the numerous publications devoted to the quantitative determination of Gp in plant materials, that gravimetric, volumetric, colorimetric, spectrophotometric, luminescent, and polarographic, and even opticomicroscopic, methods have been used for analysis [119, 120]. Officially approved are various modifications of the gravimetric and spectrophotometric methods which permit Gp to be determined when its content in plant materials is in the range of 0.005-0.01% [116-121]. Chromatographic methods — paper and thin-layer chromatographies — are more sensitive (by a factor of 5-10); with their aid it is possible to determine not only Gp but also its ethers and esters [122-125]. Gas—liquid chromatography is 50-100 times more sensitive than spectrophotometric methods [118], but certain disadvantages of this method are connected with the necessity for converting Gp into trimethyl silyl ethers.

An attempt has been made to avoid supplementary operations in the preparation of plant material for analysis, especially extraction, and for this purpose reflection spectra of the ground seeds have been taken (directly). Each calibration curve was plotted with the use of 300 points. The correlation coefficient calculated on the basis of results obtained by these and standard methods was 0.987 [126].

The method of nuclear magnetic resonance has been used to determine the level of Gp in cotton flower buds, permitting the determination not only of Gp itself but also of such minor components as hemigossypol and its ethers and esters, hemigossypolone, and the heliocides [127].

In 1981, a paper was published [128] in which it was proposed to use the method of high-performance liquid chromatography (HPLC) for the determination of Gp in seeds. A comparative consideration of the results obtained spectrophotometrically and with the aid of HPLC shows a number of advantages of the latter. The proposed method includes fewer stages, reduces technical errors to a minimum, and, which is most important, is 2500 times more sensitive than the spectrophotometric method. Even in this paper the advantage of the method was shown not only for quantitative but also for qualitative analysis. The high specificity of the method appeared in the fact that almost 35% of the amount of Gp determined in the same extract spectrophotometrically was due not to Gp but to other, gossypol-like, pigments likewise colored by the reagents used for spectrophotometry. In individual cases, the overestimation of the results reached a factor of 2-5 [129].

Initially, methanolic solutions of Gp were used for deposition on the column, and various aqueous methanolic mixtures with the addition of phosphoric acid as the mobile phase [128]; however, the well known instability of alcoholic solutions of Gp stimulated the search for more suitable systems. A. A. Nomeir et al. made a series of investigations which permitted the selection of the optimum conditions for its determination in the seeds and roots of the cotton plant [130]. According to the results of the action of solvents on the rate of decomposition of Gp, its stability in the solvents studied decreased in the sequence acetone > acetonitrile > chloroform > ethanol > methanol. It was established that in all the solvents studied, at room temperature and at 37°C, Gp underwent changes, and keeping the solutions for some time was possible only at very low temperatures of from —25 to —80°C.

In the course of the use of HPLC for the study of Gp a number of systems was proposed, such as acetonitrile—water—acetone (7:2:1), methanol—citrate buffer (55:45), tetrahydrofuran—water—phosphoric acid (60:40:0.08), hexane—chloroform—acetic acid—cyclohexane (78:20:33:2); acetonitrile—water—tetrahydrofuran (80:18:2); methanol—water—chloroform—phosphoric acid (70:30:40:0.14); methanol—water—phosphoric acid (82:18:0.1); methanol—water (87:13); and some others [131-144]. By combining the methods of HPLC and mass spectrometry it was possible to show that with alcohols Gp forms a series of complex hemiketal and ketal derivatives of its various tautomeric forms which, when an attempt was made to isolate them and during mass spectrometry, were converted into Gp or anhydrogossypol [143].

With the aid of HPLC in combination with mass spectrometry it has been possible to explain the existence of two forms — white and yellow — of gossypol hexaacetate, each of which contained six acetyl groups and was characterized by a molecular ion with *m/z* 770 although they had different melting points. It was shown that each of the two hexaacetates consisted of a mixture of stereoisomers of the three possible forms of gossypol: the white hexaacetate contained a mixture of two forms in a ratio of 3:1, while the yellow was a mixture of six forms in a ratio of 30:54:4.5:3.5:7:1 [132].

The possibility has been shown of using the HPLC method not only for analytical but also for preparative purposes, which is of particular interest when the process is performed on a chiral amino acid bound phase. Under these conditions it is possible to obtain gram amounts of (+)- and (—)-Gp from its Schiff bases [136].

In spite of the advantages of HPLC in the analysis of plant raw material, gravimetric [145] and spectrophotometric [146-156] methods have not lost their value, particularly in applied investigations. In one of the latest publications devoted to the comparative study of different species and varieties of the cotton plant a gravimetric aniline method was used, with the aid of which it was possible to establish that the species *G. barbadense* mainly accumulates (—)-gossypol, while *G. arboreum* and *G. hirsutum* contain more of the (+)- isomer [157].

In connection with the poisoning of animals that have had cottonseed meal in their fodder, an acute necessity has arisen for determining the level of Gp not only in plant raw material but also in animal tissues. And although investigations in this direction began as early as the 60s, we have found in the domestic literature no material generalizing investigations connected with the quantitative determination of Gp in tissues of animal origin.

Initially, the intensive development of studies on the quantitative analysis of Gp in animal tissues was connected with its undesirable physiological action on animals and with the development of various diets and mineral additives permitting its toxic action to be neutralized [157-169]. As also in the case of plant materials, the analysis of animal tissues presupposed the determination of free and bound Gp by the method described in [160], including the extraction of the tissues, the formation of an aniline derivative, and photometry at 440 nm.

An investigation of the luminescent determination of the level of Gp in rat organs is known in which use was made of its capacity for fluorescing intensively and specifically in concentrated sulfuric acid at 77 K [170, 171]. The method is comparatively simple and permits the determination of gossypol within the range of 10^{-5}-10^{-8} g/ml.

After the appearance of the HPLC method, work on the determination of Gp in animal tissues acquired a special boost, since the possibility appeared of performing pharmacokinetic investigations in studying metabolic transformations not only in the animal organism but also the human organism [140, 172-180]. The necessity for such investigations is connected, in the first place, with the revelation in Gp of a broad spectrum of physiological action and the creation of drugs from it. It was reported in [172] that the "detectability" of gossypol in the bile amounted to 75%. A solution of Gp in DMSO—ethanol (1:10) was used for deposition on the column, and a mixture of 10% acetic acid with ethanol as the mobile phase. Blood plasma is used most frequently for analysis, this being deposited on the column after appropriate treatment; the mobile phases used are methanol—water—acetic acid (77:20:3), methanol—citrate buffer (55:45), methanol—water—chloroform (70:30:40) + 0.1% of phosphoric acid; phosphate buffer—acetonitrile (38:62), and other systems [140, 172-180].

Various forms of Gp have been studied with the aid of the HPLC method and the efficiency of the liposomal form, which possesses a greater permeability through the "blood—testes" barrier has been shown [176]. The use of the HPLC method has enabled substantial differences to be found in the action and metabolism of (—)-stereoisomers of Gp in the rat organism [178-180].

An investigation is known of the use of the ^{31}P NMR method in the study of the action of HP on various cancer cell lines [181].

Studies on the chemical modification of gossypol and the structural—functional analysis of numerous derivatives of it have permitted the creation of drugs and their introduction into medical practice: gossypol liniments, batriden tablets, and megosin salve. A spectrophotometric method has been used for the determination of gossypol in its medical forms (liniments, aerosol) in which the optical density at 366 nm of a chloroform solution obtained after the appropriate treatment of the

medicinal form was measured. The amount of Gp was determined by comparison of a standard solution of Gp of the same concentration. A spectrophotometric method has also been used for the analysis of the substance and of megosin salve, the solvent used being acetone—water (3:1), and the optical density being measured at 385 nm; the megosin content was determined by comparison with a standard solution of megosin of the same concentration [183, 184]. To analyze the substance and batriden tablets by the same method, the UV absorption of the substance at 496 nm was measured in a mixture of dimethyl sulfoxide and alcohol (1:9) [185, 186].

PHARMACOKINETIC INVESTIGATION OF GOSSYPOL AND ITS DERIVATIVES

In connection with the creation of drugs from gossypol derivatives, the necessity has appeared for the performance of pharmacokinetic investigations, since it is impossible to develop principles of the effective and safe use of drugs without a knowledge of the rules of their circulation in the organism.

A number of investigations devoted to the study of the mechanism of the pharmacokinetics and the mechanism of the action of Gp in the organisms of various animals and of man are known [187-196]. A radioisotope method in combination with the radiochromatography of ^{14}C-labeled Gp synthesized in accordance with [188] is most frequently used for these purposes. ^{14}C-Gp has been used to study absorption in the gastrointestinal tract, excretion from the organism, and features of its distribution over the organs and tissues of the rat [189-191], chickens and laying hens [187-191], swine [187, 192-197], rabbits, cats, dogs [187], and other animals. The larger amount of ^{14}C-Gp detectable in the organs and tissues was 95% of the total administered for chickens, 16.8% for laying hens, 32.9% for swine, and 12.5% for rats [194]. Factors determining different tolerances to gossypol are different degrees of its absorption in the intestine, different values of the period of half-elimination from the organism, and the rate and degree of detoxication [187, 194, 197].

The results of a study of the distribution of Gp in the animal organism revealed an ambiguity of its localization over the organs of animals. In the main a tendency is found which permits the organs to be arranged according to the degree of accumulation of Gp in the sequence liver > muscles > blood > kidneys > lungs, and then the heart, spleen, and brain. For all species of animals the greatest radioactivity was detected in the liver, the least in the brain, while the positions of the other organs in the series could change very slightly. The accumulation of Gp in all the organs fell appreciably if such additives as fish meal [188] and iron sulfate [189] were introduced into the animals' ration. The results obtained in a study of features of the elimination of ^{14}C-Gp from the organisms of various animals are given in Table 1.

As can be seen from Table 1, the main pathway for the elimination of ^{14}C-Gp in all the animals studied is its excretion with the bile. A certain amount of it is found in the form of $^{14}CO_2$ in the exhaled air, which permits the assumption that Gp undergoes decarbonylation during metabolism. By using the samples of ^{14}C-Gp labeled in various atoms of the molecule and performing a comparative study of the air exhaled by rats, it was concluded [190] that the binaphthalene ring of Gp is not broken down and decarbonylation takes place at the expense of the aldehyde groups.

A study of the distribution of ^{14}C-gossypol over the subcellular fractions of hepatocytes of the rat liver found the largest amount of label in the microsomes and mitrochondria and the smallest amount in the nuclei [190]. The high specific activity of the microsomal fractions can be explained by the lipophilicity of Gp. It is important that the ratio of radioactivity to protein was higher in the cell fractions containing lipoprotein membranes, i.e., in the mitochondrial, lysosomal, and microsomal fractions, while its ratio was lower in the supernatant fractions containing no membranes.

As has been shown in a study of the metabolic rate of Gp in swine [194], the main metabolites of Gp are apparently gossypolone and gossypolonic and demethylated gossypolic acids, glucuronides, and sulfate esters.

The results of a pharmacokinetic study of the influence of stereoisomers of Gp on the animal organism are very interesting, showing substantial differences in the metabolism and action of the (+)- and (—)- isomers [195-199]. Thus, the half-elimination period of (—)-Gp is considerably shorter than that found for (+)-Gp and this and some other pharmacokinetic differences between the isomers are explained by the more powerful selective binding of (+)-Gp with tissue proteins [40, 195, 199]. The most interesting factor revealed in this series of investigations is that (—)-Gp does not accumulate in the organism [198].

In order to study the pharmacokinetic parameters of drugs obtained from Gp, radioactive forms of them — ^{14}C-megosin and ^{14}C-batriden — were synthesized. In experiments on mice, the distribution of both drugs in the organism and at the subcellular level, the kinetics of absorption and elimination, and binding with the macromolecules of liver hepatocytes and blood plasma were studied. It was shown that with the enteral and parenteral methods of administration, their pharmacokinetics in

TABLE 1. Elimination of ^{14}C-Gossypol (per oral administration), %

Animals	Number of days of observation	Exhaled air	Urine	Feces	Eggs	Literature
Chickens	8	3,26±0,47	—	78,55±7,45	13,67	191
Rats	13	12,1±120	3,1±0,51	77,4±7,21	—	189
Swine	20	2,1±0,15	0,69±0,003	94,6±9,7	—	194

TABLE 2. Constants and Parameters of Models of the Pharmacokinetics of Gp and Its Derivatives (single intraperitoneal injection, mouse)

Substance	Half-absorption period, h	Half-elimination, period, h	Volume of distribution, ml	Total clearance, ml/h	The area under the kinetic curve, mg·h/ml
^{14}C-Gossypol	3,28	36,47	14,80	0,78	1,28
^{14}C-Megosin	0,75	27,72	18,77	0,47	2,13
^{14}C-Batriden	4,33	46,20	36,25	0,54	1,85

TABLE 3. Amounts of Radioactivity in the Subcellular Fractions of Mouse Liver Hepatocytes (24 h after administration), %

Object of investigation	^{14}C-Gossypol	^{14}C-Batriden	^{14}C-Megosin
Liver homogenate	100,0	100,0	100,0
Nuclei	6,6	1,2	7,7
Mitochondria	23,6	17,9	40,6
Microsomes	34,4	18,2	14,6
Hyaloplasm	18,2	16,5	5,8
Cytoplasm	22,6	40,2	31,3

the blood is described satisfactorily by a one-section model with absorption [199-203]. The results of a radiometric investigation of Gp and its derivatives have been confirmed by the results of the autoradiography of the whole body of a mouse [204]. For ^{14}C-batriden and ^{14}C-megosin, just as for ^{14}C—Gp, a general tendency is retained: the greatest radioactivity is found in the liver and the least in the brain. Some pharmacokinetic parameters of Gp and its derivatives are given in Table 2.

As can be seen from Table 2, the drugs differed with respect to all the parameters concerned, i.e., modification of the structure of Gp is accompanied by qualitative changes in the manifestation of the action of the substance. At the same time, they all retained a well-defined lymphotropicity, and the results of observation of the distribution of the label over the organs during four days indicated an accumulation and prolonged presence of radioactivity in the immunocompetent organs (lymph nodes, thymus, spleen), which is of fundamental importance for characterizing gossypol derivatives used as immunomodulators.

It has been established that the elimination of ^{14}C-batriden and ^{14}C-megosin takes place through the bile, and in this case, in contrast to Gp, together with the unchanged labeled compounds a single metabolite was detected in the faeces in each case, which indicates their comparatively insignificant biotransformation in the mouse organism. About 3% of the label was eliminated with the urine, while practically no radioactivity was recorded in the exhaled air. The extrarenal pathway of the excretion of both substances, particularly batriden, favorably characterizes them as drugs, since it is assumed that a renal pathology does not lead to marked changes of their circulation in the organism.

The pronounced hepatotropicity of batriden and megosin that is shown by the active accumulation of a label in the liver was the motive for a series of experiments to study the intracellular distribution and features of the binding of the ^{14}C-labeled drugs with the macromolecules of the structures of the liver hepatocytes (Table 3). As can be seen from Table 3, for the Gp derivatives, in the main, the tendency found for Gp itself is observed: active inclusion in the microsomal and mitochondrial fraction and weak binding with the hepatocyte nuclei. It was found by further investigations that the bulk of the radioactivity in the subcellular structures of the liver cells and the blood plasma was bound to the lipids (60-80%) and the proteins (20%) with no affinity being shown for the nucleic acids. Thanks to their high affinity for lipids, gossypol and its derivatives are concentrated in the lipid layer of the membranes and, being bound with their protein components, in particular the enzyme systems, exert an action on their functional activity.

All that has been said above once more indicates not only the promising nature of the search for biologically active substances among polyphenols of plant origin but also great possibilities of the chemical modification of their structures with the aim of creating drugs with a broad action spectrum.

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