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# IMMUNOMODULATING ACTIVITY OF GOSSYPOL DERIVATIVES

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Information is given on the immunosuppressive activity of a number of gossypol derivatives, and it is shown that practically all the compounds studied are immunotropic. The structural-functional relationship and the dose-dependent nature of the action of the substances obtained are shown.

The search for methods of stimulating and depressing the immune system as a whole and individual cell populations of it is regarded as the main task of immunocorrection [1]. The search for effective immunomodulators is being carried out in the most diverse directions, but hitherto these investigations have mainly borne an empirical nature. This is due to the complexity of the organism and of the functioning of the immune system, which consists of various populations of cells interacting with one another and various substances secreted by these cells [1]. The final effect (immunostimulating or immunodepressive) depends on the integral action of a given modulator on the functional activity of the immunocompetent cells.

Both substances of protein nature and also physiologically active compounds of synthetic [2] and plant [3] origin may be immunocorrecting. At the present time, reports have appeared of the existence of immunostimulating properties in plant polyphenols [4, 5].

In the present paper we give information on the immunomodulating activity of gossypol derivatives. The basis for the study of this type of activity of gossypol was its possession of antitumoral [6] and antiviral [7] activities.

It has been found that gossypol possesses some immunosuppressive activity, and to enhance this effect it was desirable to modify its structure, which would permit the influences of various functional groups of the gossypol molecule and of the nature of substituents on its activity to be determined simultaneously.

Because of the structure of gossypol, it is possible to obtain ethers and esters, azomethines, and condensation products with compounds containing active methylene groups [8].

A consideration of the comopunds obtained in relation to their immunosuppressive action showed that the ethers (II)-(IV), although they possessed activity, did so to a smaller degree than the initial gossypol (I). Substitution of the hydroxy groups led to a fall in the activity that was almost proportional to the degree of substitution (Fig. 1).

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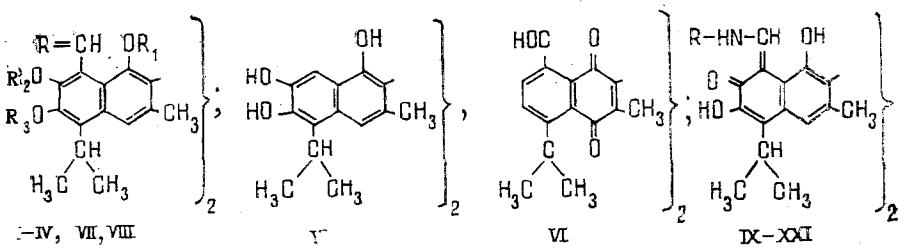


I–IV, VII, VIII V VI IX–XXI

I R = O; $R_1 = R_2 = R_3 = H$

II. R = O; $R_1 = R_2 = H$, $R_3 = CH_3$

III. R = O; $R_1 = R_2 = CH_3$; $R_3 = H$

IV. R = O; $R_1 = R_2 = R_3 = CH_3$

VII. $R_1 = R_2 = R_3 = H$; $R = C(CO-NH)(CO-NH)CO$

VIII. $R_1 = R_2 = R_3 = H$; $R = C(CO-NH)(CO-NH)CS$

IX. $R = o-C_6H_4OH$

X. $R = m-C_6H_4OH$

XI. $R = p-C_6H_4OH$

XII. $R = o-C_6H_4COOH$

XIII. $R = m-C_6H_4COOH$

XIV. $R = p-C_6H_4COOH$

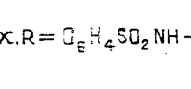
XV. $R = p-C_6H_4COOC_2H_5$

XVI. $R = CH_2CH_2SO_3OK$

XVII. $R = CH_2CH_2OH$

XVIII. $R = C_6H_4SO_2NH-C(=O)NH_2$

XIX. $R = C_6H_4SO_2NH-C(=NH)NH_2$

XX. $R = C_6H_4SO_2NH-$ 

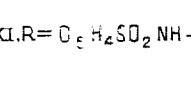
XXI. $R = C_6H_4SO_2NH-$ 




Fig. 1. Immunomodulating activity of gossypol derivatives.

The absence of aldehyde groups (V) was also accompanied by a decrease in activity, while conversion into a quinone (VI) led to low activity and high toxicity. A comparison of the results of a study of the immunodepressive activity of a number of gossypol derivatives (Fig. 1) enabled the following fundamentally important conclusion to be drawn: all the gossypol derivatives studied possessed immunotropicity to a greater or smaller degree. Here, no small role is played by unsubstituted hydroxy groups and the nature of the substituents at the aldehyde groups. The latter is clearly demonstrated with, as examples, the products of the condensation of gossypol with o-, m-, and p-aminophenols and with o-, m-, and p-aminobenzoic acids (Fig. 2). On the whole, a slight inhibiting effect was characteristic for products of condensation with aminophenols, with the exception of p-aminophenols (XI), which exhibited a stimulating action in a dose of 100 mg/kg.

An inhibiting action was characteristic for the products of the condensation of gossypol with p-aminobenzoic acid (XIV) and, particularly, with m-aminobenzoic acid (XIII), while the introduction of o-aminobenzoic acid (XII) as the amine component led to a clear stimulating effect (Fig. 2). The replacement of p-aminobenzoic acid, poorly effective in relation to imparting immunosuppressive properties, by its ethyl ester led to an increase in the inhibiting action of substance (XV). The influence of individual groups, and even atoms, in the molecule of the amine component on the immunomodulating activity is shown even more clearly by a comparison of compounds (VII) and (VIII), (XVI) and (XVII), (XVIII) and (XIX), and (XX) and (XXI) (Fig. 1).

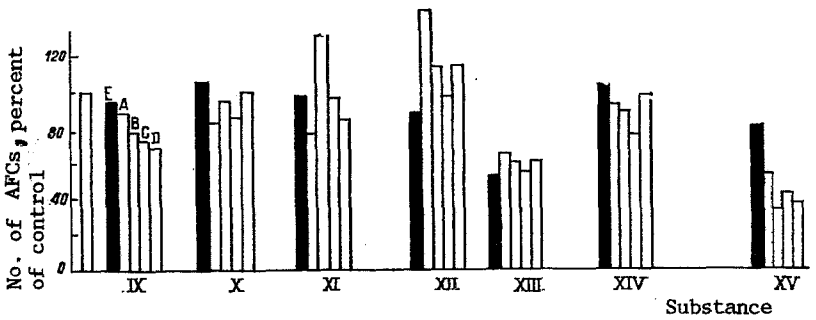



Fig. 2. Dependence of the immunomodulating activity of gossypol derivatives on the nature of the amine component, the dose, and the method of administration: A) 50; B) 100; C) 250; D) 500 mg/kg (administered after immunization); E) 100 mg/kg (administered before immunization).

A not unimportant role in achieving the effect is played by the dose and the method of administration (Fig. 2) of the substance – before or after the action of an antigen. Some of the compounds suppressed the immunity practically regardless of the scheme of administration (XIII); in some cases the time of administration influenced the magnitude of the effect (IX), (XI), (XII), (XIV); and sometimes it even changed the very type of activity: compound (XI), administered before the action of an antigen, exhibited a weak immunosuppressive effect, while when administered after the action of the antigen it revealed an immunostimulating action; a dependence of the type of activity on the nature of a substituent was also traced in the case of compound (XV).

The different degrees of correction of antibody formation (suppression or stimulation) on the administration of a preparation before or after the action of the antigen apparently show an action of particular compounds predominantly on the antigen-sensitive cells or on different stages of the inductive period during which the differentiation and division of the activated precursors and of the antibody-forming cells themselves take place.

Compound (VII), exhibiting a high immunosuppressive effect, has already been introduced into medical practice as a basic immunosuppressor in kidney transplantation and in the treatment of chronic glomerulonephritis.

Compound (XVI), which possesses a considerable immunosuppressive activity, was toxic in therapeutic doses, in view of which it was not studied further.

## EXPERIMENTAL

Schiff's bases were obtained from gossypol in accordance with [9].

The suppressive action of the preparations on the humoral factors of immunity was judged from the decrease in the number of antibody-forming cells (AFCs) in the spleens of mice of the BALB/c line immunized with ram erythrocytes in comparison with a control [10]. As the test for evaluating cellular immunity we used the model of the transplantation of allogenic skin flaps in inbred mice: the more effective the substance the longer did the transplant live [11]. The results were treated by the method of variational statistics using the Student-Fisher criterion of significance.

The majority of the substances obtained were less toxic than gossypol.

## SUMMARY

The immunomodulating activity of a number of gossypol derivatives has been studied: it was shown that practically all the compounds studied were immunotropic.

The structural-functional and dose-dependent nature of the action of the compounds obtained has been established. A dependence of the magnitude of the activity on the presence of free hydroxy groups and the nature of an amine component at the aldehyde groups has been shown.

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# STRUCTURE OF GOSSYPOL ARYLIMINES

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A number of new Schiff's bases of gossypol with aromatic amines and sulfanilamide compounds has been obtained. It has been shown by UV and PMR spectroscopy and x-ray structural analysis that in some solvents they exist predominantly in the quinoid form. For dianilinegossypol (in the solid state) the quinoid structure has been demonstrated and two polymorphic modifications have been revealed by x-ray structural analysis.

The possibility of the existence of gossypol in three tautomeric forms — dialdehyde, dilactol, and diquinoid — has been discussed in the literature [1], although the last-mentioned has been shown only in the case of its derivatives [2], including imino compounds [3]. Continuing a study of azomethines of gossypol with the aim of obtaining physiologically active compounds, we have synthesized a number of its derivatives with aromatic amines and sulfanilamide compounds (Fig. 1). In spite of the fact that compounds (I–VII) had been described previously [4], it was desirable to perform a spectral study of them in order to determine their structures more accurately.

The phenomenon of tautomerism, in which the sigmatropic transfer of a proton leads to a benzoid-quinoid equilibrium shifted in one direction or the other is characteristic for aromatic aldimines [5]. It can be seen from Table 1, which gives the parameters of the electronic spectra of Schiff's bases obtained from gossypol that absorption in the 440-480 nm region is most characteristic for them. The position and the intensity of the absorption maximum does not depend on the polarity of the solvent, which indicates their existence in practically a single tautomeric form [6]. Comparison with the spectra of 2-hydroxy-1-naphthaldimines [7] permits the absorption in the 440-480 nm region to be assigned to the quinoid form (II). This assignment was confirmed by the presence in the PMR spectra of the gossypol arylimines in $CCl_4$ of a doublet with a coupling constant J = 10-12 Hz in the 9.63-10.12 ppm region appearing as the result of the spin-spin coupling of the methine and amine protons, which is characteristic for compounds existing in the quinoid form [8].

Unfortunately, the majority of the gossypol derivatives obtained were sparingly soluble in organic solvents and it was possible to record the PMR of some of them only in DMSO. In the PMR spectra in DMSO-$d_6$, a signal in the 10.18-10.62 ppm region represented a superposition

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