BIOLOGICAL ACTIVITY OF GOSSYPOL AND ITS DERIVATIVES

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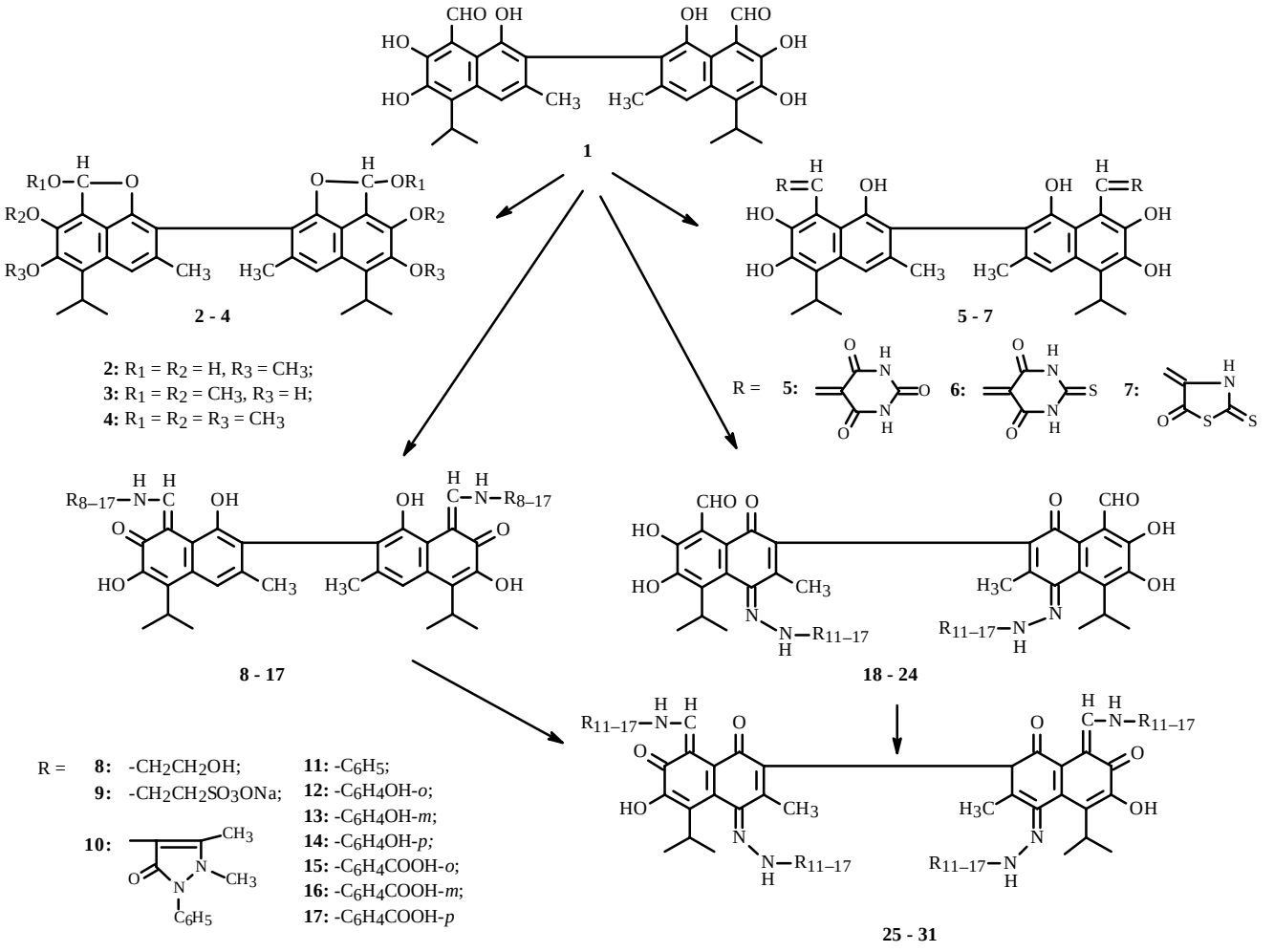
Information on gossypol and its derivatives and certain features revealed during a structure—activity analysis were presented.

Key words: gossypol; gossypol derivatives; structure—activity analysis; antiviral, interferon-inducing, immunomodulating activities.

The polyfunctional structure [1-7] of gossypol (GP) (**1**), a specific pigment of plants from the *Gossypium* L., *Cienfuegosia* Cav., *Thespesia* Sol., and *Cocia* Lewt. genera (Malvaceae) enables it to undergo esterification, azo-coupling, and condensation reactions (Scheme 1). Naturally these structural features of GP should also affect the biological activity [8].



Scheme 1. Scheme of GP transformations

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TABLE 1. Antiviral Activity of Gossypol and Its Derivatives (*in vitro*)

Compound	Virostatic activity			Virocidal activity			
	Virus			Virus			
	bird flu		Aujesky's disease*	bird flu		Aujesky's disease*	
	dose, μg/kg	degree of inhibition, log TCA_{50}	degree of inhibition, log TCA_{50}	dose, μg/kg	degree of inhibition, log TCA_{50}	dose, μg/kg	degree of inhibition, log TCA_{50}
1	100	6.5	6.3	125	5.0	125	5.5
5	100	4.5	4.0	500	6.0	100	5.0
7	125	5.0	5.5	-	-	-	-
9	50	5.5	2.0	62.5	4.75	100	5.5

*Dose 100 μg/kg.

TCA: the titre of cytotoxic action.

TABLE 2. Antiviral Activity of Gossypol and Its Derivatives (White Mice, *in vivo*)

Compound	Virus				
	bird flu		Aujesky's disease		
	dose, μg/kg	protection, %	dose, μg/kg	protection, %	protection*, %
1	250	20	250	17.0	32.7
5	125	15.0	125	17.5	-
7	-	-	125	38.5	-
9	125	12.5	125	27.1	32.7

*Dose 50 μg/kg.

The variety of activities of GP and its derivatives is indicative of several mechanisms of action on biomembranes. Thus, they can be considered polyfunctional modulators of cell processes [9].

The antiviral effect of **1** was first observed [10, 11] in experiments (*in vitro*, *in vivo*, and *in ovo*) using flu virus (PR-8 strain). The ability of GP to inactivate one type of virus while having no effect on others indicates that its action is specific. Data obtained using flu, paraflu, and polio viruses led to the conclusion that the inhibiting effect of GP is due to interaction with the proteinaceous viral shell [12].

The antiviral activity was studied further using GP derivatives as esters (**2**, **3**, **4**), imines (**8**-**11**), and condensation products with activated methylene compounds (**5**).

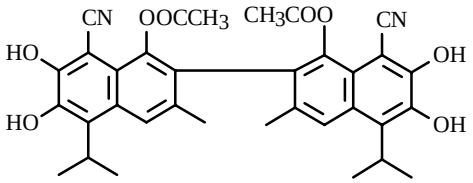
We present data for the antiviral activity of the GP derivatives (flu virus A, PR-8 strain, 100 μg/mL).

Compound	Activity
1	+++
2	±
3	±
4	±
5	++
8	±
9	+++
10	+++
11	±

It can be seen that substituting the hydroxyls (**2**, **3**, **4**) decreases the activity compared with the unsubstituted GP. Free hydroxyls are desirable for exhibiting activity. Substituting the aldehydes (**6**, **8**-**11**) also affects the activity. The compound can be more or less active than **1** depending on the nature of the substituent [13, 14].

A study of the activities of GP derivatives on a series of RNA-containing (bird hepatitis and Newcastle disease viruses) and DNA-containing viruses (Aujesky's disease virus) also showed that the activity depends on the nature of the introduced substituent. Compounds with the dibenzenoid (**1**, **5**, **7**) and diquinoid (**9**) structures (Tables 1 and 2) typically have antiviral activity.

The antiviral activity of GP and its derivatives was first determined for viruses [12] infecting integumentary epithelium cells (herpes, keratoconjunctivitis, laryngotracheitis, etc.). Later GP and its derivatives were investigated against viruses developing in blood cells, in particular, against human immunodeficient virus (HIV). As it turned out, not only GP itself but also 1,1′-dideoxygossypol and transacylated nitrile **32** inhibited HIV [15-18].



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It was found recently that the main barrier to viral infection is the system of interferon and its inductors [19]. GP was the first interferon inductor of plant origin that was of great value because of a lack of antigenic activity [20].

Effective interferon inductors are batriden [**5**), megosin (**9**), and ragosin (**10**). The interferon titers induced by each of these preparations depend on the dose, administration pathway, and duration of contact with the cell. Thus, an interferon titer of 640 $IU_{50/mL}$ was achieved using 100 μg/mL batriden in contact with cells for 30 min during a study of the interferon-inducing activities of **5** and **9** in L-926 cell culture. For megosin, 210 min at the same dose gave the same titer [21].

A study of the interferon-inducing activity of **10** has shown that it can stimulate interferon synthesis in various cell systems.

The high interferon-inducing activity of ragosin can be explained by the wide spectrum of its antiviral activity that was studied in various infection models. A high level of antiviral protection by **10** was found for flu virus (A/Aichi strain; $68/H_3T_2$, i.p.), murine encephalomyocarditis EMC virus (SK-Col-Sk strain, i.p.), and murine hepatitis virus (Mishcherin strain, oral). Ragosin also exhibits a protective effect as a prophylactic and for therapeutic administration regimes [22, 23].

At present ragosin tablets (0.05 g) are approved as a heaptoprotector for wide medical application.

The interferon-inducing compounds listed above (**5**, **9**, **10**) are GP derivatives substituted at the aldehydes. GP derivatives prepared by azo-coupling with amino-compounds at the C-4 atom are also interesting [24-26]. Positive results were obtained by comparing the structure—activity relationships for GP imines (**11**-**17**), GP azo-derivatives (**18**-**24**), and azo-derivatives of GP imines (**25**-**31**).

Determinations of the interferon-inducing activity of **11**-**31** (Fig. 1) indicate that it depends not only on the nature of the amine component but also on the condensation of the aldehydes or C-4. Imines **11**-**17** were mildly active in all versions. Azo-derivatives **18**-**24** were more active. The most active compounds were azo-derivatives of imines **25**-**31**. An examination of the effect of the position of the functional groups (*o*-, *m*-, *p*-) in the amine component of **11**-**31** showed the advantage of using the *o*-isomers for aminophenol **26** and aminobenzoic acid **29**. The *p*-isomers **28** and **31** are less active. The *m*-isomers **27** and **30** are uninteresting for use as interferon inductors.

The interferon titer induced in the organism depends also on the contact time with the compound. This is especially evident for **29**, the activity of which after 48 h increased by almost two times (1280 IU/mL) over its value after 24 h of contact (640 IU/mL) (Fig. 1*a* and *b*).

Thus, structure—activity relationships of GP derivatives enable a judicious selection of the most promising compounds for interferon induction.

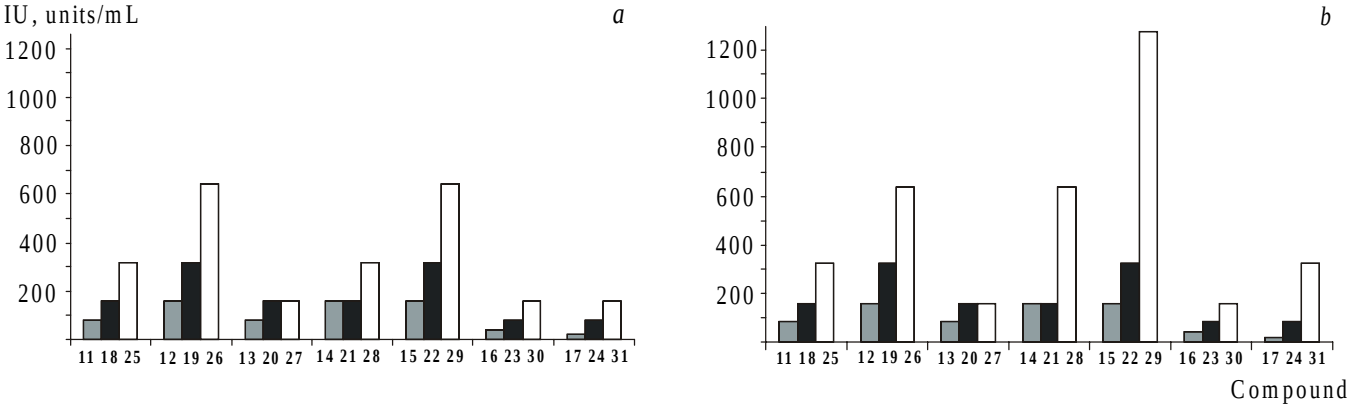


Fig. 1. Interferon-inducing activity of gossypol derivatives. Dose 200 mg/kg: after 24 (a) and 48 (b) h.

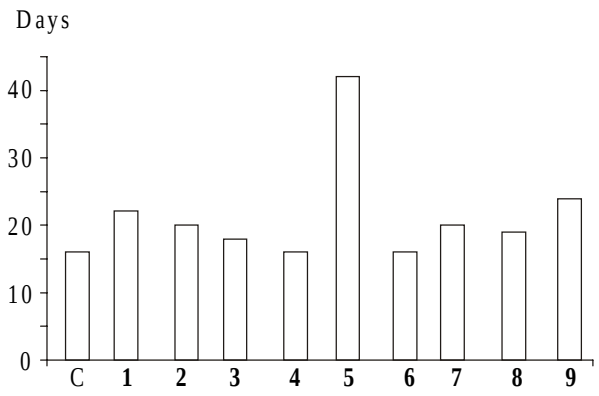


Fig. 2

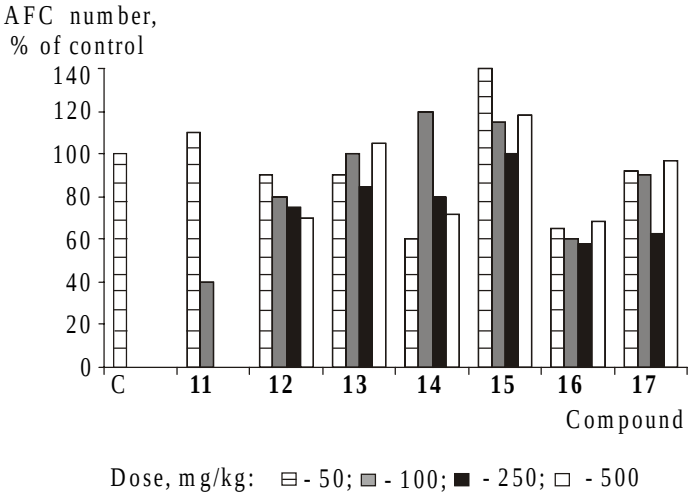


Fig. 3

Fig. 2. Survival times (days) of allotransplants using GP derivatives (**1**-**9**). C = control.

Fig. 3. Immunomodulating activity of GP arylimines (**11**-**17**) as functions of dose. C = control.

The immunomodulating activity of the interferon system is another of the numerous known effects of interferon (antiviral, antiproliferative, antimicrobial, etc.) that are indicative of its broad control—regulating functions. Furthermore, direct and indirect ties of the interferon system to the immune system have been established. A comparison of the immunosuppressive activities of more than 50 GP derivatives enabled us to conclude that they all are immunotropic to one degree or another [27-33].

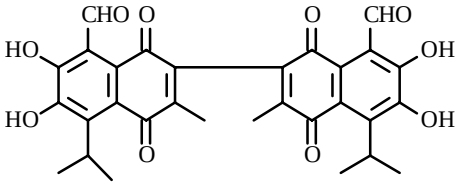
Certain approaches to eliciting and to some extent regulating the immunomodulating effect were determined (Fig. 2). Thus, the di-, tetra-, and hexamethyl ethers (**2**, **3**, and **4**, respectively) prolonged the survival of allotransplants compared with the control but not with GP. Here, as with the study of the antiviral activity, free hydroxyls are necessary for high activity.

The decisive role of the nature of the substituent on the aldehydes is seen clearly by comparing the GP condensation products with barbituric (**5**) and thiobarbituric acids (**6**). Whereas the survival time of a dermal graft averaged 42 d if **5** was used, it averaged only 16 d for **6** [33].

As in the study of interferon-inducing activity, an effect on the immunomodulating activity was observed not only for the nature of the substituent introduced at the aldehydes but also for the position of the functional groups (*o*-, *m*-, *p*-). Figure 3 shows that the *o*-isomer at all studied doses had a weak inhibiting effect. The *m*-isomer (**13**) typically had an immune response at the control level. The effect depended on the dose for the *p*-isomer. The trends were different for the aminobenzoic-acid isomers. The *o*-isomer (**15**) at all doses was an immunostimulator; the *m*-isomer (**16**), an immunosuppressor; the *p*-isomer (**17**), at the control level. This indicates that the immune response to the various compounds is complicated and depends on the

presence of free hydroxyls in GP itself, on the nature of the substituent introduced into GP, and on the position of the functional groups in the substituent. The effect is dose-dependent for all derivatives. A single compound (**11**, **14**, **17**) at one dose acts as an immunostimulator; at others, as an immunosuppressor. The dose-dependent nature of the immunomodulating activity of GP derivatives is clearly seen for batriden **5**. It was first approved for use as an immunosuppressor for kidney transplant (dose 6-7 mg/kg). Then its area of application was expanded to treatment of patients with various allergodermatitises. A dose of 3 mg/kg is recommended for stimulant activity [34-36].

It has been reported [37-39] that certain GP derivatives have antitumor activity. Thus, GP quinone, gossypolone (**33**), and GP stereoisomers are used against melanoma.



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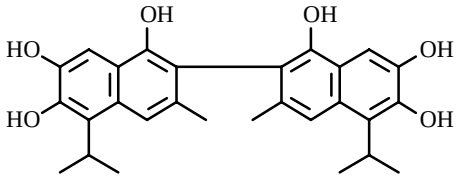
The stereospecific effect of GP isomers and **33** was studied on two melanoma lines. The (-)-isomer in all instances was found to be more active [40-43].

Studies of the antifertility activity of GP started in the 1970s are continued actively to this day [44-46]. The usefulness of GP as a male contraceptive is considered promising by several researchers. Therefore, work is being performed in this area. Publications on the antifertility activity of GP derivatives such as apogossypol (**34**) and gossypolone (**33**) have recently appeared.



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It has been proposed that the transformation products of GP may be more active contraceptives than GP itself [47, 48]. However, interesting compounds along these lines have not been found [49].

Studies on the biological activity of GP and its derivatives are continuing [50-58]. At present, it has been shown to affect the spermatozoid *Perca flavescens* [50], endogeneous opioid peptides of mouse hypothalamus [51], and the synthesis of macrophage prostaglandins [52]. Antiparasitic [53, 54] and cytogenetic [55] effects have been found.

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