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ANTIVIRAL AND INTERFERON-INDUCING ACTIVITIES OF GOSSYPOL AND ITS DERIVATIVES

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Information is given on the antiviral and interferon-inducing activities of gossypol and its derivatives.

Gossypol (GP) is one of the few polyphenolic compounds of plant origin that has found use in medical practice as an antiviral agent [1]. The antiviral action of GP is shown in a dose as low as 1 μg/ml in relation to a whole series of myxoviruses and herpes viruses. It almost completely inactivates such RNA-containing viruses as the influenza viruses A/PR-8/34 (H1NI), A2/Frunze/59, A2/Hong KongI/68 (H2N2), A2/England/42/72 (H3N2) and B/Tokyo, and the viruses of avian influenza and of Newcastle disease. GP also exhibits an inhibiting action on DNA-containing viruses — the keratogenic "YC" and "9C," and the dermatotropic "64" strains of herpes virus and the virus of Aujeszky's disease.

Some representatives of the arboviruses have proved to be highly sensitive to GP: Sindbis virus (strain Eg Ar 339), the West Nile fever virus (strain HP-94, strain Hp-94, clone 76), Japanese encephalitis virus (strain Jagar "01", the M-mutant clone 23), and the virus of tick-borne encephalitis (Sofjin strain). When 0.1-0.5% concentrations of GP were used, the index of the suppression of the viruses (difference beween the logarithms in the control and the experiment) was 3.0-5.0 [2].

A comparative study of GP with aminoadamantane in *in vitro* and *in vivo* experiments has revealed the existence of a pronounced virucidal and virus-inhibiting effect of GP in relation to the influenza virus. In an experimental influenzal infection of white mice, GP was more effective than aminoadamantane on intranasal administration but was inferior to it on internal administration [3, 4].

It has been shown in recent years that in an *in vitro* system GP inactivates the human immunodeficiency virus [5], and in a concentration of 100 μM completely inactivates cell-free preparations of HIV. In the range of concentrations from 1 to 50 μM it does not suppress viral activity completely, but the time of detecting an increase in the activity of reverse transcriptase in a culture of infected cells is prolonged, and the activity peak is lower than in an infected untreated supernatant of the culture.

The fact that GP inactivates some type of viruses and does not inactivate others shows a specificity of its action. On the basis of a study of the viruses of influenza and of parainfluenza and poliovirus the hypothesis has been put forward that the manifestation of the inactivating effect of GP requires its interaction with the viral coat [6]. Interesting is the inactivation of HIV on the use of the (−)- isomer of GP and the absence of this property in the (+)- enantiomer of GP [7].

The results of an experimental study of the antiviral properties of GP have served as a basis for the creation of a drug from it which is used in the form of a 3% liniment for the treatment of simple vesicular lichen, herpes zoster, and psoriasis [8].

Publications of recent years have shown the effectiveness of the method of chemically modifying natural compounds with the aim of increasing the specificity and efficacy of their action or reducing their toxicity.

The unique polyfunctionality of the GP molecule presents great possibilities for chemical modification, with the aid of which at the present time about two hundred GP derivatives substituted in the hydroxy and, especially, the aldehyde groups have been obtained [9].

In the present paper we give information on the antiviral and interferon-inducing activities of GP derivatives the majority of which we are the first to have synthesized (Table 1).

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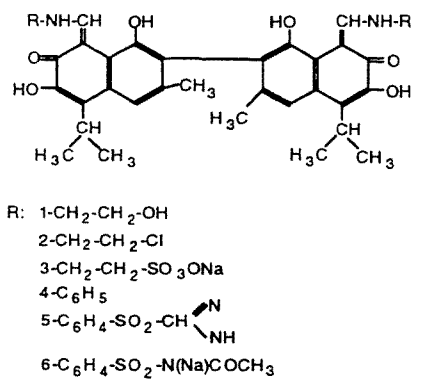
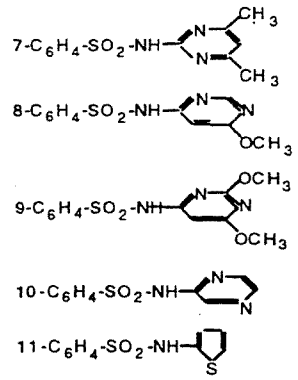
TABLE 1. Some Physicochemical Properties of Gossypol Imines

Substance	mp, °C	UV spectrum, nm, λ_{max} (log ε)	Empirical formula	N, %		Yield, %
				calculated	found	
1	255–257	382(4.36)	$C_{34}H_{40}N_2O_8$	4.63	4.83	77.8
2	>360	382(4.23)	$C_{34}H_{38}N_2O_6Cl_2$	4.55	4.34	64.04
3	>360	385(4.15)	$C_{34}H_{38}N_2O_{12}S_2Na_2$	3.60	3.75	90.8
4	302–304	440(4.13)	$C_{42}H_{44}N_2O_6$	4.16	4.23	89.11
5	254–256	450(4.69)	$C_{44}H_{46}N_8O_{10}S_2$	12.43	12.61	83.6
6	254–256	450(4.37)	$C_{46}H_{44}N_4O_{12}S_2Na_2$	5.65	5.77	48.4
7	252–253	450(4.69)	$C_{54}H_{54}N_8O_{10}S_2$	10.83	10.48	79.9
8	272–274	455(4.62)	$C_{52}H_{50}N_8O_{12}S_2$	10.76	11.08	83.3
9	260–262	455(4.70)	$C_{54}H_{54}N_8O_{14}S_2$	10.16	10.48	64.9
10	241–243	455(4.59)	$C_{52}H_{50}N_8O_{12}S_2$	10.76	10.54	78.7
11	254–256	450(4.67)	$C_{48}H_{44}N_6O_{10}S_2$	8.46	8.18	89.3

TABLE 2. Antiviral Activities of Gossypol Derivatives (influenza virus, strain PR-8, 100 μg/ml, mice)

Substance	Activity
Gossypol	+++
Gossypol dimethyl ether	±
Gossypol tetramethyl ether	±
Gossypol hexamethyl ether	±
2	±
3	+++
4	±
7	±





A study of the antiviral activities of the GP derivatives in relation to the influenza virus (strain PR-8) showed [10] that the level of activity is determined by the presence of free hydroxy groups and by the nature of the substituents in the aldehyde groups (Table 2). A lowering of activity on substitution of the hydroxy groups has also been confirmed in a study by J. P. Bader in an *in vitro* system of the anti-AIDS activity of the dimethyl ether of gossypol, which, unlike GP itself, proved to be inactive.

Investigations of the products of the modification of GP carried out with the aim of finding antiviral agents for veterinary medicine have shown that some of them, just like GP, possess virucidal and virostatic actions against DNA- and RNA-containing viruses (Table 3). Under the conditions of this experiment the greatest effect was shown by compound (**3**) and by the product of the condensation of GP with barbituric acid, which is called Batriden [11]. The latter has proved to be effective in relation to the picornavirus of poliomyelitis (strains Lugov, Smirnov, and Saukett).

Some protective action of Batriden in relation to West Nile fever virus has been demonstrated in mice [12].

A study of compound (**3**) *in vitro* and *in vivo* has shown that it possesses a broad spectrum of antiviral activity. Its high activity in a culture of human lymphocytes and human embryo fibroblasts has been established for alphaviruses — the Venezuelan equine encephalitis, Sindbis, and Semliki forest viruses [13]. In the organism of experimental animals it was active against Sindbis, herpes, and rabies viruses. Its prophylactic and therapeutic action has been established in experimental infections caused by the rabies and herpes viruses [14]. In *in vitro* experiments, when compound (**3**) was incubated with 0.5

TABLE 3. Antiviral Activities of Some Gossypol Derivatives *in vitro*

Compound	Virostatic action				Virucidal action				White mice					
	avian influenza virus		Aujeszky's disease virus		avian influenza virus		Aujeszky's disease virus		avian influenza virus		Aujeszky's disease virus		Newcastle disease virus	
	dose, μg/ml	degree of inhibition, log TCD_{50}	dose, μg/ml	degree of inhibition, log TCD_{50}	dose, μg/ml	degree of inhibition, log TCD_{50}	dose, μg/ml	degree of inhibition, log TCD_{50}	Dose, μg/kg	protection, %	Dose, μg/mg	protection, %	dose, μg/ml	protection, %
Gossypol	25	4.5	–	–	250	5.0	10	2.0	–	–	–	–	–	–
	50	6.5	–	–	–	–	25	5.5	–	–	–	–	–	–
	100	6.5	10	6.3	125	5.0	125	5.5	250	20	50	32.7		
Compound (3)	50	5.5	100	5.5	62.5	4.75	50	5.5	125	12.5	–	–	125	27.1
					125	4.75	100	5.5	–	–	–	–	–	–
	100				250	4.75	–	–	–	–	–	–	–	–
product of the condensation of GP with barbituric acid	50	2.5	–	–	250	5.0	–	–	125	12.5	50	32.7	125	27.1
	100	4.5	100	4.0	500	6.0	100	5.0						

ml of suspensions of rabies virus (strain CVS) and human acute encephalitis virus (strain FED) a considerable decrease in the activity of the rabies virus and a less pronounced action in the case of the acute human encephalitis virus were noted [15].

The antiviral action of compound (**3**) in relation to herpes simplex virus (strain "YC") was shown from a concentration of 30 μg/ml; strain L_2 of herpes virus proved to be more sensitive to the action of the preparation — a fall in the activity of the virus was observed from concentrations as low as 15-20 μg/ml. This compound possessed a more pronounced virucidal effect than GP, ensuring the survival of 40% of the animals at LD_{50} [16]. A series of experiments using various schemes of administration (intraabdominally, intraperitoneally, *per os*) in various doses (500, 750, 1000 μg/mouse) showed that compound (**3**) protected animals from infection caused by herpes simplex virus, the greatest effect being observed when the preparation was administered 24 h before the virus.

The results obtained have served as a basis for the development from compound (**3**) of an antiherpetic agent — the salve Megosin 3% [17], approved for medical use on adults (genital herpes, simple vesicular lichen, herpes zoster). Antiviral activityagainst herpes simplex (type II) virus has been detected for peracylated GP nitriles [18], which possess a lower toxicity than GP [19].

Ever more frequently in recent years, reports have been appearing of the antiviral and immunomodulating actions of one compound or another connected with its capacity for inducing interferon. Knowing the broad spectrum of antiviral activity and high immunotropicity of GP itself and its derivatives, it is possible to assume that they possess interferon-inducing capability. In actual fact, GP has proved to be the first substance of plant origin for which a capability of inducing the formation of interferon in cell cultures has been shown [20].

Numerous experiments have shown that the introduction of GP and some of its derivatives into animal organisms, just like the treament of cell cultures with these preparations, is accompanied by the formation of interferon and the development of antiviral resistance [21, 22]. The results of a study of the interferon-inducing activities of a number of GP derivatives have revealed, as in the case of the study of antiviral activities, that compound (**3**) and Batriden are the most active. When compound (**3**) was used in a dose of 50 μg/g, the production of interferon after 24 h amounted to 320 IU/ml [21], while in the case of Batriden the same amount of interferon was formed on the use of a dose of 100 μg/g. Compounds (**1**), (**2**), and (**5-11**) in doses of 25-50 μg/g induced only 16-128 IU/ml [23].

The results of a study of the interferon-inducing activities of GP derivatives substituted in the hydroxy groups revealed the same characterisic features as were established in the investigation of the antiviral and immunomodulating activities: substitution of the hydroxy groups lowers the capability of inducing interferon, the level of which depends on the nature of a substituent introduced into the aldehyde groups [24, 25].

The high protective action of GP and its derivatives for such interferon-sensitive viruses as the vesicular stomatitis virus, Sindbis virus, and West Nile fever virus permit the assertion that their antiviral effect is determined to a considerable degree by their capacity for inducing interferon in the organism [26].

EXPERIMENTAL

The UV spectra of the substances were taken on a SF-26 spectrophotometer at a concentration of 0.002% and a cell thickness of 1 cm. TLC was conducted on Silufol UV-254 plates using the acetone–toluene (4:6) system.

The di-, tetra-, and hexamethyl ethers were obtained by the method of Adams et al. [27].

Compounds (**1**), (**2**), and (**4**)-(**11**) were obtained by the method of [28].

Disodium Salt of bis-2,2′-{[(7,7′, 8,8′-tetrahydro-1,1′,6,6′-tetrahydroxy-5,5′-diisopropyl-3,3′-dimethyl-7,7′-dioxo)-2,2′-binaphthyl]-8,8′-methylenimino}ethanesulfonic Acid (3). To a solution of 15.0 g (0.37 mole) of caustic soda in 375 ml of absolute ethyl alcohol was added 47.2 g (0.37 mole) of β-aminoethanesulfonic acid, and the mixture was heated in the water bath at 80°C. The resulting solution was treated with a solution of 65.0 g (0.12 mole) of GP in absolute ethyl alcohol, and the reaction mixture was heated in a current of nitrogen at 60°C for 3 h. The precipitate that had formed was filtered off and was washed with ethyl alcohol, and diethyl ether, and dried. This gave an amorphous yellow powder with a greenish tinge: mp >360°C, R_f 0.35. UV spectrum: λ_{max} (acetone–water (3:1)), nm: 384, 404 (log ε 4.12, 4.61).

Found, %: N 3.75; $C_{34}H_{38}N_2O_{12}S_2Na_2$. Calculated, %: N 3.46.

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