COMPLEX COMPOUNDS OF GLYCYRRHIZIC ACID WITH ANTIMICROBIAL DRUGS

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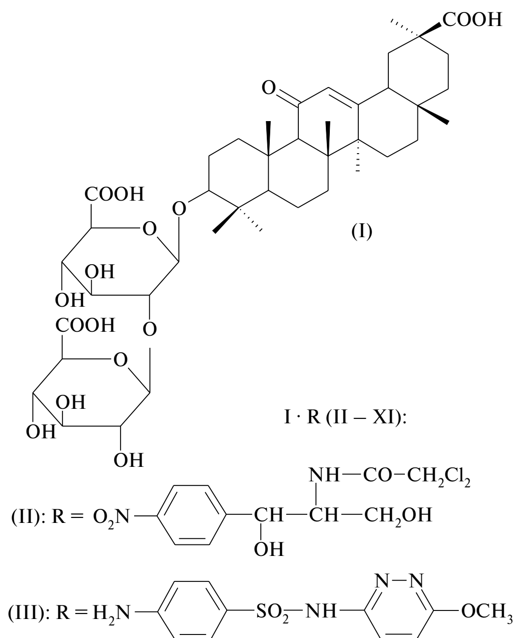
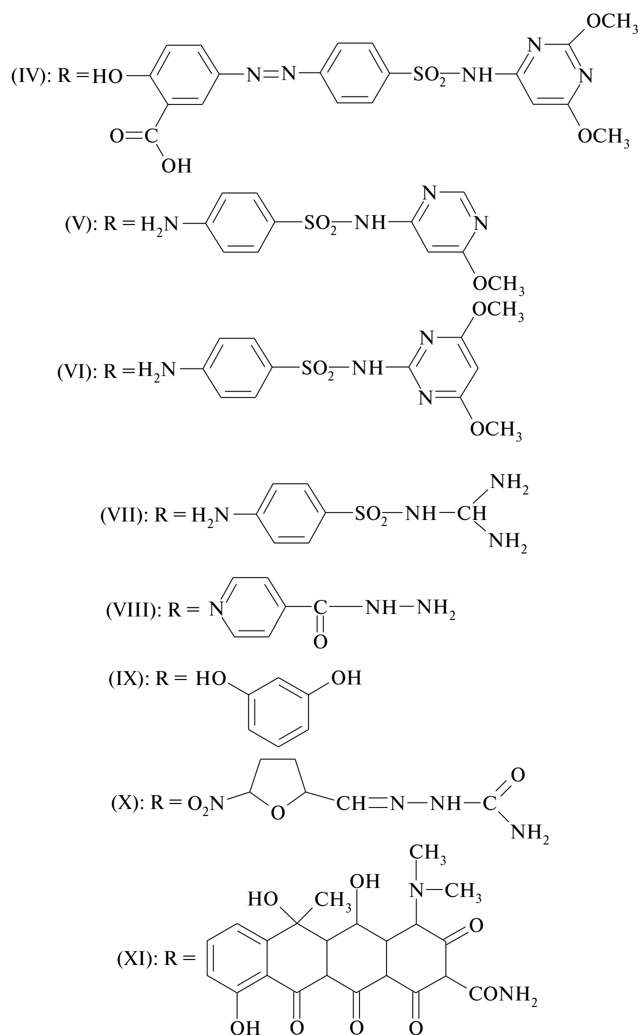
A promising direction in the development of new effective drugs is the synthesis of molecular complexes, for example, with cyclodextrins, which can protect parent substances from premature metabolic decay and provide for their transmembrane transport [1]. Previously, we suggested using 18β-glycyrrhizic acid (GA, I) as a complex-forming agent for the synthesis of new transport forms of the well-known drugs (nonsteroidal antiinflammatory agents, prostaglandins, uracils, etc.) and other biologically active substances [2 – 7].

In continuation of our work in the R&D of new GA-based preparations, we have synthesized a series of new molecular 1 : 1 complexes (II – XI) between antimicrobial drugs and GA (92 ± 2 %) [8]





and studied their acute toxicity, antimicrobial activity, and immunomodulant properties.

Complex compounds II – XI between GA and the well-known antimicrobial drugs (levomycetin, sulfapyridazine, salazodimethoxine, sulfamonomethoxine, sulfadimezine,

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TABLE 1. The Properties of Complex Compounds of Glycyrrhizic Acid with Antimicrobial Drugs

Compound (appearance)	$[\alpha]_D^{20}$	IR spectrum: ν_{max}, cm^{-1}	UV spectrum: λ_{max}, nm (lg ε) (solvent)	Empirical formula; yield, %/ *T*, °C
II (amorphous powder of yellowish color)	+ 34° (*c* 0.04; MeOH)	3400 (OH); 1642 (COOH),1640 (C=O); 1520 (Ph)	257 (4.04) (50% EtOH)	$C_{42}H_{62}O_{16} \cdot C_{11}H_{12}N_2O_5Cl_2$ 90.3/22 °C
III (white powder)	+ 60° (*c* 0.02 dioxane)	3376 (OH); 3250 (NH_2); 1728 (COOH); 16.44 (C=O); 1592 (Ph); 1572, 1528, 1512 (NH, NH_2, –C=N–)	232 (4.46) 262 (4.56) (dioxane)	$C_{42}H_{62}O_{16} \cdot C_{11}H_{12}N_4O_3S$ 91.2/50 °C
IV (amorphous powder of orange color)	+ 20° (*c* 0.02 dioxane)	3600 – 3200 (OH, NH); 1750 – 1700 (COOH); 1660 (C=O); 1620, 1590 (Ph, –C=N–, –N=N–)	250 (4.23) 354 (4.19) (dioxane)	$C_{42}H_{62}O_{16} \cdot C_{18}H_{17}N_5O_7S$ 91.3/50 °C
V (amorphous powder of cream color)	+ 30° (*c* 0.02 dioxane)	3600 – 3200 (OH, NH_2); 1730 (COOH); 1640 (C=O); 1600 (Ph); 1580 (NH), –C=N–	269 (4.27) (dioxane)	$C_{42}H_{62}O_{16} \cdot C_{11}H_{12}N_4O_3S$ 92.5/50 °C
VI (amorphous powder of cream color)	+ 47.5° (*c* 0.02 dioxane)	3346 (OH); 1736, 1710 (COOH); 1640 (C=O); 1596 (Ph); 1555 (NH, –C=N–)	232 (4.30) 262 (4.32) (dioxane)	$C_{42}H_{62}O_{16} \cdot C_{12}H_{16}N_4O_4S$ 92.0/50 °C
VII (amorphous powder of cream color)	+ 45° (*c* 0.02 dioxane)	3600 – 3200 (OH, NH_2); 1730 (COOH); 1650 (C=O); 1600 (Ph); 1550 (NH); 1520 (–C=NH–)	257 (4.3) (dioxane)	$C_{42}H_{62}O_{16} \cdot C_7H_{10}N_4O_2S$ 94.6/50 °C
VIII (amorphous powder of light-yellow color)	+ 50° (*c* 0.04; 50% EtOH)	3344 (OH); 1716 (COOH); 1656 (C=O); 1552 (NH_2, Py)	246 (4.26) (50% EtOH)	$C_{42}H_{62}O_{16} \cdot C_6H_7N_2O$ 92.5/50 –C
IX (amorphous powder of cream color)	+ 50° (*c* 0.02; dioxane)	3600 – 3200 (OH); 1728, 1700 (COOH); 1640 (C=O); 1600 (Ph)	244 (4.20) (dioxane)	$C_{42}H_{62}O_{16} \cdot C_6H_6O_2$ 92.0/50 °C
X (fine crystalline powder of yellow color)	+ 40° (*c* 0.01; MeOH)	3600 – 3200 (OH, NH_2); 1705 (COOH); 1660 (C=O); 1590 (NO_2, NH-, $CONH_2$); 1500 (Ph)	250 (4.50) 363 (4.28) (MeOH)	$C_{42}H_{62}O_{16} \cdot C_6H_{10}N_4O_4$ 92.7/50 °C
XI (amorphous powder of dark brown color)	– 15° (*c* 0.02; MeOH)	3360 (OH); 1724 (COOH); 1640 (C=O); 1616 (Ph); 1584 ($CONH_2$)	222 (4.45) 251 (4.76) (MeOH)	$C_{42}H_{62}O_{16} \cdot C_{22}H_{23}N_2O_8$ 91.5/50 °C

sulgin, isoniazid, resorcinol, furacilin, and oxytetracycline) were obtained by mixing components in a 1 : 1 molar ratio in a water – ethanol medium or dioxane at room temperature or at ~50°C.

The IR spectra of complexes II – XI exhibit a shift of the absorption peaks of OH and C=O groups by 10 – 20 cm^{-1} toward lower frequencies as compared to their positions in the spectra of initial glycosides. This fact confirms the formation of intermolecular hydrogen bonds between drug and GA molecules (Table 1). In the IR spectrum of complex III (GA – sulfapyridazine), the absorption peak of OH groups is shifted by 24 cm^{-1}, the peak of C=O groups by 22 cm^{-1}, and the peak of COOH by 12 cm^{-1}. The UV spectra of complexes containing aromatic chromophores display additional absorption maxima in the regions of 222, 232, and 262 nm (complexes III, VI, XI), 354 nm (IV), or 369 nm (X) (Table 1). The purity of the synthesized compounds was checked by TLC; the proposed compositions were confirmed by the results of elemental analyses (Table 1).

As can be seen from the results of biological tests presented in Table 2, the maximum percentage of mice (9 of survived 10) in the group of staphylococcal infection was observed for animals treated with complex II in a dose of 50 mg/kg (1/10 LD_{50}). In contrast, the fractions of animals that survived in the groups treated with GA or levomycetin alone were 1/10 and 3/10, respectively. The average life in the group of animals treated with complex II was also significantly longer than that in the other groups.

Analogous results were obtained in cases of infections caused by *Ps. aeruginosa*, *Pr. vulgaris*, and *E. coli*.

Investigations of the effect of complex II on the humoral immunity component showed that this preparation induces (like GA) an increase in the antibody-forming cell (AFC) production in the spleen (Table 3), in contrast to a decrease in this production caused by levomycetin. On the other hand, the GA – levomycetin complex (as well as levomycetin alone) enhances the delayed type hypersensitivity (DTH) response, while GA suppresses this response (Table 4).

Thus, the GA complex with levomycetin (complex II) increases the resistance of mice with respect to infections caused by *St. aureus*, *Ps. aeruginosa*, *Pr. vulgaris*, and *E. coli* to a greater extent than do levomycetin or GA alone. The complex stimulates both humoral and cell immunity components.

EXPERIMENTAL CHEMICAL PART

Equimolar complex of [beta]-glycyrrhizic acid with levomycetin (II). A mixture of 1.64 g (2 mmole) GA, 0.65 g (2 mmole) levomycetin, 100 ml of 96 % ethanol, and 25 ml of water was stirred at 20 – 22°C for 1 h (until complete dissolution of the components). Then, the solvent was evapo-

TABLE 2. Antimicrobial Properties of the Complex between Glycyrrhizic Acid and Levomycetin in Mice Studied on Various Infections

Compound	Dose, mg/kg	Number of animals	Number of survived animals			Average lifetime, days
			3 days	7 days	10 days	
Staphylococcus aureus						
II	50	10	10	9	9	9.6 ± 0.1*
Levomycetin	50	10	9	4	3	6.1 ± 0.7*
GA	50	10	8	2	1	4.8 ± 0.8*
Control	–	10	7	–	–	2.7 ± 0.8
Pseudomonas aeruginosa						
II	50	10	9	8	8	8.7 ± 0.2*
Levomycetin	50	10	8	3	2	5.4 ± 0.2*
GA	50	10	6	1	1	2.8 ± 0.6
Control	—	10	3	—	—	1.4 ± 0.5
Proteus vulgaris						
II	50	10	9	8	8	7.9 ± 0.2**
Levomycetin	50	10	9	5	4	5.3 ± 0.5*
GA	50	10	7	3	2	6.5 ± 0.5**
Control	—	10	6	—	—	3.2 ± 0.7**
Escherichia coli						
II	50	10	9	8	7	8.0 ± 0.3**
Levomycetin	50	10	9	3	3	4.3 ± 0.3*
GA	50	10	5	4	2	5.2 ± 0.5**
Control	—	10	7	—	—	3.0 ± 0.5**

* $p < 0.05$; ** $p < 0.01$ relative to control.

rated in vacuum and the residue was dried to constant weight. This yielded 2.06 g (90.3 %) of complex II (Table 1).

General method for the synthesis of equimolar complexes of β-glycyrrhizic acid with sulfanylamides (III – VII). To a solution of 1.64 g (2 mmole) of GA in 200 ml of dioxane was added 2 mmole of the corresponding sulfanylamide preparation (sulfapyridazine, salazodimethoxine, sulfamonomethoxine, sulfadimezine, or sulgin) and the mixture was stirred at 50°C for 1 h (until complete dissolution of the components). Then, the solution was filtered, the filtrate was evaporated in vacuum at 50°C, and the residue was dried to constant weight. The yields and properties of the target complexes are listed in Table 1.

General method for the synthesis of equimolar GA – drug complexes (VIII – X). To a solution of 1.64 g (2 mmole) of GA in 100 ml of 96 % ethanol was added 2 mmole of the corresponding drug (isoniazid, resorcinol, or furacilin) and the mixture was stirred at 50°C for 2 h (until complete dissolution of the components). Then, the solvent was evaporated in vacuum at 50°C, and the residue was dried to constant weight. The yields and properties of the target complexes are listed in Table 1.

Equimolar complex of [beta]-glycyrrhizic acid with oxytetracycline (XI). A mixture of 0.82 g (1 mmole) GA, 0.6 g (1 mmole) of oxytetracycline, 50 ml of 96 % ethanol, and 25 ml of water was stirred at room temperature for 2 h (until complete dissolution of the components). Then, the solvent was evaporated in vacuum and the residue was dried to constant weight. This yielded 1.3 g (91.5 %) of the target complex (Table 1).

EXPERIMENTAL PHARMACOLOGICAL PART

The antimicrobial properties of the equimolar GA – levomycetin complex (II) in comparison to GA and the drug alone were studied *in vivo* on white mice infected with

TABLE 3. Effect of the Complex of Glycyrrhizic Acid with Levomycetin on the Humoral Immunity Component in Mongrel Mice

Compound	Dose, mg/kg	Number of animals	Number of AFC in spleen
II	25	20	592.5 ± 40.0*
II	50	20	608.0 ± 36.0*
II	100	20	605.3 ± 52.5*
Levomycetin	50	20	140.0 ± 10.0**
GA	50	20	528.6 ± 27.0**
Control		20	235.0 ± 16.0

* $p < 0.05$; ** $p < 0.02$ relative to control.

TABLE 4. Effect of the Complex of Glycyrrhizic Acid with Levomycetin on the Cell Immunity Component (Delayed Type Hypersensitivity Response)

Compound	Dose, mg/kg	Number of animals	Test foot weight, g	Control foot weight, g
II	50	10	4.62 ± 0.11*	1.75 ± 0.11
Levomycetin	50	10	4.36 ± 0.32*	1.74 ± 0.21
GA	50	10	2.14 ± 0.01*	1.75 ± 0.23
Control		10	2.49 ± 0.02	1.75 ± 0.29

* $p < 0.05$ relative to control.

standard strains of *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*.

In every series of tests, the doses of daily-grown cultures of a given pathogenic microbe were titrated on a group of mice. The bacterial culture suspensions were intraperitoneally injected with 0.5 ml of physiological solution. The species of *St. aureus* were introduced in the amount of 2.5×10^9 microbial cells per 20 g body weight, *E. coli* was loaded to 1.5×10^6, *Pr. vulgaris* to 2.0×10^9, and *Ps. aeruginosa* to 2.5×10^9 microbial cells per 20 g body weight. In the control groups, these doses of pathogenic microbes led to a 50 % loss of animals within 3 – 5 days after inoculation.

Animals in the test groups were treated with the synthesized compounds in a dose of 1/10 LD_{50} (50 mg/kg, p.o.). On the fifth day, 2 h after the last treatment, the animals were inoculated with daily-grown microbe cultures as described above. The treatment with GA – drug complexes continued for the next 3 days. The state of animals in these groups was monitored for 10 days. The efficacy of the treatment was evaluated in terms of the percentage survival and lifetime of experimental animals.

Immunomodulant activity of the synthesized compounds. The effect of GA – drug complexes upon the humoral immunity response was studied on mongrel and CBA line mice weighing 18 – 22 g. The tests were performed using the Jerne – Nordin [9] method in the Cunningham – Czenberg modification [10]. The synthesized complexes were injected intraperitoneally in a single daily dose of 50 mg/kg over five days. The first injection was combined with the intraperitoneal immunization treatment. The test antigen was represented by goat erythrocytes in an optimum dose of 2×10^8. The humoral immunity response was evaluated on the 5th day by determining the number of AFCs in the spleen of immunized test animals. The test results obtained in each experimental series were compared to data for the control groups.

The cell immunity component was evaluated by the delayed-type hypersensitivity reaction to 2,4-dinitrofluorobenzene, monitored using a drop test for goat erythrocytes [11]. The DTH development was evaluated by comparing the model foot edema growth in animals of the control and test groups. The test compounds were introduced perorally in a dose of 1/100 LD_{50} over a period of five days beginning with the first day of sensitization.

REFERENCES

1. *Inclusion compounds*, *V. 3, Physical Properties and Applications*, S. L. Atwood, I. E. D. Davis, and D. D. NacNicol (eds.), Academic Press, London (1984).
2. G. A. Tolstikov, Yu. I. Murinov, L. A. Baltina, et al., *Khim.-Farm. Zh.*, **24**(8), 26 – 27 (1990).
3. G. A. Tolstikov, L. A. Baltina, Yu. I. Murinov, et al., *Khim.-Farm. Zh.*, **25**(2), 29 – 32 (1991).
4. G. A. Tolstikov, Yu. I. Murinov, L. A. Baltina, et al., *Khim.-Farm. Zh.*, **25**(3), 42 – 44 (1991).
5. G. A. Tolstikov, V. A. Myshkin, L. A. Baltina, et al., *Khim.-Farm. Zh.*, **30**(5), 36 – 38 (1996).
6. L. A. Baltina, R. M. Kondratenko, Kh. M. Nasyrov, et al., USSR Inventor’s Certificate No. 1616925; *Byull. Izobret.*, No. 48 (1990); *Ref. Zh. Khim.*, 240, 118P (1991).
7. V. N. Maistrenko, V. N. Gusakov, V. A. Rusakov, et al., *Dokl. Ross. Akad. Nauk*, **335**(3), 329 – 331 (1994).
8. L. A. Baltina, R. M. Kondratenko, S. R. Mustafina, et al., *Khim.-Farm. Zh.*, **35**(1), 38 – 41 (2001).
9. N. K. Jerne and E. A. Nordin, *Science*, **140**, 405 (1963).
10. A. J. Cunningham and A. Czenberg, *Immunology*, No. 2, 599 – 600 (1968).
11. L. A. Baltina, S. A. Ryzhova, E. V. Vasil’eva, et al., *Bioorg. Khim.*, **22**, 926 – 930 (1996).