SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME GOSSYPOL DERIVATIVES

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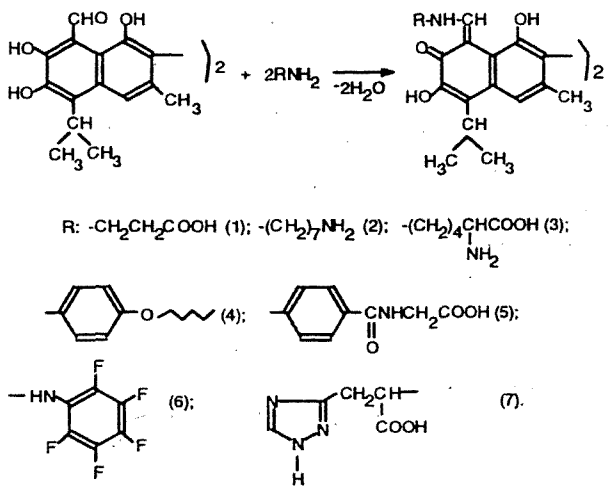
The establishment of a link between modified structures of known natural compounds and their physiological activity is basic for the creation of drugs with high efficiency and selective biological action. Thus, the synthesis and study of the biological activity of gossypol and its derivatives has revealed a number of characteristic features of the influence of chemical structure on biological action [1]. Gossypol derivatives obtained by substituting the carbonyl groups proved to be less toxic both for animals and for cell cultures, and the biological activity of these compounds was more pronounced, while derivatives with respect to the hydroxy group of the gossypol molecule possessed a lower activity [2].

In order to obtain new polyfunctional substances based on gossypol, we have synthesized a number of gossypol derivatives as shown in the scheme.



It has been reported previously [3, 4] that imino derivatives of gossypol undergo benzoid-quinoid tautomerism.

The existence of benzoid-quinoid tautomerism for the compounds that we had synthesized was studied by PMR and IR spectroscopies. The PMR spectra of the compounds synthesized, taken in $CDCl_3$ or Py-d_5 solution, contained the signals of gossypol itself [5] and also signals of the corresponding functional groups of the substances synthesized.

The PMR and IR spectra showed that for the alkylimines the predominating form in solution was the quinoid form, while for arylimines it was the benzoid form [6, 7]. The antiviral and anticholinesterase activities of the gossypol derivatives synthesized have been studied. The results of the study of their anticholinesterase activities are given in Table 1.

In a determination of the influence of the compounds obtained on the kinetics of the enzymatic hydrolysis of acetylcholine by the cholinesterases, the substances were divided into two classes. The first class (from 1 to 3) included compounds the R groups of which consisted of aliphatic fragments, while the second class (from 4 to 7) contained substances with aromatic and heterocyclic residues. With respect to their action on acetylcholinesterase (AChE) and butyrylcholinesterase

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TABLE 1. Anticholinesterase Activities of the Gossypol Compounds Synthesized.

Compound	pK_i		K_i(BuChE)
	AChE	BuChE	K_i(AChE)
1	2.12	2.06	1.146
2	2.32	2.28	1.083
3	2.43	2.18	1.784
4	2.09	2.02	1.160
5	2.95	2.77	1.545
6	2.48	2.29	1.545
7	2.92	2.34	3.750

(BuChE), all the substances were reversible inhibitors with a predominantly noncompetitive type of action. This shows that these substances do not interact with the active centers of cholinesterases. The structures of the substances including both aliphatic and aromatic radicals had no great influence on the inhibition of the activities of AChE and BuChE, although the efficiency of inhibition of AChE was somewhat higher than that of BuChE.

A change in the radical for representatives of the first class of compounds caused a change in their efficiency for AChE, which was not the case for BuChE. For the gossypol derivatives containing aromatic fragments, the effect of a change in R was of similar nature. For AChE and BuChE a substance with a conjugated carbamoyl group (compound **5**) was the weakest among the gossypol derivatives synthesized: it was five times less active than compound (**4**) for BuChE and 7.3 times for AChE. The selectivities of the action of compounds (**4**) and **5**) in relation to the two types of cholinesterases were, respectively, 0.86 and 0.64. It must be mentioned that the first members of both classes of compounds were the most effective inhibitors of AChE and BuChE.

The results of the experiments show that the compounds obtained enter into interaction with both types of cholinesterase. Their efficacy is apparently connected with a hydrophobic interaction of the voluminous gossypol molecule with sections of the enzymes not present in the active center. Such an interaction may cause conformational changes of the protein molecule of the enzyme, as a result of which the access of the substrate to the active center is hindered, and this causes a fall in the rate of hydrolysis of the substrate. According to the literature, a hydrophobic interaction [8, 9] with ligands is more characteristic for BuChE than for AChE. The fall in the activity of the enzymes under the influence of gossypol derivatives may be connected with a screening of the cholinesterase active centers by the more voluminous inhibitor molecule.

Thus, a comparison of the structures and anticholinesterase activities of the substances synthesized has shown that they are reversible inhibitors of cholinesterases, and their effect depends on the chemical nature of the radicals linked to the basic gossypol fragments.

We have studied the toxic effect of the preparations on the monolayer of a culture of chick embryo fibroplasts (FCEs) as an influence on the multiplication of the vesicular stomatitis virus (VSV). The treatment of a monolayer of a culture of CEF cells with various concentrations of the preparations showed a slight toxicity of them for this culture. After contact with the cells for 24 h, compound (**1**) showed the highest toxicity — 150-180 μg/ml. Other compounds were less toxic — 250-400 μg/ml.

The antiviral action of the preparations (used mainly in the form of suspensions because of their poor solubility in water or in dimethyl sulfoxide) appeared in concentrations 3-4 times lower than the toxic dose. Thus, for compound (**1**) the antiviral activity after preliminary treatment of a culture of CEFs for 24 h before the addition of the virus amounted to 40-45 μg/ml. The other compounds inhibited the infectious activity of the virus in a dose of 60-100 μg/ml. Simultaneous treatment of cell cultures with the preparation and with the virus lowered the infectious activity of the virus and protected the cells from cytodestructive action.

It must be mentioned that the dose of substance suppressing the infectious activity of the virus was higher than on preliminary treatment with the preparation for 24 h before the introduction of the virus, and amounted to 80-125 μg/ml. Compound (**7**) exhibited a comparatively high antiviral activity, suppressing the infectious activity of the virus in a dose of 50 μg/ml (the cytotoxicity for FCEs amounted to 350-400 μg/ml).

EXPERIMENTAL

The individuality of the compounds synthesized was checked by TLC on Silufol UV-254 plates in the following systems: 1) chloroform—ethanol (2:1), and 2) acetone—benzene (3:2). The revealing agent was iodine vapor. Melting points

were determined on a PTP instrument, and IR spectra were taken on a Specord 71-IR instrument in the range of 3700-750 nm^{-1} in KBr tablets. PMR spectra were taken on an XL-200 instrument (Varian) with a working frequency of 200 MHz in $CDCl_3$ or Py-D_5 solutions. Anticholinesterase activities were determined on a Specord-221 instrument in the KIN regime. The analyses of all the compounds corresponded to the calculated figures.

Synthesis of Gossypol Derivatives. 1. (1,1',6,6'-Tetrahydroxy-5,5'-diisopropyl-3,3'-dimethyl-7,7'-dioxo-2,2'-binaphthyl)-8,8'-dimethyleneiminopropionic Acid. With heating on the water bath, 0.5 g (0.001 mole) of gossypol was dissolved in 15 ml of ethyl alcohol, and a solution of 0.19 g (0.0022 mole) of 3-aminopropionic acid in 10 ml of acid was added to the clear solution obtained. The mixture was heated with continuous boiling of the solvent and with stirring for 3 h and was left overnight at room temperature. The yellow precipitate that had deposited was filtered off, and, to eliminate unchanged gossypol and 3-aminopropionic acid, the residue was washed successively with alcohol and ether. The substance was dried in a vacuum drying chest at 50-60°. Empirical formula $C_{36}H_{40}N_2O_{10}$, yield 49.6%, mp 197-199°C, R_f 0.54.

IR spectrum (cm^{-1}: .2840 (CH_2), 1690 (C=O), 1618 (CH−NH), 1432 (CH_2CO).

PMR spectrum ($CDCl_3$, ppm): 13.84-14.35 (br, 2H, NH), 10.21 (d, 2H, COOH), 7.91 (d, 2H, CH−NH), 4.55 (d, 2H, OH-6), 4.21 (s, 4H, CH_2), 3.86 (s, 2H, CH_2CO), 3.45 (d, 2H, OH-1), 2.86 (m, iso-C_3H_7), 2.35 (s, 4H, CH_2), 2.10 (dd, 6H, CH_3-3).

The following were obtained similarly:

Compound 2. $C_{44}H_{62}N_4O_6$, yield 98.7%, mp. >350°C, R_f 0.45. IR spectrum (cm^{-1}): 1615, 1608, 725. PMR spectrum ($CDCl_3$, ppm): 13.89-14.40 (br, 2H, NH), 7.94 (d, 2H, CH−NH), 4.25 (d, 4H, NH_2), 3.15 (s, 4H, CH_2), 2.64 (m, iso-C_3H_7), 1.5 (m, 10H, CH_2).

Compound 3. $C_{42}H_{54}N_4O_{10}$, yield 43.3%, mp, 247-248°C, R_f 0.40. IR spectrum (cm^{-1}): 1695, 1660, 1628. PMR spectrum ($CDCl_3$, ppm): 13.80-14.50 (br, 2H, NH), 10.8 (d, 2H, COOH), 7.93 (d, 2H, CH−NH), 3.85 (s, 4H, NH_2), 2.95 (d, 2H, $CHNH_2$), 1.7 (m, 6H, CH_2).

Compound 4. [10].

Compound 5. $C_{48}H_{46}N_4O_{12}$, yield 92.3%, mp >350°C, R_f 0.56. IR spectrum (cm^{-1}): 1685, 1680, 1650. PMR spectrum (Py-d_5, ppm): 11.2 (s, 2H, COOH), 9.81 (s, 2H, NHCO), 9.26 (d, 2H, CH=N), 7.8 (d, 4H, H-2), 7.46 (dd, 4H, H-3), 7.28 (d, Ar), 5.1 (d, 4H, CH_2).

Compound 6. [10]

Compound 7. $C_{40}H_{42}N_8O_{10}$, yield 39.2%, mp. 271-272°C, R_f 0.48. IR spectrum (cm^{-1}): 3490, 1705, 1646. PMR spectrum (Py-d_5, ppm): 12.1 (dd, 2H, COOH), 9.43 (d, 2H, CH=N), 7.94 (s, 2H, H-4), 6.1 (d, 2H, CH), 2.98 (d, 2H, NH), 1.85 (m, 4H, CH_2).

Biological Activities of the Products. Anticholinesterase Activities. To determine anticholinesterase activities, the sources of AChE and BuChE used were purified water-soluble preparations of human blood erythrocyte AChE and horse blood BuChE with specific activities of 1.2 and 9.6 U/mg produced by the Perm Scientific−Research Institute of Vaccines and Sera. The catalytic activities of the cholinesterases were determined by Ellman's colorimetric method at 25°C in 0.05 M phosphate buffer, pH 7.5 [11]. The cholinesterase substrate used was acetylthiocholine iodide from Chemapol (Czechoslovakia). The chromogen used in the experiments was 5,5-dithiobisnitrobenzoic acid from Koch-Light Lab. (United Kingdom). The efficiency of the reversible inhibitors was determined by Dixon's graphical method [12] and was expressed in the form $pK_i = \log K_i$. Each value given in Table 1 was obtained as the mean of five measurements.

The determination of toxicity was made by the method of Sadykov et al. [13]. The minimum concentration of preparations causing a 50% cytodestructive action (++) was regarded as the cytotoxic dose (CTD_{50}).

From the results obtained we determined the maximum acceptable concentration (MAC) of the preparations [14]. For many compounds, the MAC amounted to 95-100 μg/ml.

Determination of the Antiviral Activities of the Preparations. Various dilutions of the preparations were added to 2- to 3-day cultures of CEFs. After contact of the cells with the preparations for 30 minutes, the cells were washed and were infected with the virus, and fresh nutrient medium was added. After 24 h, when in a control experiment in which cells were treated with the virus alone a cytodestructive action of the virus was observed, the culture liquid was decanted off for a determination of the infectivity of the virus [13].

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