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WATER-SOLUBLE COMPLEX OF GOSSYPOL: PRODUCTION, STRUCTURE AND BIOLOGICAL ACTIVITY

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The article provides information on the preparation and study of the structural and biological properties of water-soluble complexes of gossypol with N-polyvinylpyrrolidone. The most suitable method for obtaining complexes of N-polyvinylpyrrolidone with gossypol derivatives turned out to be a method that represents the co-precipitation of the starting gossypol with a polymer in non-aqueous solvent systems with stirring. In this way, a water-soluble complex of gossypol with N-polyvinylpyrrolidone was obtained. The resulting complex is a brightly colored powdery substance, completely soluble in water. Its physicochemical parameters are characterized. The structure of the resulting complex was confirmed by spectroscopy (UV, IR).

In the course of the work, the relaxing effect of the PGN-02 polyphenol on the preparation of rat aorta was studied for the first time. Biological studies have shown that polyphenol PGN-02 has a strong relaxing effect and significantly reduces the force of contraction of the aorta caused by KCl (50 mM). It was shown that as a result of blocking the voltage-dependent L-type Ca^{2+} channels located in the plasmalemma, muscle relaxation occurred. The relaxing effect of PGN-02 polyphenol is associated with the blocking of L-type Ca^{2+} channels, as evidenced by the results of experiments conducted with the specific Ca^{2+} channel blocker verapamil.

Keywords: gossypol, modification, complex, N-PVP, UV-, IR-spectroscopy, biological activity, *in vitro*.

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Introduction

It is known that one of the main directions in the chemistry of natural and physiologically active compounds is the study of the relationship between the chemical structure and biological activity. These systematic studies make it possible to develop a scientific basis for the targeted synthesis of effective and specific biologically active substances [1–5]. The first known low molecular weight interferon inducer is gossypol (Fig. 1), the yellow pigment of cottonseed. Gossypol is a natural plant polyphenol that is unique not only in its structure, but also in its biological activity. Based on gossypol its and some of its derivatives, a number of antiviral drugs have been developed [6–8].

The antiviral activity of gossypol and its derivatives was first determined for viruses [1] that infect cells of the integumentary epithelium (herpes, keratoconjunctivitis, laryngotracheitis, etc.). Later, gossypol and its derivatives were tested against viruses that develop in blood cells, in particular against the human immunodeficiency virus (HIV). As it turned out, not only gossypol itself, but also its derivatives 1,1'-dideoxygossypol and transacylated nitrile (Fig. 2) inhibit HIV [9–12].

It has been reported [13, 14] that some gossypol derivatives have antitumor activity. Thus, the quinone gossypol, gossypolone (Fig. 3) and some stereoisomers of gossypol are used against melanoma.

The stereospecific effect of gossypol and gossypolone isomers was studied in two melanoma lines. The (-)-isomer proved to be more active in all cases [15–18].

Studies of the antifertility activity of gossypol, begun in the 1970s, are actively continuing to this day [19]. The feasibility of using gossypol as a male contraceptive is seen as promising.

The antifertility activity of gossypol derivatives such as apogossypol (Fig. 4) and gossypolone has been reported [19].

Gossypol modification products have been shown to be more potent contraceptives than gossypol [1].

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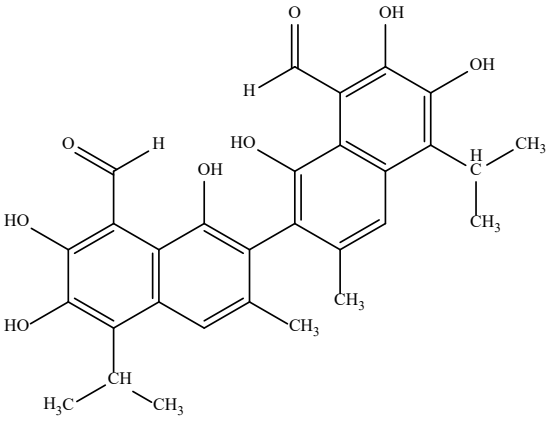

Fig. 1. The structural formula of gossypol

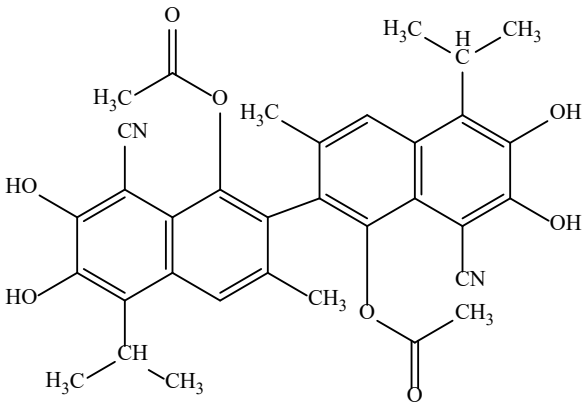

Fig. 2. The structural formula of transacylated nitrile 1,1'-dideoxygossypol

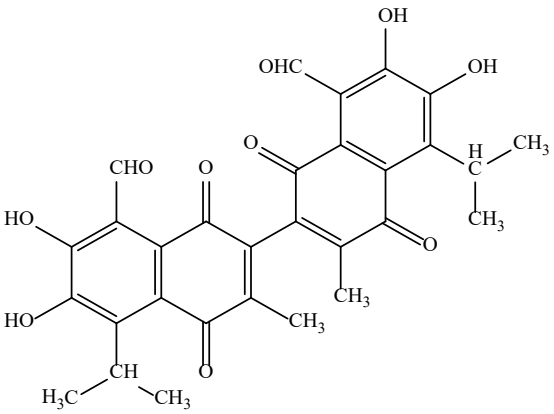

Fig. 3. The structural formula of gossypolone

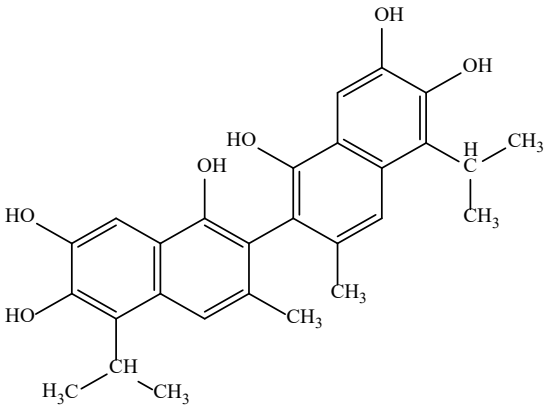

Fig. 4. The structural formula of apogossypol

However, some drawback of drugs created because of gossypol is the complete lack of solubility in water. One of the methods for increasing the solubility of substances is their chemical modification. One of such methods of giving them the ability to dissolve in water is complex formation. It should be noted that this reaction is carried out under "mild" conditions, under which the activity of the drug and the ease of its desorption in the body are preserved.

In connection with the above, the purpose of this study was to purify gossypol, obtain its water-soluble derivative, analyze its structure, and determine its specific biological activity.

Experimental part

Object of study. The study used a water-soluble complex of gossypol with N-PVP, obtained by chemical modification in the laboratory of low molecular weight biologically active compounds of the Institute of Bioorganic Chemistry named after academician A.S. Sadykov, Academy of Sciences of the Republic of Uzbekistan.

Research methods. To study the spectral characteristics, the following equipment was used: a Shimadzu UV-1280 UV spectrophotometer (Japan) (1×1 cuvette), an IRTracer-100 IR spectrometer (FTIR spectrometer). The individuality of the obtained complex was controlled by thin-layer chromatography on Silufol-UV-254 plates (Czechoslovakia). The melting point of the substance was determined in open capillaries in an air bath heated by an electric coil on a PTP TU 25-11-1144 instrument.

Preparation of water-soluble gossypol complex PGN-02. Extraction and purification of gossypol [20] was carried out according to. The complex was obtained according to the procedure [20–24]. To a solution of 1.0 g (0.01 mol) of gossypol in 50 ml of freshly distilled chloroform was added 9.0 g (0.005 mol) of N-polyvinylpyrrolidone and stirred for 24 hours at room temperature. Then the reaction mixture was distilled at 35-40 °C under vacuum to dryness. Yield: 8.0 g (80.0% of theory). The complex is named PGN-02 and is a powdery substance of light yellow color, odorless. Soluble in water, chloroform, dimethylformamide, practically insoluble in benzene, diethyl ether, petroleum ether. With concentrated sulfuric acid gives a bright crimson color.

Biological activity. The experiment was carried out on preparations of the aorta of outbred male rats (weighing 200–250 g). Euthanasia of experimental animals was carried out by displacement of the cervical vertebrae. The chest was opened; the aorta was surgically isolated and then placed in a perfused special chamber (5 ml) with saline Krebs-Henseleit (mM): NaCl 120.4; KCl 5; $NaHCO_3$ 15.5; NaH_2PO_4 1.2; $MgCl_2$ 1.2; $CaCl_2$ 2.5; $C_6H_{12}O_6$ 11.5, HEPES pH 7.4.

Discussion of results

Spectral Characteristics Analysis. A complex of water-soluble gossypol with N-PVP was obtained. Some physicochemical parameters of the obtained complex are determined and its proposed structure is presented, obtained because of the results of spectrometric studies.

During the interaction of N-PVP with gossypol, apparently, the inclusion of a molecularly dispersed substance into the molecule of the polymer matrix occurs due to the formation of hydrogen and coordination bonds between the functional groups of the reagents. This can be indirectly confirmed by the shifts in the main absorption maxima in the UV spectra of the complex with N-PVP compared to the initial substances (Table).

To confirm the structure of the water-soluble complex of gossypol with N-PVP, a comparative IR spectroscopic study of the compound PGN-02 with gossypol was carried out (Fig. 5). The infrared spectrum of PGN-02, pre-dried to constant weight, in the range from 400 to 4000 cm^{-1} is fully compatible with the absorption bands of the spectrum of the gossypol standard sample.

The absorption bands of stretching vibrations in the regions of 3420 and 2962 cm^{-1} in the IR spectrum of gossypol indicated the presence of dimers formed due to OH-OH bonds, and the inflection on the low-frequency slope at 3330 cm^{-1} indicated the presence of polymeric structures involving these hydrogen bonds.

The spectrum of N-PVP has a broad absorption band at 3413 cm^{-1} with an inflection at 2953 cm^{-1}, which occurs due to vibrations of the hydrate bond.

In the spectrum of the PGN-02 complex, an absorption band at a frequency of 2955 cm^{-1} is more pronounced than the same band in the spectrum of N-PVP itself, while the band of the carbonyl group does not shift. This suggests that some of the hygroscopic water normally present in N-PVP was more strongly bound to gossypol.

Based on the results of the above studies, we proposed the following structural formula for the resulting PGN-02 complex (Fig. 5).

Relaxant effect of PGN-02 polyphenol on rat aortic preparation [25].* In the experiment, it was found that the contraction of aortic preparations induced by KCl (50 mM) is significantly relaxed by the PGN-02 polyphenol. It was found that PGN-02 polyphenol at dose-dependent concentrations (5–100 μM) attenuated KCl-induced (50 mM) aortic contraction by 16.8±3.4% and 75.2±3.1% compared with the control (Fig. 7).

As shown in Figure 7, PGN-02 has a significant effect on the activity of voltage-gated Ca^{2+} channels induced by KCl (50 mM).

A subsequent experiment showed that the addition of PGN-02 polyphenol (100 μM) and Ca^{2+} ions to Krebs solution in the presence of 50 mM KCl significantly reduced the contraction of aortic preparations compared to the control (Fig. 8).

Some characteristics of gossypol with N-polyvinylpyrrolidone

Name of compounds	Melting temperature, °C	Rf* System 1, 2	UV spectrum, nm, λ_{max}, (log ε)	Basic oscillation frequencies in the IR spectrum, cm^{-1}	Yield, %
N-PVP	[20]			3412.98, 2952.99, 2325.64, 1651.50, 1492.86, 1460.71, 1422.17, 1373.79, 1285.88, 1074.06, 844.37, 734.59, 647.10, 571.43	[20]
Gossypol	178–81	0.39[1]	366 (4.27) chloroform	3419.11, 2962.22, 1709.02, 1611.30, 1578.51, 1439.82, 1379.29, 1338.64, 1266.48, 1123.71, 1051.79, 913.66, 841.12, 772.95, 699.79, 641.74, 609.67, 589.59, 569.78, 478.28, 423.80	83
PGN-02	169–71	0.47[2]	380 (4.12) water	3397.27, 2955.50, 1647.09, 1492.97, 1461.28, 1422.68, 1373.32, 1286.80, 844.51, 735.12, 647.08, 574.91	79.6

**System 1.* acetone : benzene : acetic acid (9 : 1 : 0.6); *System 2.* acetone.

*Biological studies were carried out by academicians of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan, Academician T.F. Aripov and his students.

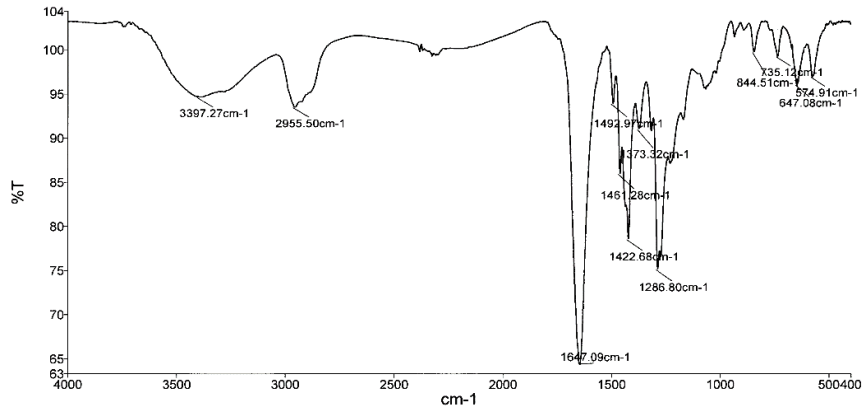

Fig. 5. IR spectrum PGN-02

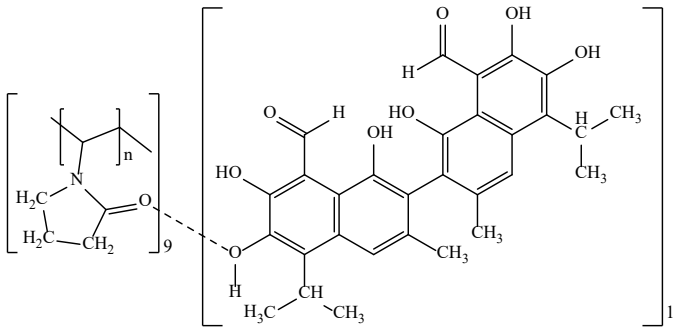

Fig. 6. The structural formula of PGN-02

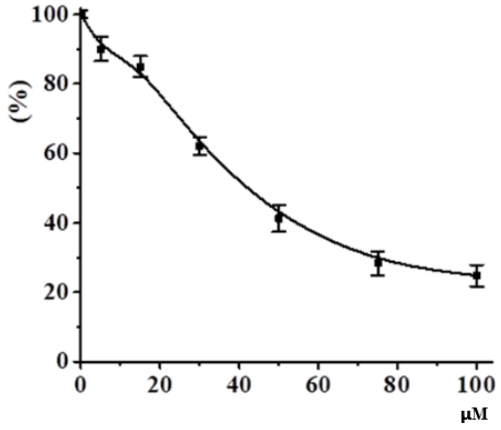

Fig. 7. Effect of PGN-02 polyphenol on contraction of rat aortic smooth muscle preparations induced by KCl (50 mM)

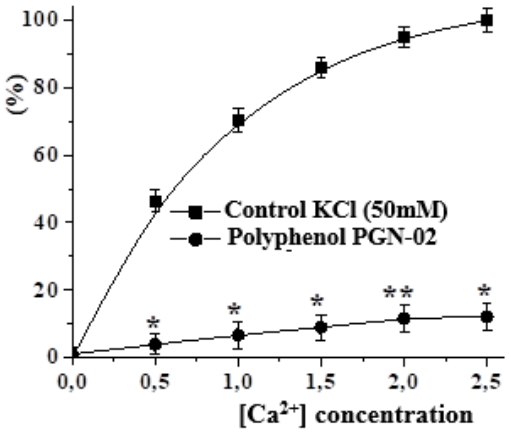

Fig. 8. Effect of [Ca^{2+}] concentration in the medium on the relaxant activity of PGN-02 polyphenol

In the next experiment, to prove that the relaxant effect of the studied polyphenol PGN-02 depends on the Ca^{2+} channels of the L-type, an analysis of the conductivity of the aortic preparation was performed with the simultaneous addition of a specific blocker verapamil. For this, a concentration of verapamil (0.1 μM) was used, causing a half-maximal contraction of aortic preparations caused by KCl (50 μM). It was found that under these conditions, the PGN-02 polyphenol further weakens the contractile activity of the aorta by 12.9±2.8%.

Conclusions

A water-soluble complex of gossypol with N-PVP was obtained by complexation. Its physicochemical, structural properties and biological activity have been studied. Using spectral analysis (UV, IR), the structure of the PGN-02 complex was analyzed and its general structural formula was proposed.

For the first time, the relaxant effect of PGN-02 polyphenol was studied on a preparation of rat aorta. The test polyphenol PGN-02 was found to have a significant effect on contraction of the aortic preparation induced by KCl (50 mM). It has been suggested that the weakening of the activity of the aortic preparation caused by the PGN-02 polyphenol is due to the blockade of voltage-dependent Ca^{2+} channels.

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Conflict of Interest

The authors of this work declare that they have no conflicts of interest.

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