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Isolation of Gossypol from the Bark of Cotton Roots

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Abstract:
In this research, following activities were carried out as the isolation of the gossypol substance which belong to the class of polyphenols, on the basis of the roots of cotton brought from various regions of the republic, purification, comparison of their products, determination of some physico-chemical constants, study of the structure using UV and IR spectroscopy methods.

Key words: *gossypol, cotton root, cotton variety, polyphenol, rotary evaporator, UCG (gossypol acetic acid), UV and IR spectra.*

Introduction. Polyphenols - natural compounds with biological activity, constitute the bulk of many higher plants. During the period of plant growth and development, they perform metabolic, regulatory and protective functions in many oxidation-reduction processes.

Polyphenols are the main raw material in the creation of broad-spectrum drugs due to their high biological activity and low toxicity. On their basis, antiviral, anti-inflammatory, antiulcer and antitumor drugs have been created.

Among polyphenolic compounds, gossypol is distinguished by its structure and unique biological activity. Mostly found in plants of the Gossypium family. Currently, some of its derivatives are used in medicine as an antiviral agent. The study of the activity of gossypol showed that even at a dose of 1 μm/ml, gossypol is active against various microviruses and herpes viruses [1-3].

Experimental part. The individuality of the synthesized compounds was controlled by thin layer chromatography on UV-254 Silufol plates. Developer - iodine vapor or 1% alcohol solution of $SnCl_4$.

IR absorption spectra of compounds were recorded in the range of 400-4000 cm^{-1} on an Avatar System 360 FT-IR spectrophotometer and a Protege 460 Magna-IR technology (Nicolet Instrument Corporation, USA) using samples in the form of tablets with KBr 7 mm in diameter and with a resolution of 4 cm^{-1}.

The bark of cotton roots isolated in a wet state was dried in air. The dried and crushed bark was placed in a flat-bottomed flask and extracted with diethyl ether. The ether extract was left overnight and filtered through 3-4 layers of gauze. The filtrate solution was concentrated on a rotary evaporator. To the concentrated extract was added 10 ml of glacial acetic acid and left while one day to precipitate. The formed precipitate was filtered off and dried in air at room temperature. The isolated crystals of yellow-lemon color were weighed.

Depending on the cotton variety, the amount of gossypol ranges from 0.02-6.64%, however, as a result of studies, it was found that, in general, the amount of gossypol can be

from 0.39 to 1.7%. The melting point of pure gossypol is 180-181 °C. Depending on the cotton variety, the amount of gossypol ranges from 0.02-6.64 % however, as a result of studies, it was found that, in general, the amount of gossypol can be from 0.39 to 1.7%. The melting point of pure gossypol is 180-181 °C.

Results and their discussion. The amount of gossypol in the seeds and other organs of cotton will change as the cotton grows and depends on the variety, climate, soil characteristics, water supply to the region, agrotechnical measures, the amount and composition of fertilizers applied to the soil.

It was found that the content of gossypol in the bark of cotton roots is 0.56-3.0 %. A smaller amount is contained in the leaves, stem bark, bite layers, seed husks, the layer enveloping the seeds, more in cotton flowers, mainly in the seed core. Ismailov A.I. [4] in his studies cited data on the areas of cotton cultivation. The amount of gossypol in the seed nucleus significantly depends on the areas where the cotton is grown. For example, the amount of gossypol in absolutely dry and fat-free seeds, the kernel of cotton variety 108-F, harvested per year, is as follows: in Turkmenistan - 1.36%, Uzbekistan - 1.98%, Azarbayzhan - 2.09%.

Nature does not create anything simple so? Any organ and physiological product, as well as any secretion, animals or plants is a product of adaptation to living conditions for the benefit of oneself. Consequently, gossypol also occupies an important place in the development of cotton.

Gossypol is an antioxidant in oxidation processes [5-6] prevents the multiplication of DNA and RNA-containing viruses, as well as arboviruses is a raw material for obtaining medicines with immunosuppressive and other biological effects and most importantly, on the basis of gossypol it is possible to create a large number of interferon inducers [7-9].

Isolation of gossypol remained a laborious and complex technological process [10]. In addition, gossypol obtained in this way may not meet medical requirements [11-14].

From the above data, it follows that gossypol is found in large quantities in cotton seeds and all previously conducted studies were aimed at isolating it from cotton seed oil.

We studied the isolation of gossypol from cotton roots, which were considered waste.

It is known that the regions of the republic are distinguished by their climate, soil, agrotechnical measures, research, varieties of cotton as mentioned above, the studies were aimed at comparing the yield and physico-chemical properties of gossypol isolated from the roots of cotton grown in different regions of the republic.

Cotton roots were collected in different regions (Khorezm, Syrdarya, Andijan, Jizzakh) after the cotton harvest (November-December).

The bark was removed from the peeled roots, crushed to a size of 0.6-0.8 cm and air dried. Dry crushed bark was weighed and extracted with diethyl ether in a flask, the filtered extracts were concentrated by distilling off the solvent and precipitated with 7-8 ml of glacial acetic acid. Precipitated lemon-yellow crystals of acetic acid gossypol (UKG-1) were filtered off and the yield was determined.

In order to obtain purified gossypol, the received UKG-1 was transferred to UKG-2.

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For this, a small amount of UKG-1 was dissolved in acetone and stirred, 2 times more glacial acetic acid was added, after that the solution was left for a day for precipitation. The filter cake was dried at room temperature (yield 90%).

According to this method, cotton roots collected in different regions were processed and the yield of gossypol was determined (Table 1).

Table 1

The amount of gossypol (40%) isolated from different varieties of cotton harvested in different areas (ω%)

№	Region	Variety	Mass of bark. g.	Ether volume ml.	UKG-1 gr *(ω%)*	UKG-2 gr *(ω%)*
1	Khorezm	Khorezm-127	100 gr	400 ml	0.6	0.54
2	Jizzakh	Gulbahor	100 gr	400 ml	0.89	0.8
3	Andijan	Andijan-27	100 gr	400 ml	0.80	0.72
4	Syrdaryn	Boyaut	100 gr	400 ml	0.87	0.78

Some physico-chemical constants of gossypol were determined, isolated from different varieties of cotton (Table-2). The color of the obtained substances changed from yellow-lemon to dark yellow. The solubility of the obtained substances in organic solvents: acetone, benzene, diethyl ether and hexane was determined. The results obtained showed good solubility of gossypol in acetone, diethyl ether and poor solubility in hexane.

Table 2

Some physico-chemical constants of gossypol obtained from different varieties of cotton.

№	Variety	$T_{boiling}$, ^0C	R_f		Output, *(ω%)*	Color
			hexane: acetone (3: 1)	benzene: acetone (5 : 1)		
1	Khorezm-127	187-185	0,33	0,71	0.50	Yellow-lemon
2	Gulbahor	186-187	0,29	0,79	0.74	Light yellow
3	Andijan-27	184-185	0,26	0,80	0.67	Dark yellow
4	Boyaut	185-187	0,31	0,70	0.72	Yellow
5	Sample (gossypol derived from oil)	184-186	0,31	0,77		Dark yellow

Systems: 1. Hexane: Acetone (3: 1); 2. Benzene: Acetone (5: 1)

It can be seen from the table that the yield of gossypol obtained from measles of cotton roots harvested in Jizzakh and Syrdarya regions is higher than in Khorezm and Andijan, this difference can be explained as follows.

The climate of Khorezm region is hot, the soil is saline, Andijan region is moderately hot, but high humidity; the climate of Jizzakh and Syrdarya regions differs from the Khorezm and Andijan regions, the humidity is lower than in Andijan, but higher than in Khorezm

region, therefore, it can be argued that climatic conditions and air humidity dramatically affect the development of cotton, which in turn affects the structure and the yield of substances in the composition of the plant.

Consequently, in order to increase the amount of gossypol in the roots of cotton, the soil must be fertile, non-saline and the plant must undergo agrotechnical treatment.

In addition to physico-chemical parameters, the structure of the obtained gossypols was studied using UV and IR spectra.

In the UV spectra of gossypol obtained from cotton roots, 3 maxima were observed: 235.92, 289.59 and 374.91 nm, which corresponded to the UV spectra of gossypol obtained earlier from cotton seed oil. According to the research data of Ziyaev Kh.L. [6], these at a maximum in the region of 240-245, 280 and 385-390 were observed in the spectra of a freshly prepared chloroform solution of gossypol.

IR - spectra of the isolated gossypol in the region of 3495.79 cm^{-1} , 3424 cm^{-1} corresponded to the absorption bands of stretching vibrations of hydroxyl groups; at 1614.06 cm^{-1}, absorption bands of vibrations of conjugated bonds and the carbonyl group were observed; 1503 cm^{-1} absorption bands corresponding to the aromatic nucleus; 1165.71 cm^{-1} vibrations of the isopropyl group at the C5 position; stretching vibrations of the CH_3 group in the range of 2851-2957 cm^{-1} and bending vibrations in the range of 1379.99-1441.74 cm^{-1}.

Conclusions

1. Gossypol was isolated from the roots of different types of cotton, harvested in different areas and a comparison of the yield of gossypol, as well as physical and chemical properties was carried out.

2. When isolating gossypol from the roots of cotton, it was established that long-term storage of measles from horses of cotton has a negative effect on the yield and quality of gossypol.

3. As a result of the research, it was determined that for the isolation of gossypol, the optimal harvesting of cotton roots is at the end of November and the first weeks of December.

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