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MOLECULAR COMPLEXES OF MONOAMMONIUM SALT OF GLYCYRRHIZIC ACID WITH SOME UREA AND THEIR ANTIBIOTIC ACTIVITY

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Abstract: *This article reports on obtaining the inclusion compounds of the monoammonium-glycyrrhizin salts with urea, thiourea and methylolthiourea. The compounds were obtained by the liquid phase way, and their structures were characterized by the UV- and IR-Spectroscopic methods. Proceeding from the spectral data, it was concluded that the complexation occurs through the interaction of polar groups of the components. The composition of the complexes was studied using the method of isomolar series. Equilibrium constants and changes in Gibbs free energy of the complexation process were also calculated. The biological activity of complex compounds was studied on the example of fungicidal activity of one of the most common pathogenic fungus Fusarium. The obtained compounds can be used as as fungicides and stimulants in agriculture.*

Keywords: *glycyrrhizic acid, thiourea, methylolthiourea, biological activity, fungicides*
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1. Introduction

Increasing the productivity of agricultural crops, their protection from pests, including from pathogenic fungi, is an urgent task to this day.

The chemical methods are often used to solve this problem. In particular, an increase in productivity is achieved through applying biostimulants obtained on the basis of synthetic preparations. One of the most effective ways to protect plants from pests is pre-sowing seed treatment with pesticides and various seed treatment agents. However, this application does not always provide the desired results. Recently, the use of their compositions with biologically active natural substances is proposed in order to increase the effectiveness of the applied pesticides [1].

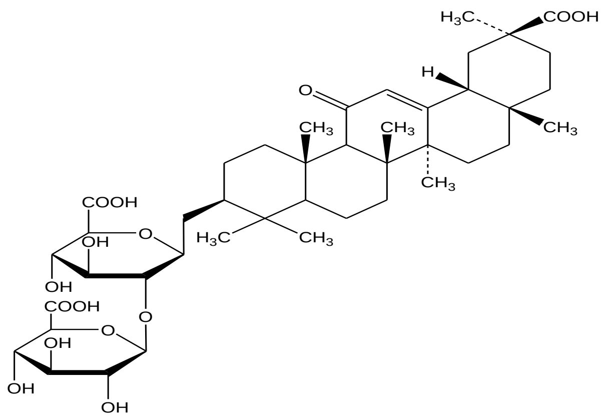
In this regard, triterpene glycoside - glycyrrhizic acid as an important component of licorice root is a very promising. This acid can form stable compounds (complexes) of the "host -guest" type due to its chemical structure and unique physicochemical properties. It should be added that the solubility and effectiveness of influence can be increased by this way; as well as the effective doses of most drugs and other biologically active substances can be reduced [2-4].

2. Methods and materials.

2.1. Reagents

As is known, urea (U) and thiourea (TU) are used in organic synthesis for obtaining the polymers, pesticides and a number of other organic compounds. In addition, urea and thiourea are utilized as organic fertilizers. Their methylol derivatives, on the basis of which various biological active substances have been obtained, are of considerable importance [5].

From a chemical point of view, urea and thiourea are very active reagents. Their molecules are extremely polar to have significant dipole moments. Thus, it has been experimentally established that thiourea has an ability to form stable aggregates with various, even non-polar organic substances [6]. Urea has similar properties too.



2.2. Obtaining complexes

According to the above data, we obtained the complex compounds of the monoammonium glycyrrhizin salts (MAGS) with urea, thiourea and methylol-thiourea in order to search out new highly effective biologically active substances for agriculture. The target compounds were obtained by preparative liquid-phase method in the ratio of reagents 1: 1, 2: 1 and 4: 1 in aqueous ethanol.

The nature of the intermolecular interaction was characterized by UV and IR spectroscopy. The ratio of the components has been analyzed by the methods of isomolar series, and some physicochemical parameters of the obtained compounds were identified as well (Table 1).

Table 1. Some physicochemical parameters of the MAGS-R complexes

R	Ratio of the reagents	Yield, %	Melting point, °C	R_f^*
Urea	1:1	86	186-187	0.68
	2:1	91	187-188	0.75
	4:1	90	180-182	0.94
Thiourea	1:1	91	190-191	0.53
	2:1	94	195-196	0.67
	4:1	90	200-201	0.70
Methylolthiourea	1:1	91	188-189	0.53
	2:1	93	195-196	0.40
	4:1	90	205-206	0.43

**The system of solvents: ethanol-chloroform (1:1)*

3. Results and their discussion.

3.1. ***UV spectroscopic analysis***

A change in the state of electrons and valence bonds in a molecule is reflected in the absorption of electromagnetic rays by them.

Thus, from comparison of UV spectra of MAGS and complexes, the hypsochromic shift of the absorption maximum of the aglycone glycoside group conjugated with the C=O double bond (Table 2) is observed. This means that this group is involved in the formation of the complex.

3.2. ***IR spectroscopic analysis***

When analyzing the vibrational spectra of MAGS and the obtained complexes, it is obvious that the stretching vibrations of the associated –OH groups of MAGS form a broad absorption band in the spectral region at 3500-3204 cm^{-1}; while the stretching vibrations of –CH_3, –CH_2- groups are apparent in the form of low-intensity bands at 2930-2874 cm^{-1}. The absorption bands specific to the stretching vibrations of the carbonyl groups of the carbohydrate part unique to the glycoside appear at 1722 cm^{-1} and the absorption bands specific to the stretching vibrations of the carbonyl group belonging to the aglycone was apparent at 1656 cm^{-1}. Vibrations of the carbonyl group of carboxylic acid ions and the deformation vibrations of NH_4^+ appear in the form of absorption bands of average intensity close to 1590 cm^{-1} and at 1387 cm^{-1}, respectively.

Table 2. UV and IR spectral data of MAGS-R complexes

R	The ratio of the reagents	UV spectrum, nm, λ_{max} (lgε)	IR spectrum, ν, cm^{-1}	
			OH, NH	>C=O
Urea	1:1	255 (4.2)	3404	1723
	2:1	253 (4.1)	3397	1720
	4:1	253 (4.1)	3375	1715
Thiourea	1:1	240 (4.2)	3381	1712
	2:1	240 (4.3)	3394	1718
	4:1	245 (4.2)	3379	1714
Methylolthiourea	1:1	240 (4.1)	3368	1714
	2:1	254 (4.1)	3382	1719
	4:1	253 (4.2)	3392	1723

3.3 Isomolar series method

The compositions of the obtained complexes were analyzed by the method of isomolar series (Fig. 1) This method is based on determining the ratio of the isomolar concentrations of the reacting substances corresponding to the maximum yield of the resulting complex compound. The dependency graph of the yield of the complex on the solution composition is characterized by an extreme point [7].

The optical densities of solutions of the isomolar series were determined in a phosphate buffer medium at these wavelength values: 259 nm for the MAGS-U complex, 254 nm for the MAGS-TU complex, and 252 nm for the MAGS-MTU complex. The stability constant K_S of the complex was calculated by the Babko method [8] according to the optical density (Fig.2).

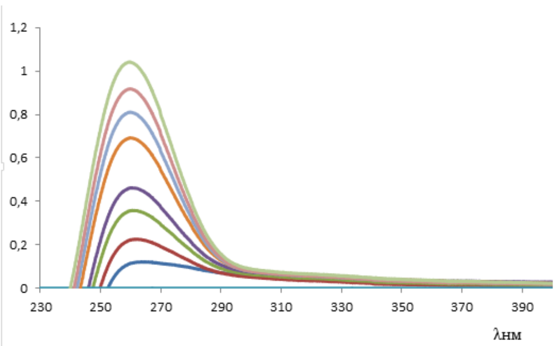


Fig. 1. The absorption curves of the isomolar solution series of the complex c = 10^{-4} M; pH = 7.2 (MAGS-U).

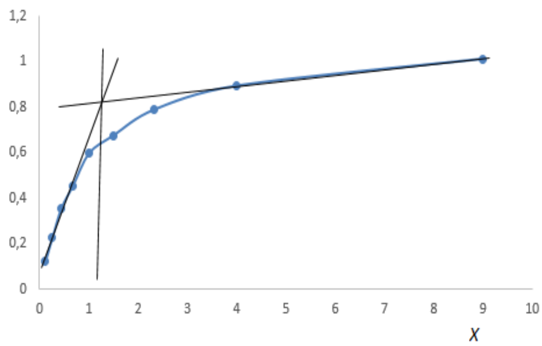


Fig. 2. The dependence of the optical density on the ratio of components in the isomolar series: λ = 259 nm, c = 10^{-4} M; pH =7.2 (MAGS-U).

According to the following equation, the change in Gibbs energy of the complexation process was revealed from the obtained K_S value:

$$\Delta G = -RT\ln K_S$$

The analogical experiments were performed with the MAGS-TU and MAGS-MTU complexes also. The obtained data are summarized in Table 3.

Table 3. K_S and ΔG values of the MAGS complexes

№	Complexes	Ratio	Isosbestic point, nm	K_S, M^{-1}	Δ*G*, kJ/mol
1	MAGS-U	1:1	>300	$3{,}4\cdot10^4$	$-2.8\cdot10^5$
2	MAGS -TU	2:1	235	$8{,}35\cdot10^6$	$-3.97\cdot10^4$
3	MAGS -MTU	1:1	242	$7{,}2\cdot10^5$	$-3.3\cdot10^4$

As is seen from the determined values of K_S and ΔG, the complex obtained with thiourea proved more stable. This can be explained by the greater electron-donating ability of the sulfur atom. The smallest value of the change in Gibbs energy during the MAGS-U complexation shows that urea is more reactive than thiourea.

4. Assessment of antibiotic activity.

As is known, pathogenic fungi damage greatly agriculture, in particular grain crops. GA (glycyrrhizic acid) and its derivatives have a wide spectrum of biological activity, especially, they have antibiotic activity in comparison to pathogenic fungi. The preparations were registered all over the world to contain components of licorice roots, including GA with about 1800 nominations. However, their fungicidal activity was poorly investigated.

It should be noted that certain studies are being carried out in this direction; these studies show the promising nature of GA and its derivatives as a basis for obtaining new effective biologically active compounds. Thus, the compositions of tebuconazole (TBA) with glycyrrhizic acid and its sodium salt significantly increase the penetration of TBA into the internal volume of the processed grain, which contributes to the recovery from seeds of soft spring wheat and spring barley from infection; also, they reduce the development and spread of common root rot at the first stages of organogenesis of spring wheat and spring barley [1].

A complex compound of MAGS with salicylic acid (SA) was proposed as antifungal compound for *Verticillium dahliae* Klebhan - the active substance of verticillium wilt. It found that this compound ensured the resistance of cotton to stress factors, stimulated its growth and promoted the maturation of cotton fiber. The effective fungicidal activity of the complex compound with copper obtained on the basis of technical GA was also noted. This compound has a fungicidal effect against the cereal rust pathogen. The composition of copper- and cobalt-diglycyrrhizinates in the experiments raised the resistance of wheat to fungal diseases, and also stimulated the plant development. The complex compound of MAGS with indolyl butyric acid was presented as a phytohormonal preparation [9-11]. The fungicidal activity of copper diglycyrrhizinate against some types of pathogenic fungi and rust pathogens was indicated in [12] as well.

From abovementioned data, we studed the antibiotic (fungicidal) activity of the obtained MAGS compounds with urea and thiourea in vitro (under laboratory conditions). The objects of study were pathogenic fungi of the *Fusarium* family - *F.culmorum, F.solani, F.poae, F.graminarium* and *F.oxsporium*, selected from the Collection of the Institute of Genetics and Experimental Biology of Plants of Uzbekistan Academy of Sciences; as well as isolated from infected organs of *Triticum aestivum*. The culture tests of the most common pathogenic mycomycetes prevailing in Uzbekistan were used in studies.

The antibiotic activity of MAGS complexes was studied by comparing the diameters of zones of incubation growth in nutrient medium [13-15]. The drug P-4 permitted for use in Uzbekistan was used as a control drug. Observations of changes in development zones continued for 7 days, measurement of zones was carried out on the 5th and 7th days of observation.

Table 4. Influence of the obtained complexes of MAGS on the development of pathogenic fungi

№	Fungus	Fungicidal and fungistatic zones of preparations, d, mm			
		Control	1	Control	2
1	*F. culmorum*	21.6**	9.4*	14.6**	26.7*
2	*F. graminearum*	15.4*	4.4**	-	12.5**
3	*F. oxysporum*	-	8.4*	-	11.5*
4	*F. poae*	-	-	-	6.2**
5	*F. solani*	35.4*	10,7*	24,7**	27.8**

Notes: 1-MAGS-U; 2-MAGS-TU, control - P-4.
"-"lack of influence.
**zone of fungicidal action - the width of the zone of complete suppressing the development of the analyzed microorganisms (fungi), mm;*
***zone of fungistatic action - the width of the zone of partial or strong suppressing the development of the analyzed microorganisms (fungi), mm.*

The results of the experiments showed that the analyzed drugs have no antagonistic effect against *F.poae*. However, these drugs in the controlled variants had a suppressive effect against pathogenic fungi *F.culmorum*, *F.graminearum*, *F.oxysporum* and *F.solani*.

The data obtained on the determination of the biological activity of complexes of MAGS with urea and thiourea showed the possibility of using these compounds in the development of new, effective means of protecting plants from pathogenic fungi.

5. Experimental part

5.1. Used equipment

UV and IR spectra were recorded on Shimadzu-1280 and Cary 60 UV-Vis spectrophotometers in quartz cuvettes with a thick of 10 mm and on an IR Fourier spectrometer IRTracer-100 (Shimadzu, Japan), respectively. The starting MAGS and methylolthiourea were obtained by known methods [2 (p. 279), 16].

***5.2. Obtaining complex MAGS-U* (1:1).**

1.68 g (0.002 mol) of MAGS was dissolved in 100 ml of 50% ethanol (v/v) with intensive stirring. 0.12 g (0.002 mol) of urea dissolved in 25 ml of the same solvent was added to this solution. The resulting mixture was stirred with a magnetic stirrer for 4-5 hours at 40-50^{0}C. After that, the alcohol was distilled off on a rotary evaporator; the residue was dehydrated by freeze drying way. The obtained complex was pale-yellow amorphous substance.

The complexes MAGS-TU, MAGS-MTU were obtained in a similar way in appropriate molar ratios.

***Obtaining MAGS-U* (2:1) complex.** 1.68 g (0.002 mol) of MAGS was dissolved in 100 ml of 50% ethanol (v/v) with intensive stirring. Also, 0.12 g (0.002 mol) of urea dissolved in 25 ml of the same solvent was added to this solution. The resulting mixture was stirred with a magnetic stirrer for 4-5 hours at 40-50^{0}C. After that, the alcohol was distilled off on a rotary evaporator; the residue was dehydrated by freeze drying way. Note that MAGS-TU complexes are yellowish needle crystals.

The MAGS-TU, MAGS-MTU complexes were obtained in a similar way in 2:1 molar ratios.

***Obtaining MAGS-U* (4:1) complex.** 3.36 g (0.004 mol) of MAGS was dissolved in 100 ml of 50% ethanol (v/v) with intensive stirring. 0.06 g (0.001 mol) of urea dissolved in 25 ml of the same solvent was added to this solution. The resulting mixture was stirred with a magnetic stirrer for 4-5 hours at 40-50^{0}C. After that, the alcohol was distilled off on a rotary evaporator; the residue was dehydrated by freeze drying way. Complexes obtained are yellowish needle crystals.

It should be added that the MAGS-TU, MAGS-MTU complexes were obtained in a similar way in 4:1 molar ratios.

Yields of the complexes, the melting temperatures and R_f value, as well as IR- and UV-spectral data are given in Tables 2 and 3.

5.3. Growing the culture tests.

The samples of fungi were grown in artificial chambers with a temperature of +25 +26°C in KDA and Czapek nutrient medium. During the growing process the contaminated samples were removed. 1 g of the test substances was added into the nutrient medium placed in Petri dish and evenly leveled with a spatula. Then, 4 holes were made in each dish. A control preparation (P-4) was added to 2 holes in each dish, the remaining 2 holes were loaded with a culture of pathogenic fungi in a liquid state.

6. Conclusions

Thus, molecular complexes of MAGS with urea, thiourea, and methylolthiourea were obtained in molecular ratios of 1:1, 2:1, and 4:1. The molecular structure and composition of the complexes were analyzed by UV and IR spectroscopy, as well as by means of isomolar series. On the basis of spectral data, it revealed that molecular complexes are formed due to intermolecular dipole-dipole bonds. The fungistatic activity of the obtained complexes revealed in experiments on culture tests of pathogenic fungi *Fusarium*. These compounds can be used in the development of new fungicides and stimulants for agriculture.

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МОЛЕКУЛЯРНЫЕ КОМПЛЕКСЫ МОНОАММОНИЙНОЙ СОЛИ ГЛИЦИРРИЗИНОВОЙ КИСЛОТЫ С НЕКОТОРЫМИ МОЧЕВИНАМИ И ИХ АНТИБИОТИЧЕСКАЯ АКТИВНОСТЬ

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В данной статье сообщается о получении соединений включения моноаммонийной соли глицирризиновой кислоты с мочевиной, тиомочевиной и метилолтиомочевиной, Соединения получены жидкофазным способом, их строение охарактеризованы УФ- и ИК-спектроскопическими методами. На основе спектральных данных сделан вывод о том, что комплексообразование осуществляется за счет взаимодействия полярных групп компонентов. Состав комплексов исследован с помощью метода изомолярных серий. Также рассчитаны константы равновесия и изменение свободной энергии Гиббса процесса комплексообразования. Биологическая активность комплексных соединений изучена на примере фунгицидной активности в отношении некоторых наиболее часто встречающихся патогенных грибов Fusarium. Полученные соединения могут использованы в качестве фунгицидов и стимуляторов в сельском хозяйстве.

Ключевые слова: *глицирризиновая кислота, мочевина, тиомочевина, метилолтиомочевина, биологическая активность, фунгицид.*

QLİSİRRİZİK TURŞUSUNUN MONOAMMONİUM DUZUNUN BƏZİ KARBAMİDLƏRLƏ MOLEKULYAR KOMPLEKSLƏRİ VƏ ONLARIN ANTİBIOTİK AKTİVLİYİ

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Bu məqalədə qlisirrizik turşusunun monoammonium duzunun karbamid, tiokarbamid və metiloltiokarbamid ilə daxilolma birləşmələrinin hazırlanması haqqında məlumat verilir. Birləşmələrin strukturu UF və İQ spektroskopik üsullarla xarakterizə edilmişdir. Spektral məlumatlara əsasən, kompleksləşmə komponentlərin polyar qruplarının qarşılıqlı təsiri nəticəsində baş verir. Komplekslərin tərkibi izomolyar seriya üsulu ilə tədqiq edilmişdir. Kompleksəmələgəlmə prosesinin tarazlıq sabitləri və Gibbs sərbəst enerjisinin dəyişməsi də hesablanmışdır. Birləşmələrin funqisid aktivliyi ən çox yayılmış Fusariumun patogen göbələklərinə qarşı tədqiq edilmişdir. Alınan birləşmələr kənd təsərrüfatında funqisid və stimulant kimi istifadə oluna bilər.

Açar sözlər: *glisirrizik turşusu, karbamid, tiokarbamid, metiloltiokarbamid, funqisid*