ACADEMICIA

ISSN: 2249-7137 Vol. 11, Issue 9, September 2021 Impact Factor: SJIF 2021 = 7.492

ACADEMICIA
An International Multidisciplinary Research Journal
(Double Blind Refereed & Peer Reviewed Journal)

DOI: 10.5958/2249-7137.2021.02025.5

THE MOLECULAR COMPLEXES OF THE MONOAMMONIUM SALT OF GLYCYRRHIZIC ACID WITH UREA, THIOUREA, METHYLOLTHIOUREA AND THEIR FUNGICIDAL ACTIVITY

Yusup Tojimamatovich Isaev*; Ibragim Raxmanovich Askarov*; Sandjar Ashiralievich Rustamov*; Egamberdiev Doston Usmondjon ugli*; Xabibjon Xojibekovich Kushiev**

* Department of Chemistry,
Andijan State University, Andijan, UZBEKISTAN

**Department of Biology,
Gulistan State University, Gulistan, UZBEKISTAN
Email id: yusufjon_67@inbox.ru

ABSTRACT

This article reports on the preparation of inclusion compounds of the monoammonium salt of glycyrrhizic acid with urea, thiourea and methylolthiourea. Based on the spectral data, it was concluded that complexation occurs due to the interaction of polar groups of the components. The composition of the complexes was studied using the isomolar series method. Equilibrium constants and the change in the Gibbs free energy of the complexation process were also calculated. The biological activity of complex compounds has been studied using the example of fungicidal activity against some of the most common pathogenic fungi Fusarium. The obtained compounds can be used as stimulants in agriculture.

KEYWORDS: *Pathogenic Fungi, Stimulants In Agriculture, Crops, Highly Effective Biologically Active Substances.*

INTRODUCTION

Increasing the productivity of agricultural crops, their protection from pests, including pathogenic fungi, is an urgent task to this day. To solve this problem, chemical methods are often used. In particular, an increase in productivity is achieved by the use of biostimulants obtained based on synthetic preparations. One of the most effective ways to protect plants from pests is the pre-sowing treatment of seeds with pesticides and various dressing agents. However, this practice does not always give the desired results. Recently, in order to increase the effectiveness of the applied pesticides, it is proposed to use their compositions with biologically active natural substances [1].

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

MATERIALS AND METHODS

As you know, urea (U) and thiourea (TU) are used in organic synthesis in the preparation of polymers, pesticides and a number of other organic compounds.

In addition, urea and thiourea are used as organic fertilizers. Of no small importance are their methylol derivatives, on the basis of which various biologically active substances have been obtained [5].

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

Urea has similar properties. Based on the above data, to search for new highly effective biologically active substances for agriculture, we have obtained complex compounds of the monoammonium salt of HA (MASGK) with urea, thiourea and methylol-thiourea. The target compounds were obtained by preparative liquid phase 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 was analyzed by isomolar series methods, and some physicochemical parameters of the obtained compounds were determined (Table 1).

TABLE 1. SOME PHYSICOCHEMICAL PARAMETERS OF THE MASGK-R COMPLEXES

R	Reagent ratio	Output %	M.p. °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

*** Solvent system: ethanol-chloroform (1: 1)**

RESULTS AND DISCUSSION

A change in the state of electrons and valence bonds in a molecule is reflected in the absorption of electromagnetic rays by them. Thus, when comparing the UV spectra of MASGA and complexes, a hypsochromic shift of the absorption maximum of the aglycone glycoside group conjugated with the C = O double bond is observed (Table 2). This suggests that this group contributes to the formation of the complex. When analyzing the vibrational spectra of MASGA and the obtained complexes, it can be seen that the stretching vibrations of the associated –OH groups of MASGA form a broad absorption band in the spectral region at 3500-3204 cm^{-1}, the stretching vibrations of – CH_3, -CH_2- groups appear as low-intensity bands at 2930-2874 cm^{-1}. The absorption band characteristic of the stretching vibrations of the carbonyl groups of the carbohydrate moiety of the glycoside appears at 1722 cm^{-1}, the carbonyl group of the aglycone at 1656 cm^{-1}. Vibrations of the carbonyl group of carboxylic acid ions appear in the form of absorption bands of average intensity about 1590 cm^{-1}, deformation vibrations of NH4 + at 1387 cm^{-1}.

TABLE 2. UV AND IR SPECTRAL DATA OF MASGK-R COMPLEXES

R	Ratio	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

The compositions of the obtained complexes were analyzed by the method of isomolar series. This method is based on determining the ratio of the isomolar concentrations of the reactants corresponding to the maximum yield of the resulting complex compound. The curve of the dependence of the yield of the complex on the composition of the solution is characterized by an extreme point [7].

The optical densities of solutions of the isomolar series were determined at a wavelength of 259 nm for the MASGK-M complex, 254 nm for the MASGK-TM complex, and 252 nm for the MASGK-MTM complex in a phosphate buffer medium. The optical density was used to calculate the stability constant KS of the complex by the Babko method [8].

.

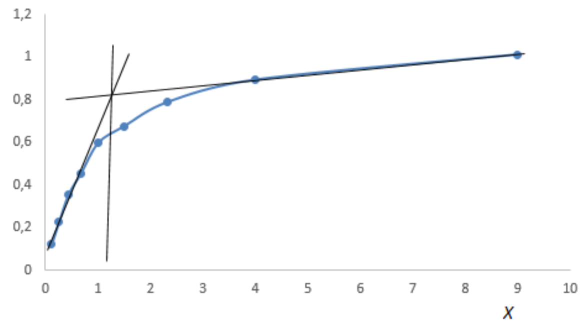

Fig. 1. The dependence of the optical density on the ratio of components in the isomolar series: λ = 259 nm, c = 10^{-4} M; pH = 7.2 (MASGK-M).

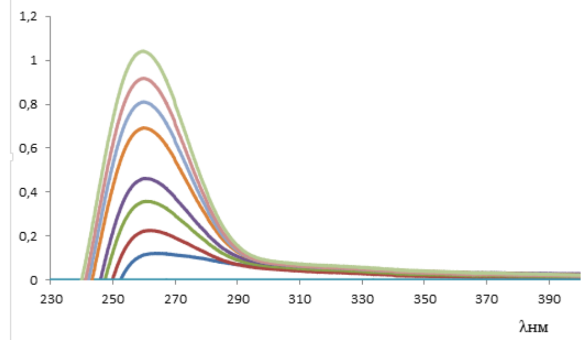

Fig. 2. Absorption curves of the isomolar series of solutions of the complex c = 10^{-4} M; pH = 7.2 (MASGK-M).

From the obtained KS value, the change in the Gibbs energy of the complexation process was found according to the following equation:

$$\Delta G = -RTlnK_S$$

Similar experiments were carried out with the MASGK-TM and MASGK-MTM complexes. The obtained data are summarized in the table. 3.

TABLE 3. KS AND ΔG VALUES OF MASGK COMPLEXES

№	Complex	Component ratio	Isobestic point, nm	K_S, M^{-1}	ΔG, kJ/mol
1	MASGK-M	1:1	>300	$3{,}4\cdot10^4$	$-2{,}8\cdot10^5$
2	MASGK-TM	2:1	235	$8{,}35\cdot10^6$	$-3.97\cdot10^4$
3	MASGK-MTM	1:1	242	$7{,}2\cdot10^5$	$-3{,}3\cdot10^4$

From the determined values of KS and ΔG, it can be seen that the complex obtained with thiourea is more stable. This can be explained by the greater electron-donating ability of the sulfur atom. The smallest value of the change in the Gibbs energy of complexation of MASGK-M shows that urea is more reactive than thiourea.

Assessment of antibiotic activity

As known, pathogenic fungi cause great damage to agriculture, in particular to grain crops. HA and its derivatives have a wide spectrum of biological activity, in particular, they have antibiotic activity.

The preparations registered all over the world containing components of liquorice roots, including HA, number about 1800 names. However, their fungicidal activity is poorly understood. A complex compound of MASGA with salicylic acid (SA) has been proposed against the fungus *Verticillium dahliae* Klebhan, the causative agent of verticillium wilt (wilt). It has been shown that this compound causes cotton resistance to stress factors, stimulates growth and promotes the maturation of cotton fibre. The effective fungicidal activity of the complex compound with copper obtained based on technical HA was noted. This compound has a fungicidal effect against the cereal rust pathogen. The composition of copper and cobalt diglycyrrhizinates in the experiments increased the resistance of wheat to fungal diseases, and also stimulated the development of the plant. The complex compound of MASGA with indolylbutyric acid is presented as a phytohormonal preparation [9-11]. The fungicidal activity of copper glycyrrhizinate against some types of pathogenic fungi and rust pathogens was also noted [12].

Since the purpose of this study was to obtain new biologically active derivatives of MASGK, we studied the antibiotic (fungicidal) activity of the obtained MASGK compounds with urea and thiourea in the laboratory conditions. The objects of study were pathogenic fungi of the Fusarium family - *F.culmorum, F.solani, F.poae, F.graminarium* and *F.oxsporium*, obtained from the collection of the Institute of Genetics and Experimental Biology of Plants of the Academy of Sciences of the Republic of Uzbekistan, as well as isolated from infected organs of wheat Triticum aestivum. The studies used test cultures of the most common pathogenic myxomycetes in Uzbekistan. The antibiotic activity of MASGK complexes was studied by comparing the diameters of incubation growth zones in nutrient media [13-15].

The drug P-4 approved for use in Uzbekistan was used as a controlled drug. Observations of changes in development zones were carried out 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 MASGK ON THE DEVELOPMENT OF PATHOGENIC FUNGI

№	**fungus**	**Fungicidal and fungistatistic 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-MASGK-M; 2-MASGK-TM, control - P-4

"-" lack of influence.

**** zone of fungicidal action - the width of the zone of complete suppression of the development of the studied microorganisms (fungi), mm;***

***** zone of fungistatic action - the width of the zone of partial or strong suppression of the development of the studied microorganisms (fungi), mm.***

The results of the experiments showed that the studied drugs do not have an antagonistic effect against F.poae. However, these drugs in control 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 MASGK with urea and thiourea show the possibility of using these compounds in the development of new, effective means of protecting plants from pathogenic fungi.

EXPERIMENTAL PART

UV spectra were recorded on Shimadzu-1280 and Cary 60 UV-Vis spectrophotometers in quartz cuvettes 10 mm thick, IR spectra were recorded on an IR Fourier spectrometer IRTracer-100 (Shimadzu, Japan). The starting MASGA and methylolthiourea were obtained according to known methods [16,17].

Obtaining complex MASGK-M (1: 1). 1.68 g (0.002 mol) of MASGA was dissolved with vigorous stirring in 100 ml of 50% ethanol (v/v). To this solution was added 0.12 g (0.002 mol) of urea dissolved in 25 ml of the same solvent. The resulting mixture was stirred on a magnetic stirrer for 4-5 hours at 40-50 °C. After that, the alcohol was distilled off on a rotary evaporator, the residue was dehydrated using freeze-drying. Pale yellow amorphous substance. The complexes MASGK-TM, MASGK-MTM were obtained in a similar way in the corresponding molar ratios. Complexes MASGK-TM, yellowish needle crystals. Complex MASGK-MTM, small yellowish crystals.

Growing test cultures. Mushroom samples were grown in artificial chambers with a temperature of +25 +26 ℃ in KDA and Czapek nutrient media. During cultivation, the contaminated samples were removed. In the nutrient medium, placed in Petri dishes, add 1 g of the test substances and evenly levelled with a spatula. Then, 4 holes are made in each dish. A control preparation (P-4) is added to 2 holes in each dish, the remaining 2 holes are inoculated with a culture of pathogenic fungi in a liquid state.

Composition of KDA nutrient medium:
Potatoes - 200 g;
Sucrose - 20 g;
Agar - 20 g;
Water - 1 *l*;
pH - 6.0

The composition of the Czapek culture medium:
KNO_3 – 2,0 g;
K_2HPO_4 – 1,0 g;
$MgSO_4$ – 0,5 g;
KCl – 0,5 g;
$FeSO_4$ – 0,001 g;
Sucrose - 20 g;
Water - 1 *l*;
pH - 6.0

CONCLUSION

Thus, molecular complexes of MASGK 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 the method of isomolar series. On the basis of spectral data, it was revealed that molecular complexes are formed due to intermolecular dipole-dipole bonds. In experiments on test cultures of pathogenic fungi Fusarium, the fungistatic activity of the obtained complexes was revealed. These compounds can be used in the development of new stimulants for agriculture.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the senior researcher of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan, Doctor of Chemical Sciences. M.B. Gafurov and a researcher of the Institute, PhD R. Esanov for the rendered practical assistance in the performance of this work.

REFERENCES

1. Dushkin A.V., Meteleva E.S., Khomichenko N.N., Vlasenko N.G., Teplyakova O.I., Khalikov M.S., Khalikov S.S. A new pesticide preparation based on complexes of tebuconazole and glycyrrhizin derivatives. Journal. The successes of modern natural science. - 2016. - No. 11 (part 2). - S. 296-300. DOI: 10.17513 / use.36227.

2. G.A. Tolstikov, L.A. Baltina, V.P.Grankina, R.M.Kondratenko, T.G. Tolstikova. Licorice: Biodiversity, Chemistry, Medical Applications. Novosibirsk. Publishing house GEO. 2007.S. 123.

3. Yakovishin L.A. Molecular complexes of triterpene glycosides with biologically active substances: preparation, chemical and pharmaceutical properties and biological activity. Diss. doctor farm. sciences. Sevastopol. 2018.351 p.

4. Nafisi S., F. Manouchehri, M. Bonsaii. A comparative study of glycyrrhizin and glycyrrhetinic acid complexes interactions with DNA and RNA // Iranian J. Org. Chem. – 2012. – Vol. 4, № 2. – P. 841-849.

5. Isakov H. Synthesis, classification and development of technology for producing biologically active compounds with multifunctional action, based on formaldehyde and furfural. Diss. doctor tech. sciences. Tashkent. 2019.200 s.

6. Chemistry of guest-host complexes. Editors F. Vögtle and E. Weber. Per. from English Moskava. Peace. 1988 p. 384-385.

7. M.I.Bulatov, I.P. Kalinkin. A practical guide to photometric methods of analysis. Leningrad. Chemistry. 1986. p. 241.

8. Babko A.K. Physicochemical analysis of complex compounds in solutions. Publishing house of the Academy of Sciences of the Ukrainian SSR. 1955.328 s.

9. Navruzov S.V., Khashimova N.R., Akhunov A.A., Kuldosheva K.M. Influence of natural preparations on cotton yield and fiber quality during soil salinization. Universum: Chemistry and Biology. No. 11 (77), 2020. p. 49-52.

10. Khashimova N.R. The mechanism of the formation of cotton resistance to phytopathogens and the ways of its regulation. Abstract dissertation. Doctor of Biol. sciences. Tashkent. 2016.77 p.

11. Patent RUz No. IAP 05090. Method of combating cotton wilt / Akhunov A.A., Khashimova N.R., Pshenichnov E.A., Avtonomov V.A., Dalimov D.N., Matchanov O.D., Gafurov M.B. // Official Bulletin. - 2015. - No. 10.

12. Ablakulova N.A. Assessment of the effect of natural triterpenoids on wheat fungal diseases. Abstract dissertation. Doctor of Philosophy in Biol. sciences. Tashkent. 2019.42 p.

13. Booth C. Methods in microbiology. Vol.4. 1971. Academic Press London and New York. PP. 137-149, 404-421.

14. Leslie J.F., Summerell B.A. The Fusarium laboratory manual. Copyright 2006. Blackwell Publishing.

15. Kirai Z., Clement Z. et al. Phytopathology methods. M., 1974.S. 180.

16. G.A.Tolstikov, L.A. Baltina, V.P.Grankina, R.M.Kondratenko, T.G. Tolstikova. Licorice: Biodiversity, Chemistry, Medical Applications. Novosibirsk. Publishing house GEO. 2007.S. 279.

17. Isaev Yu.T., Rustamov S.A., Khozhimatov M.M., Otakhonov K.K. Study of the structure and composition of the supramolecular complex of the monoammonium salt of glycyrrhizic acid with methylolthiourea. Bulletin of NUUz. Natural Sciences. 2020.3 / 1. S.292-296.