CHEMISTRY SCIENCES

BIOORGANIC CHEMISTRY

DOI - 10.32743/UniChem.2021.89.11.12507

COMPLEX COMPOUND OF THE MONOAMMONIUM SALT OF GLYCYRRHIZIC ACID WITH THIOUREA AND THE OF ITS TOXICITY

Yusuf Isaev
Candidate of Science, assistant professor, Department of Chemistry of Andijan state university, Uzbekistan, Andijan
E-mail: yusufjon_67@inbox.ru

Doston Egamberdiev
PhD student, Department of Chemistry of Andijan state university, Uzbekistan, Andijan

Ibragim Askarov
Doctor of Chemistry, Professor of the Department of Chemistry of Andijan state university, Uzbekistan, Andijan

Sanzhar Rustamov
Doctor of Philosophy in Chemistry, Senior Lecturer Department of Chemistry of Andijan state university, Uzbekistan, Andijan
E-mail: sanjar.rustamov.2018@mail.ru

Natalya Vypova
Candidate of Biological Sciences, Senior Researcher at the Institute of Bioorganic Chemistry, Uzbekistan, Tashkent

КОМПЛЕКСНОЕ СОЕДИНЕНИЕ МОНОАММОНИЙНОЙ СОЛИ ГЛИЦИРРИЗИНОВОЙ КИСЛОТЫ С ТИОМОЧЕВИНОЙ И ЕГО ТОКСИЧНОСТЬ

Исаев Юсуп Тожимаматович
канд. хим. наук, зав. кафедрой химии Андижанский Государственного Университета, Республика Узбекистан, г. Андижан
E-mail: yusufjon_67@inbox.ru

Эгамбердиев Достон Усмонджон угли
докторант кафедры химии Андижанского Государственного Университета, Республика Узбекистан, г. Андижан

Аскаров Иброхим Рахмонович
д-р хим. наук, профессор Андижанского Государственного Университета, Республика Узбекистан, г. Андижан

Библиографическое описание: COMPLEX COMPOUND OF THE MONOAMMONIUM SALT OF GLYCYRRHIZIC ACID WITH THIOUREA AND THE OF ITS TOXICITY // Universum: химия и биология : электрон. научн. журн. Isaev Y. [и др.]. 2021. 11(89). URL: *https://7universum.com/ru/nature/archive/item/12507*

Рустамов Санжар Аширалиевич
ст. преподаватель кафедры химии
Андижанского Государственного Университета,
Республика Узбекистан, г. Андижан
E-mail: sanjar.rustamov.2018@mail.ru

Выпова Наталья Леонидовна
канд. биол. наук, ст. науч. сотр.
Института Биоорганической химии,
Республика Узбекистан, г. Ташкент

ABSTRACT

The development of new dosage forms based on local herbal raw materials is urgent. Currently, much attention is paid to one of the main active ingredients of licorice roots - glycyrrhizic acid and its derivatives. Due to its peculiar structure and properties, glycyrrhizic acid forms inclusion compounds with various organic substances. The article summarizes the results of the work carried out on the preparation of a supramolecular complex of the mono-ammonium salt of glycyrrhizic acid with thiourea, the study of some physicochemical parameters and the determination of the acute toxicity of the obtained compound. Thus, UV -, IR-spectroscopy and mass spectrometry characterized the nature of the intermolecular interaction of the components, conclusions about the composition of the complex were made using the methods of isomolar series and high-performance liquid chromatography. It follows from the spectral data that complexation occurs due to the formation of hydrogen bonds between hydrophilic groups of molecules. The stability constant and the change in the Gibbs energy of complex formation are calculated. Based on the results of the acute toxicity determination, it was revealed that the resulting complex belongs to safe substances of class IV. The resulting complex compound can be used as a starting substance in the development of new biostimulants for agriculture.

АННОТАЦИЯ

Разработка новых лекарственных форм на основе местного растительного сырья является актуальным. В настоящее время большое внимание уделяется одному из основных действующих веществ корней солодки - глицирризиновой кислоте и ее производным. Благодаря своеобразной структуре и свойствам глицирризиновая кислота образует соединения включения с различными органическими веществами. В статье обобщены результаты проведенных работ по получению супрамолекулярного комплекса моноаммониевой соли глицирризиновой кислоты с тиомочевиной, изучению некоторых физико-химических показателей и определению острой токсичности полученного соединения. Таким образом, методами УФ-, ИК-спектроскопии и масс-спектрометрии охарактеризован характер межмолекулярного взаимодействия компонентов, сделаны выводы о составе комплекса с использованием методов изомолярных рядов и высокоэффективной жидкостной хроматографии. Из спектральных данных следует, что комплексообразование происходит за счет образования водородных связей между гидрофильными группами молекул. Рассчитаны константа устойчивости и изменение энергии Гиббса комплексообразования. По результатам определения острой токсичности установлено, что полученный комплекс относится к безопасным веществам IV класса. Полученное комплексное соединение может быть использовано в качестве исходного вещества при разработке новых биостимуляторов для сельского хозяйства.

Keywords: licorice, glycyrrhizic acid, isomolar series method, acute toxicity.
Ключевые слова: солодка, глицирризиновая кислота, метод изомолярных рядов, острая токсичность.

Medicinal plants are a rich source of biologically active substances, including medicinal ones. Some plant substances are not directly used in medical practice, they are used as starting substances for the synthesis of effective medicines.

As you know, licorice is a medicinal plant, the roots of which are used to treat inflammatory diseases of the upper respiratory tract and gastrointestinal tract. One of the main active components of licorice root is a triterpene glycoside-glycyrrhizic acid (HA). The unique physical and chemical properties of this acid have made it a valuable object of research by specialists in various branches of science [1, 2].

Experimental. IR spectra were recorded on an IR Fourier spectrophotometer IRTracer-100 (Shimadzu), UV spectra were recorded on a Shimadzu-1280 spectrophotometer. Chromatography was performed on an Agilent 1200 chromatograph (Germany); the melting point of substances was determined using an SMP10 instrument.

The stability constant and the ratio of the complex components were determined in a phosphate buffer medium (Na_2HPO_4-NaH_2PO_4, pH = 7.2).

Receiving the complex. 1.68 g (2 mmol) of MASGA was dissolved in 50 ml of 50% ethyl alcohol, 0.152 g of crystalline thiourea was added to the resulting solution, and the mixture was kept under constant stirring on a magnetic stirrer for 4-5 hours at 40-50 ° C. After that, the alcohol was distilled off in a rotary evaporator, the residue was dried on a freeze dryer. Pale yellow needles. Output 92%. T.m. = 186 ± 2 ºC (with expansion).

For HPLC analysis, a sample of a standard solution prepared as follows: 1.0 g (accurately weighed) of thiourea was dissolved in 50 ml of warm water, the solution was quantitatively transferred into a volumetric flask with a volume of 100 ml. The volume of the solution

was brought to the mark with distilled water: solution A. 1 ml of solution A was diluted with the mobile phase. The solution thus obtained was centrifuged at 7000 rpm for 5 minutes before chromatography. A standard monoammonium salt (MASGA) solution was prepared in a similar way.

The most widely used derivative of GA is its (MASGA), a drug based on which called Glycyram is used in medicine for eczema, allergic dermatitis. In addition, this salt is part of some other medicines.

In the IR spectrum of the complex at 3381 cm^{-1}, there is a low-intensity absorption band related to associated OH and NH groups, and the absorption band of the C=O group at 1712 cm^{-1}. The absorption band characteristic of NH_2 bending vibrations was not found. In addition, it was found that the intensity of the absorption band of stretching vibrations of this group sharply decreases (Fig. 1).

The changes noted above confirm that the COOH, OH, and NH_2 groups of MASGA and thiourea are involved in the intermolecular interaction in the complex. In addition to hydrogen bonds, ion-dipole bonds are likely to form.

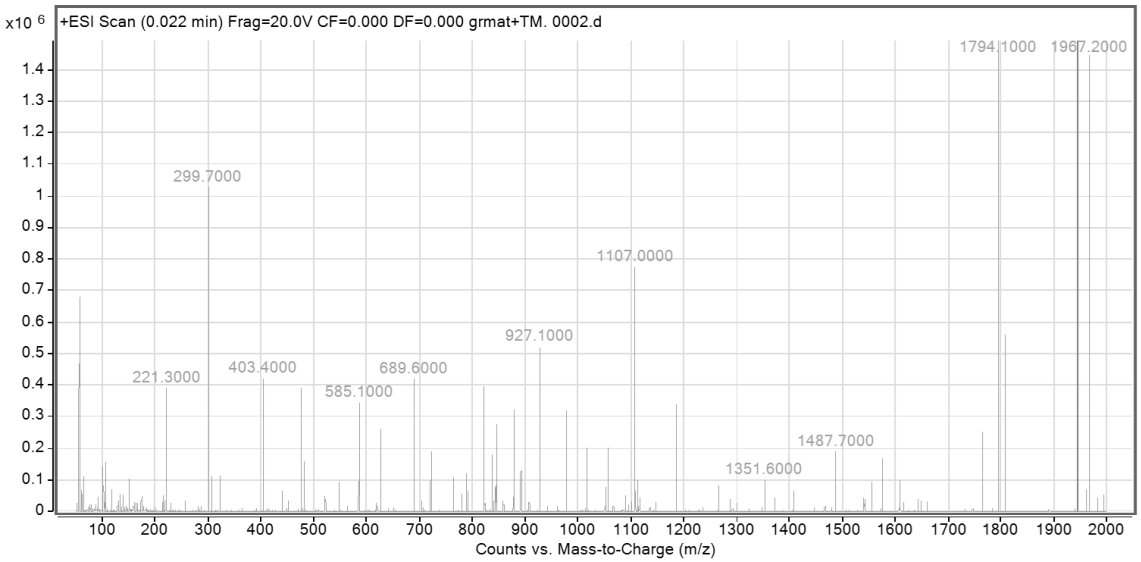

Figure 1. Mass spectrum of the complex compound MASGA with thiourea

In the mass spectrum of the complex, one can see the signals of the ions of the complex in the form of a monomer, dimer, and tetramer, and the signal of the ion in the form of a tetramer has the highest intensity (Table 1).

Table 1.

Mass Spectral Data of the MASGK complex with Thiourea

Ion	*m/z*	Intensity %
$[2M^{GC}+4M^{TM}-M^{NH_3}+H]^+$	1967,2000	95,0
$[4M^{GC}+4M^{TM}-2M^{NH_3}-4H]^{2+}$	1794,1000	100,0
$[5M^{GC}+5M^{TM}-NH_3+H]^{3+}$	1487,7000	13,2
$[2M^{GC}+M^{GA}+M^{TM}-6H]^+$	1107,0000	52,4
$[2M^{GC}+2M^{TM}+Na+H]^{2+}$	927,1000	35,3
$[M^{GC}+M^{TM}-M^{NH_3}+H]^{3+}$	299,7000	70,0

The composition of the complex was determined by the isomolar series method (Ostrmyssensky-Zhob method) and by HPLC.

The method of isomolar series is based on determining the ratio of isomolar concentrations of reagents corresponding to the maximum yield of the compound [4,5]. To determine the isomolar series, the optical density of each series is measured and a graph of the optical density versus $\frac{C_1}{C_1+C_2}$, is plotted, the point of intersection of tangent lines is marked, which corresponds to the ratio of the components of the complex.

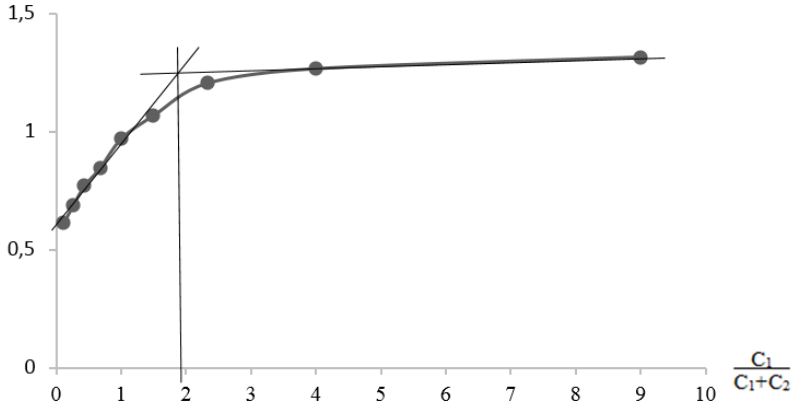

Figure 2. Change in the optical density of the components of the complex in the isomolar series at λ = 254 nm.

Using the found concentrations from the optical density, the equilibrium constant of complexation is calculated according to the following equation:

$$K = \frac{[MASGA - TU]}{[MASGA][TU]}$$

From the found value of the equilibrium constant, the change in the Gibbs energy can be calculated using the equation: $\Delta G = -RT\ln K$.

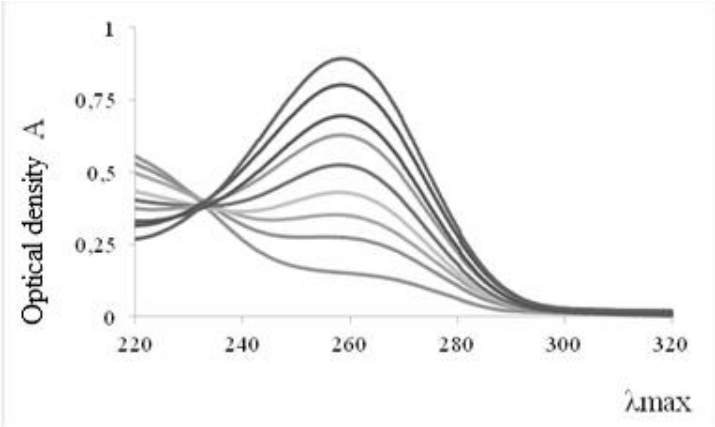

Figure 3. Curves of the optical density of solutions of the isomolar series

The composition of the resulting complex was determined by HPLC. The chromatographic analysis results are shown in the following table:

Table 2.

Quantitative composition of the MASGA complex by HPLC

Mass fraction, theoretical %		Mass fraction found %		Retention time (min)
MASGA	Thiourea	MASGA	Thiourea	
91,7	8,3	90,8	8,2	7,529

The systematic study of the biological activity of GA and its derivatives began in the 40s of the last century, when their anti-inflammatory activity was discovered in a combination of low toxicity [6]. The problem of low solubility and undesirable side effects of drugs can be solved with the help of substances that form inclusion compounds or molecular complexes of the "guest-host " type. It has been established that the pharmaco-therapeutic and physico-chemical properties of drugs are significantly improved in the composition of such complexes [7,8]. Thus, it was noted that complex compounds of GA with antiviral and antibacterial drugs, such as levomycitin, sulfapiridazine, salazodimethoxine, sulgin, resorcinol, increased the resistance of mice to diseases caused by *Staphylococcus*, *Escherichia coli*, *Pseudomonas aeruginosa* bacteria and strengthen the immune system of animals [9].

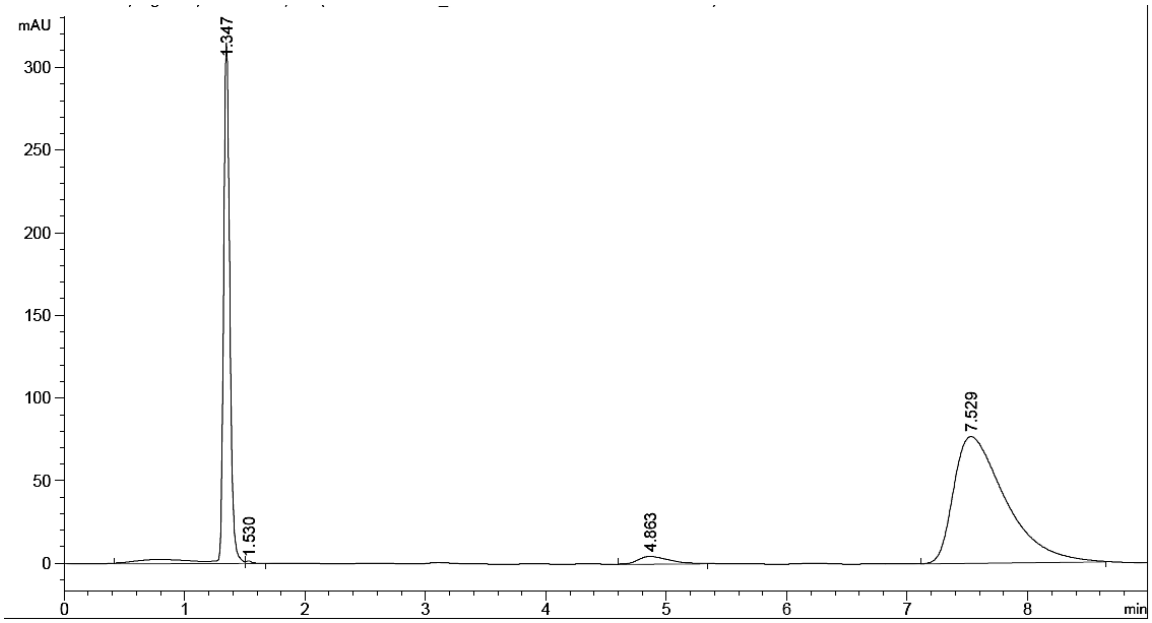

Figure 4. HPLC chromatogram of the complex of MASGA with thiourea

Due to the peculiar chemical structure of GA and its monoammonium salt, they can form molecular complexes with various low-molecular pharmacones. It should be noted that MASGA can be easily obtained from licorice root extract, it dissolves better, and also MASGA is less toxic than GA.

In previous works, we obtained supramolecular complexes of MASGA with urea, methylolurea, and some sulfa drugs [10, 11]. In experiments to identify biological activity, antioxidant and antifungal activities of the above complexes were noted. In order to identify the pharmacological and biological characteristics of the obtained complex, its acute toxicity was studied by the Lichfield-Wilcoxon method [12]. Crystalline thiourea was used for the reference drug. The drugs were administered orally at 200, 250, 300, 400 and 600 mg / kg. With the introduction of thiourea at a dose of 200 and 250 mg / kg, after 5-10 minutes, excitation, a decrease in pupils and an increase in respiration were observed in the animals. After 2-3 hours, the condition of the animals returned to normal. No deaths were observed at a dose of 200 mg / kg. At a dose of 250 mg / kg, the death of animals was noted every other day (2/6). With the introduction of thiourea at a dose of 300, 400, and 500 mg / kg, similar changes in the state of the animals were observed. The lethal outcome was observed after 1-3 days: at a dose of 300 mg / kg 3/6, at a dose of 400 mg / kg 4/6, at a dose of 500 mg / kg 6/6.

When thiourea was administered orally to mice, the LD_{50} value was 306 mg/kg, LD_{16} 250 mg / kg, and LD_{84} 360 mg / kg. From these data, it follows that thiourea belongs to the 3rd class in terms of toxicity.

After oral administration of the complex compound MASGA with thyurea at a dose of 2000 and 2500 mg / kg on the third day, as a result of tachycardia, the lethal outcome of the animals was 5/6 and 6/6, respectively. at doses of 1300 and 1600 mg / kg, tachycardia was observed less frequently, and the lethal outcome was 2/6 and 3/6, respectively. At a dose of 1000 mg / kg, no lethal outcome was noted.

The results are presented in table 3.

Table 3.

The degree of toxicity of the complex compound MASGA with thiourea (I)

Drug	**Dose, mg / kg**	**Number of dead animals**	**LD_{16} -м+м mg/kg**	**LD_{50} -м+м mg/kg**	**LD_{84} -м+м mg/kg**
Thiourea	200	0/6	250	306 III group	360
	250	2/6			
	300	3/6			
	400	4/6			
	500	0/6			
I	1000	0/6	1300	1530 IV group	1800
	1300	2/6			
	1600	3/6			
	2000	5/6			
	2500	6/6			

Thus, it was revealed that the toxicity of the complex compound of MASGA with thiourea is 5 times less than the toxicity of thiourea.

Conclusions. For the first time a supramolecular compound of MASGA with thiourea was obtained and some of its physicochemical parameters were studied.

The composition of the obtained compound was determined by the method of isomolar series, and the change in the change in the Gibbs energy of the complexation process was also determined.

The nature of the intermolecular interaction of the components of the obtained complex was characterized by UV and IR spectroscopy.

The acute toxicity of the complex obtained (IV class) was determined.

Reference:

1. V.I. Litvinenko, T.P. Popova. [Electronic resource]. Pharmacom. (2004). http://farmacomua.narod.ru/licorice.
2. G.A. Tolstikov, L.A. Baltina, V.P. Grankina, R.M. Kondratenko. Licorice Biodiversity, chemistry, application in medicine. Academic Publishing House "GEO". Novosibirsk. Russia. 2007. c. 311. (In Russian).
3. T.G. Tolstikova, A. G. Tolstikov, G. A. Tolstikov. Bulletin of the Russian Academy of Sciences. 77 (10); 2007: p. 867-874. (In Russian).
4. M.I. Bulatov, I.P.Kalinkin. Practical guide to photometric methods of analysis. 5th ed. Leningrad: Chemistry. 1986. 432 c. (in Russian).
5. Babko A.K. Physico-chemical analysis of complex compounds in solutions. Kiev, Publishing House of the Acad. of Sci. of the Ukr. SSR, 1955. 328 c. (in Russian).
6. Brovchenko B.V. Improvement of quality control methods for crushed raw materials and licorice preparations. Dissertation for the degree of candidate of pharmaceuticals sciences. Moscow. Russia. 2020. 169 p.
7. L.A.Yakovishin, V.I.Grishkovets. Studies in Natural Products Chemistry. 2017. V.55, Chapter 11, P.351-383. DOI: 10.1016/B978-0-444-64068-0.00011-5
8. Yakovishin L.A., Grishkovets V.I., Korzh E.N. Scientific notes of the Crimean University named after V.I. Vernadsky. Biology. Chemistry. 2017; 3 (3); 270-277. (In Russian).
9. Kondratenko R.M. Glycyrrhizic acid and related licorice triterpenoids in the synthesis of promising biologically active compounds. Abstract of the dissertation of the doctor of chemical sciences. (2006). Ufa, Russia. 48 p. https://rusneb.ru/catalog/000200_000018_RU_NLR_bibl_1097850/
10. I.R.Askarov, Yu.T.Isaev, S.A. Rustamov, U.M.Yakubov. XIII Int. Symp. on the Chemistry of Natural Compounds (Shanghai, October 16-19, 2019). Shanghai, China. 2019. P. 62.
11. Isaev Yu. T., Rustamov S. A., Askarov I. R. "Bulletin of the Perm State Pharmaceutical Academy". Perm. Russia. 22 (2018). 34-35. (in Russian).
12. Mironov A.N., Bunyatyan N.D. and other. Guidelines for conducting preclinical studies of drugs. Part I, Grif and K. Moscow. Russia. (2012). p. 80-93.