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Analysis of the Supramolecular Complex of Kinetin with Glycyrrhizic Acid Using the Chromatography Mass Spectrometry Method

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***Abstract*—**Supramolecular complexes of glycyrrhizic acid with kinetin in various molar ratios were obtained, physico-chemical parameters and spectral properties of the resulting complexes were studied (UV, IR, mass spectrometry.

***Keywords*—**Monoammonium salt of glycyrrhizic acid, glycyrrhizic acid, supramolecular complex, isomolar series, IR spectroscopy.

I. Introduction

TRITERPENOIDS are a class of natural compounds widespread in the plant world and animals. They are represented by a great structural diversity and biomedical properties. One of these natural compounds is glycyrrhizic acid (GA) - the main active principle of licorice root (*Glycyrrhiza glabra* L.), which grows on the territory of the Uzbekistan Republic and is a renewable natural source. Natural biologically active compounds capable of self-organization, recognition of other particles and molecules, are the subject of many years of research by chemists on modeling various biochemical processes.

The biological activity properties (wounds, viruses, inflammation, various tumors, allergies, antioxidant effects) of GA and its derivatives have been studied [1]. The anti-inflammatory effect of GA is related to the effect of immunity on humoral and cellular factors. GA slows down the release of quinine and has membrane-protective properties of the inflamed area connective tissue cells. Adenine and kinetin slow down the rate of lipid oxidation by binding to free radicals and toxins of the oxidation process. By acting on the skin of the adrenal glands, it increases the production of corticoids and androgens by the adrenal glands [2].

It is known from the literature that the quality and yield of winter wheat and cotton plants improved in the areas planted with sweet corn [3], [4]. The biologically active substance in the root is mainly GA, which has stimulating and hormonal properties [5], [6]. In addition, a number of supramolecular complexes based on GA and its salts were obtained and their effect on plant growth and development processes were studied [7]. In particular, the supramolecular complex of GA with salicylic acid at low concentrations (10^{-7} M) had a beneficial effect on cotton seed germination and cotton growth under the influence of antioxidant system enzymes, and as a result the yield of cotton fiber increased by 5-7 quintals/hectare.

The rapidly developing supramolecular chemistry has made it possible to create methods for molecular encapsulation of a number of drugs and to study the structure of the obtained complexes in order to determine their stability. One emerging way to create low-dose drugs is to prepare clathrates in the presence of glycosides from these locally available plant materials. Molecular complexes obtained in the presence of plant glycosides lead to an increase in water solubility, bioavailability of drugs and the formation of a broad spectrum of biological activity [7], [8].

The biological activity of modifications with GA is determined by the peculiarities of their chemical structure. In order to create new pharmaceutical products, research is being conducted to obtain complexes of GA amino acids, alkaloids, antibiotics, etc. Among them, there are known drugs that have anti-inflammatory, analgesic, anti-allergic, hypolipidemic, antioxidant, antitoxic, hepatoprotective, immunotropic, antimicrobial and antitumor activities [9], [10].

Yakovishin and Grishkovets obtained supramolecular complexes of GA and its monoammonium salt (MASGA) with L-histidine, streptocide, caffeine, quercetin, and a number of other biologically active substances. The structures were studied using spectroscopic methods. In addition, the stability constants of the obtained complexes and the Gibbs free energy values were determined by the isomolar series method [11].

Adenine is a component of adenine DNA and RNA; it is involved in the formation of adenosine triphosphate, the energy source of the cell [12]. Kinetin (N6-furfuryladenine) is a growth factor and is one of the most widely used ingredients in many skin care cosmetics. There is also some information about the antiplatelet aggregation factor, which reduces the formation of blood clots in the human body, and its ability to correct RNA-related genetic diseases [13].

It is very difficult to study the nature of intermolecular interaction when GA is the host in "guest-host" complexes. In order to study these interactions, complexes obtained with

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phytohormones were studied using the NMR-spectroscopy method. Complexes of GA with phytohormones were studied by this method, and it was found that complexes are formed due to hydrophobic interaction and also hydrogen bonds. It was concluded that the carbohydrate part of GA is involved in the complex formation of phytohormones with GA.

The complexation of GA with phytohormones has also been studied by quantum chemical calculations.

When the Amber approximation was modelled in water and room temperature using the molecular dynamics method, hydrophobic interactions were unlikely to dominate. GA and phytohormones molecules were observed to be oriented with their hydrophobic part relative to each other. This orientation is variable and lacks a clear, more convenient geometry [14].

Given the above data, the purpose of this work is to obtain supramolecular complexes of GA with kinetin and to study some of their physicochemical properties [15].

II. Materials and Methods

A. Chemicals and Reagents

Organic solvents: acetone (purity, ≥ 99,5%), ethyl alcohol (purity, ≥ 99,8%), glacial acetic acid (purity, ≥ 99,9%), benzene (purity, ≥ 99%), acetonitrile (purity, ≥ 99,9%), chloroform (purity, 99,7%), ammonium hydroxide (25%), hexane (purity, 99,5%) and sodium hydroxide (purity, ≥ 99,8%). To compose the isomolar series, we used 10^{-4} M aqueous solutions of fitogormons and MASGA (buffer Na_2HPO_4-NaH_2PO_4 pH 7.2).

B. Instruments

IR spectra of the complexes formed in KBr tablets were obtained on a Perkin Elmer spectrophotometer (USA). A UV-Shimadzu 12.80 spectrophotometer was used to obtain UV spectra. The mixing of solutions was carried out on the magnetic stirrer MM-5, and the processes of distillation of volatile substances were carried out using the rotary evaporator IR-1M2. Molecular masses were determined on a Q-TOF LC-MS device (Agilent Technologies series 6520V), dehydration on a Lyophilic device Automatic FREEZE-Dryer 10-010, and melting point on a P5-TU 25-11-1144 device.

III. Experiment Section

Supramolecular complexes of kinetin with GA were prepared in a 1:2 ratio. The weighed portion of 1.646 g GA (2×10^{-3} mol) was dissolved in 25 ml of 50% aqueous ethanol solution at 60 °C. Then 0.22 g (1×10^{-3} mol) of kinetin was added and then vigorously stirred for 6–7 h in a magnetic stirrer at room temperature. The organic part was then separated from the reaction mixture using a rotor evaporator and the aqueous part was dried by freezing.

The same method was used to synthesize molecular complexes of adenine, kinetin and GA, in a 1:2, 1:4, 1:9 ratio.

To obtain the complexes, GA was dissolved in 96% ethyl alcohol and then a solution of kinetin was added to it with vigorous stirring. The alcoholic part was distilled under a vacuum and the aqueous part was dried by the lyophilic method. The optical spectra (UV, IR) of the obtained molecular complexes were studied in order to determine the known physicochemical characteristics and to study the process of complex formation.

IV. Results and Discussions

In this work, the molecular complexes of GA and kinetin are analyzed in the water: ethanol (1:1) system [16]. Some physicochemical quantities of the obtained complexes were determined and the processes of complex formation were analyzed on the basis of optical spectroscopic (UV, IR) and mass spectrometry methods.

IR spectra of the obtained compounds, the frequencies of valence vibrations of -OH groups in the GA molecule were observed in the form of a wide shoulder in the region of 3368 cm^{-1}. The frequencies of valence vibrations of the CH_3 and CH_2 groups were observed in the region of 2924–2868 cm^{-1}, as well as the frequencies of valence vibrations of the carbonyl part of the carboxyl groups in the GA molecule were observed within 1713 cm^{-1} (Fig. 1).

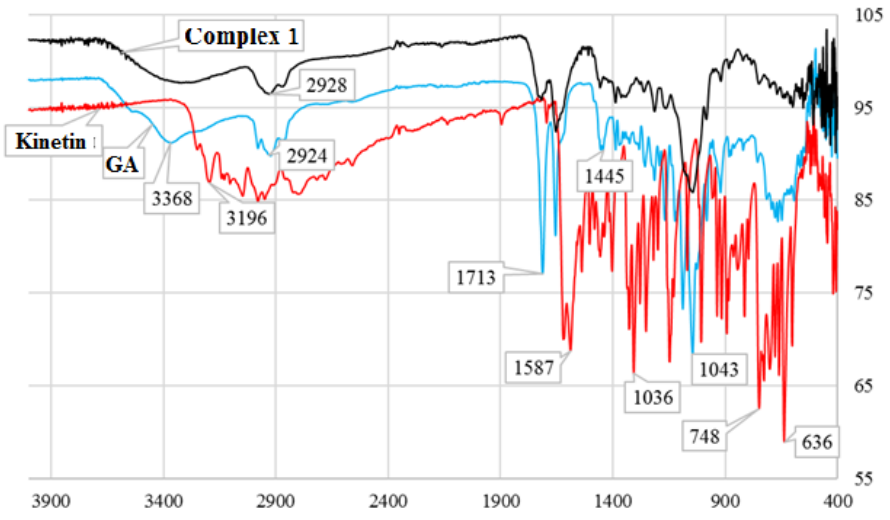

Fig. 1 IR spectrum of GA complex

The frequency of valence vibrations of the carbonyl group located on C-11 in the aglycone part of the GA molecule was intense in the region of 1656–1653 cm^{-1}.

The frequency of the deformation vibrations of the CH_3, CH_2 groups are formed in the range of 1446–1143 cm^{-1}. The frequency of valence vibrations of C-O-C and C-OH bonds in the molecule was observed in the ranges of 1087–1043 cm^{-1}, and the frequencies of deformation vibrations of the group (=CH) – in the field of 985–975 cm^{-1}.

The frequencies of valence vibrations belonging to the -NH- group in the kinetin molecule were observed at 3198–3193 cm^{-1}, and the frequencies of vibrations belonging to the -C=N bonds were observed in the region of 1592–1588 cm^{-1}.

Based on the change in the basic frequencies of the functional groups in the IR spectra of the starting substances, we can assume what types of interactions exist between the molecules in the formation of supramolecular complexes. Since, the frequencies of valence vibrations of -OH groups in the GA molecule were observed in the region of 3370-3364 cm^{-1}, and in the complex of 3342-3335 cm^{-1}.

The difference in the frequency of valence vibrations of OH groups was 29 cm^{-1}, which indicates that hydrogen bonds are involved in the complex formations. In addition, a sharp decrease in the intensity of the -NH- group vibrations in the region of 3395-3055 cm^{-1} shows the formation of a complex ion-dipole ($–NH_3^+ \cdots$O-H, H^+ OH, $-COOH^-$, except hydrogen bonds) interactions [16]. The resulting substance is a pale yellow powder. The melting temperature was found to be 206 ± 1 ºC.

UV spectra of GA, kinetin and the resulting complex were obtained and compared. The maximum peak of the GA-specific UV spectrum was seen at 252 nm, the maximum peak of the kinetin-specific was seen at 270 nm, and that of the complex was seen at 267 nm (Fig. 2).

In the UV spectrum of GA, the maximum absorption was observed in the wavelength range of 252 nm, associated with the transition of n→π* electrons between the carbonyl group in the C-ring of the GA molecule and the conjugate in the conjugate state.

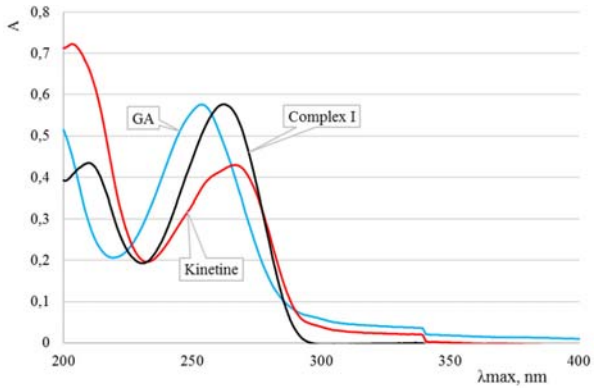

Fig. 2 UV spectra of the supramolecular complex of GA:Kinetin and GA, kinetin

The above-mentioned changes are also observed in the heterocyclic bonds in the kinetin molecule, and specific absorption of UV rays is observed. It can be observed that the maximum light absorption in the UV spectrum of the compound derived from GA and kinetin is relatively different. These differences in UV spectra indicate the formation of a complex. Because there are noticeable differences in UV spectra.

The stability constant and Gibbs free energy of complexes are determined in solutions of two components ("guest" and "host") with the same molar concentration (10^{-4} M) using the isomolar sequence method. The starting materials are mixed in a ratio of 1:9 to 9:1. This, in turn, maintains a constant solution volume and total reagent concentration.

A buffer system (phosphate buffer Na_2HPO_4 - NaH_2PO_4, pH 7.2) is used to keep the ionic strength and pH of the solutions constant. Solutions prepared for isomolar series were mixed in an incubator mixer at constant temperature (20 °C) for 40 min. The obtained data are shown in Fig. 3. As can be seen from the figure, there are isobestic points at 227 and 262 nm. The presence of these isobestic points indicates the formation of similar complexes in solution. The equilibrium constant for the process can be expressed as:

$$K = \frac{[GA - Kinetin]}{[GA] \cdot [Kinetin]} \quad (1)$$

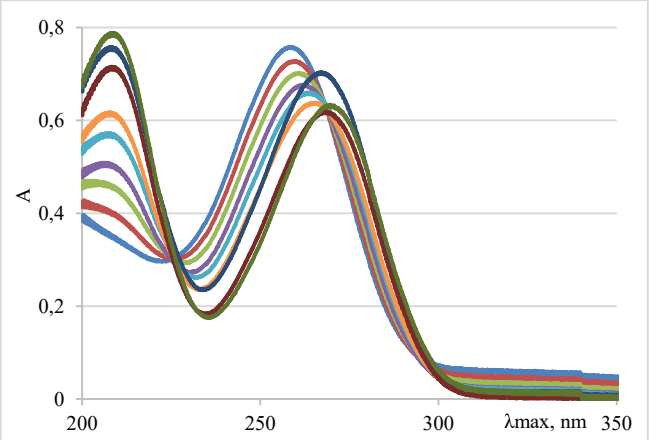

Fig. 3 UV spectra of the supramolecular complex GA and kinetin

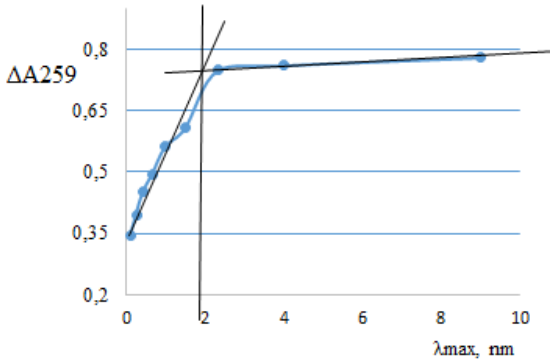

Fig. 4 Plot of the relationship between the optical density of the isomolar series of the GA complex and the concentration of reactants between kinetin

It is possible to determine the molar ratio of the initial substances in the complex composition from the graph of the relationship between the UV rays absorbance of the obtained isomolar series and the concentration ratio of the initial reactants (Fig. 4).

As can be seen from Fig. 4, GA and kinetin achieved the maximum yield of complex formation at a ~2:1 mole ratio. For complexes with a molar ratio of 2:1, the stability constant of the complex was calculated using (2) [17]:

$$K = \frac{\Delta A_0 \cdot \Delta A_1}{c(\Delta A_0 - \Delta A_1)^2} \quad (2)$$

Here, the concentration of C-substance, ΔA_0 is the change in absorbance of UV light of the fully dissociated complex, ΔA_1 is the change in absorbance of UV light corresponding to the value on the curve.

As a result, the stability constant of the GA:kinetin complex was determined to be $7.90 \cdot 10^5$ $K \cdot M^{-1}$. Using the value of these stability constants (K), the Gibbs free energy of the formed complex was calculated in (3) [18]:

$$\Delta G = -2.3RT \lg K \quad (3)$$

Here T is the temperature (K), and R is the universal gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$).

As a result of calculations, it was determined that the Gibbs free energy of the complex is equal to $3.30 \cdot 10^{-4}$ J/mol.

In addition to the above methods, the mass spectrum of the GA:kinetin 2:1 complex was obtained on a Q-TOF LC-MS (Agilent Technologies series 6520V) device in order to reflect the formation of the obtained complexes and there, in solution and to more accurately analyze the composition of the complex (Fig. 5).

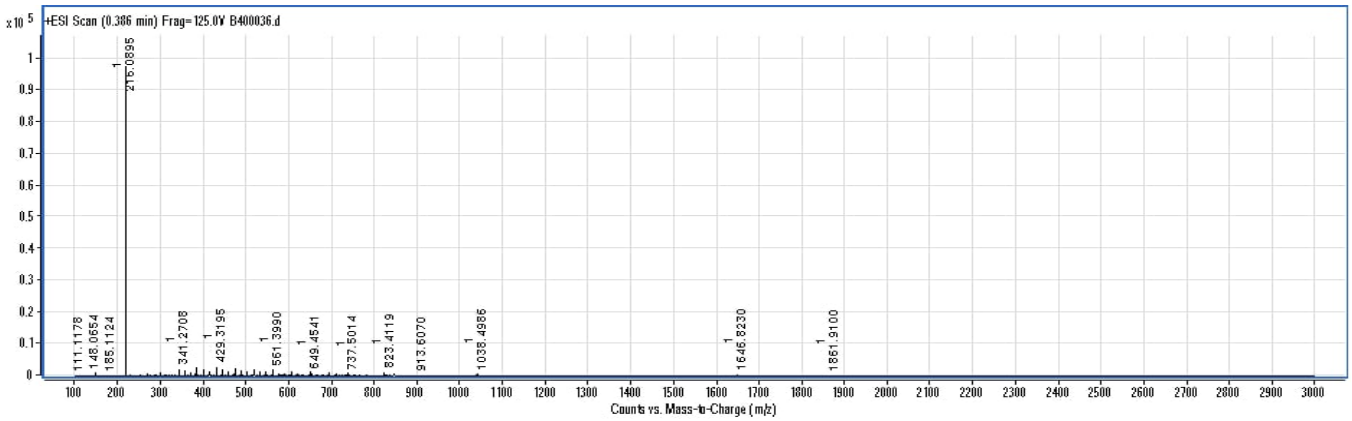

Fig. 5 GA:Kenitin 2:1 chromatomass spectrum

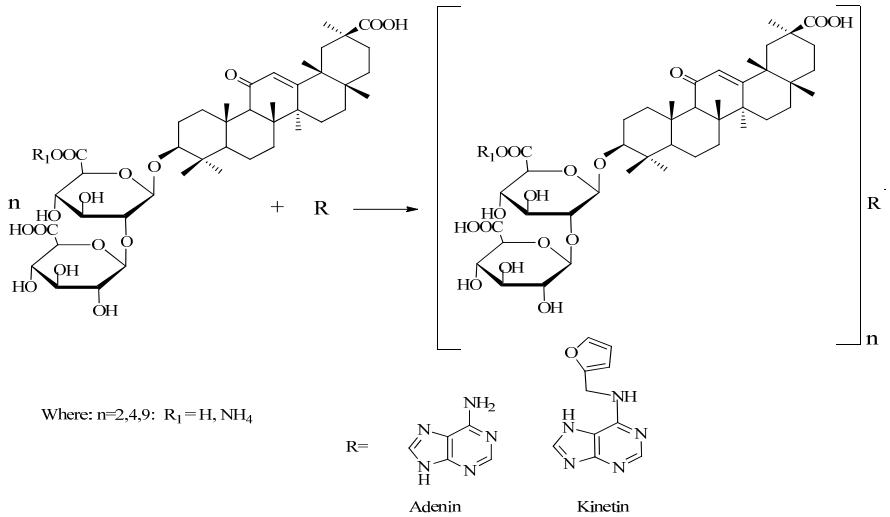

Fig. 6 Scheme of the reaction of GA with Adenine and Kinetin

GA:Kinetin 2:1 Monomer of the starting materials of the complex in the mass spectrum, except for the masses (m/z) of molecular ions belonging to the dimers and trimers, 1038.4986 and 1861.9100 masses (m/z), $[M^{GA}+M^{Kinetin}+H]^+$ and $[2M^{GA}+M^{Kinetin}+H]^+$ ions were observed and kinetin molecules show the formation of "guest-host" complexes due to non-covalent interactions.

TABLE I
THE MAIN CHARACTERISTIC OF IONS IN THE MASS SPECTRUM OF THE GA:KINETIN 2: 1 COMPLEX

The structure of ions	m/z
$[M^{kinetin}+H]^+$	216.0895
$[2M[^{kinetin}+H]^+$	429.3195
$[3M^{kinttin}+H]^+$	649.4541
$[M^{GA}+H]^+$	823.4119
$[2M^{GA}+H]^+$	1646.8230
$[M^{GA}+M^{Kinetin}+H]^+$	1038.4986
$[2M^{GA}+M^{Kinetin}+H]^+$	1861.9100

V. CONCLUSION

GA: Kinetin 2:1 complex contains 1038.4986 and 861.9100 ions of $[M^{GA} + M^{Kinetin} + H]^+$ and $[2M^{GA} + M^{Kinetin} + H]^+$ ions in addition to the masses (m/z) of monomers, dimers and trimers of the starting materials. The presence of masses (m/z) was determined based on chromatomass spectral data. In this complex, it was concluded that the molecules of GK and kinetin formed complexes of the "guest-host" type due to non-covalent interactions.

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