Synthesis and Chemical Identification of the Supramolecular Complex of Glycyrrhizin Acid and B-Indolyl-3-Acetic Acid

Shakhodat Khakimboevna Ibragimova¹, Tulkin Arzikulovich Djuraev², Khabibjon Khadjibabaevich Kushiev³

¹ Master, Gulistan State University
² PhD, Gulistan State University
³ Doctor of Biological Sciences, Professor, Gulistan State University

ABSTRACT: The study synthesized a supramolecular complex of glycyrrhizin acid and β -indolyl-3-acetic acid in a 4:1 ratio isolated from the root of the plant licorice (Glycyrrhiza glabra L.). The resulting GA:IAA (4:1) complex was chemically identified based on the comparison of IR – Fure spectra of the starting agents.

KEY WORDS: Glycyrrhiza L., glycyrrhizin acid, β -indolyl-3-acetic acid, IR-spectroscopy, Supramolecular complex.

INTRODUCTION


The study of the mechanisms of formation of “guest-host” type complexes is one of the most important theoretical / practical directions of supramolecular chemistry [Polovyanenko, 2009; 4–19], GA’s “host:guest” type auto-association has been confirmed by many researchers [Borisenko et al., 2013; Pp. 85–92; Tolstikova et al., 2007; 867–874-p.].

In the supramolecular complex, GA is estimated to form ring-shaped dimer structures with a hydrophobic void due to intermolecular hydrogen bonds. It is noted that the resulting space can provide the formation of a complex of the "guest-host" type [Groen et al., 1952; 87–91-p.].

The reaction property of GA in the supramolecular complex has been studied in detail by some researchers [Tolstikov et al., 1991; Pp. 29–33; Maistrenko et al., 1994; 329–331-b ; Gusakov et al., 2001; 1307–1310-b ; Kornievskaya, 2008; Pp. 5–20; Borisenko et al., 2015; Pp. 89–94; Xaitbaev, 2015; Pp. 155–158].

In particular, studies have shown an increase in auto-association of GA: streptomycin supramolecular complex in different ratios (1: 1; 2: 1; 3: 1; 3: 2) with increasing GA concentration in the formation of "host: guest" type structures [Vetrova et al. et al., 2016; 27–34-p.].

At present, a wide range of positive biological activity of supramolecular complexes of GA with different agents has been confirmed and the prospects for use in various sectors of the economy are highly valued. In particular, studies have shown that the combined use of GA and its disodium salt with tebuconazole increases the penetration of wheat into the grain before sowing, which in turn increases resistance to various pathogenic infections in the early stages of organogenesis, and significantly increases productivity in the final stage [Dushkin et al., 2016; 296–300-p.]. It is estimated that GA molecules (~ 60–100) form vesicles / micelles and increase the degree of transmembrane permeability when they contain the main drug molecules involved [Dushkin et al., 2016; 296–300-p.].

It has been noted that GA significantly increases the permeability of the cell membrane and serves as a promising source in the development of pharmacological agents. It is noted that the supramolecular complexes of the "host-host" type of GA have an effect that increases the biological activity of many drugs. It is assumed that GA provides a change in the physical properties of lipids in the biological membrane by forming a supramolecular complex with cholesterol in the cell membrane, forming a bribe with ionic permeability in the membrane.


Corresponding Author: Shakhodat Khakimboevna Ibragimova

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 05 Issue 03 March 2022
DOI: 10.47191/ijcsrr/V5-i3-33, Impact Factor: 5.825
IJCSRR @ 2022
www.ijcsrr.org

Available at: ijcsrr.org
Page No.:863-869
In the molecular complexes of GA formed with pharmaceutical ingredients (pharmacon), a decrease in the therapeutic effect of pharmacons was found [Tolstikova et al., 2007; 870-b.].

The complex-forming properties of GA isolated from the root of the licorice plant have been studied in detail by some researchers. In particular, the amino acids of GA, nitrogenous bases of nucleic acids, cholesterol, cyclodextrin, pharmaceutical substrates and others. More than 70 complexes were formed and the physicochemical properties of the "guest-host" type of interaction were analyzed [Yakovshin, 2018; 10–238-p.].

Studies have been conducted to obtain a supramolecular complex with glycyrrhizic acid from the root of licorice (Glycyrrhiza L.) and lagoxilin diterpenoid isolated from the plant Lagochilus intoxicating [Matchanov et al., 2001; 38–42-p.].

It is also assumed that the prospects for use in agricultural purposes are high - the synthesis and chemical identification of supramolecular complexes of GA and phytohormones is relevant from a theoretical / practical point of view.

The purpose of this study is to isolate glycyrrhizic acid from the root of the licorice plant (Glycyrrhiza glabra L.) and to synthesize the supramolecular complex of glycyrrhizic acid and β-indolyl-3-acetic acid from chemical identification.

MATERIALS AND METHODS

According to the chemical structure of glycyrrhizinic acid, triterpene-glycyrrhizic acid is a glycoside formed by glucuronic acid residues [Yakovshin, 2018; Pp. 10–19].

The physical / chemical properties of GA have been studied in detail by many researchers, including the hydrophobic (triterpene fragment) and hydrophilic (2 glucoronide residues) fragments present in the molecule, which are thought to determine the possibility of micelle formation in the complex [Marsh and Lewy, 1955; 9–14-p.].

Spectrophotometric methods are effectively used in the quantitative / qualitative chemical identification of GA in biometric compositions [Brezhnev et al., 2007; 142-143-b.; 2009; Pp. 19-23; Krakhmalev et al., 2012; 431-435-b.].

Separation of GA from the species of licorice (Glycyrrhiza L.) by researchers, methods of chemical identification in the High-performance liquid chromatography (HPLC) method are presented, [Garmaeva, 2007; Pp. 3–20].

The researchers also recommended the method: extraction of GA extract from the root of the licorice plant in NH4OH solution (1%) and acid precipitation in H2SO4 solution (conc.), in the next stage: recirculation in acetone H2SO4 solution (1%), precipitation of GK ammonium salt (25%) in NH4OH solution and crystallization in icy CH3COOH medium. [Stolyarova et al., 2008; 256–258-p.]

In the experiments, the preparation of the root extract of the local licorice (Glycyrrhiza glabra L.) and the separation of GA from its composition, chemical identification was carried out using standard methods. [Astafeva et al., 2013; Pp. 261–263; Schlotgauer, 2013; 553–556-p.].

In the experiments, licorice root extract was analyzed in the range of 4000–500 cm⁻¹ using IR – Fure spectrometry “PerkinElmer Spectrum IR” (Germany; version 10.6.1). KBr tablets were used in the preparation of test samples. In the experiments, the IR spectra of GK and β-indolyl-3-acetic acid were compared with the spectra described in the description of the library "Biochemica".

RESULTS AND ANALYSIS

Modern physicochemical methods currently used in the extraction of GA are the extraction of plant roots in high-temperature water [Abjalelov et al., 2016; Pp. 100–104; Mukhopadhyay and Panja, 2008; Pp. 539–545; Tikhomirova et al., 2008; Pp. 71–74], aqueous solution of NaOH [Rybalchenko et al., 2002; 55–59-p.], use of methanol [Kim et al., 2004; 447–453-p.], ethanol [Khabibrakhmanova et al., 2016; 97–102-p.; Gagieva et al., 2011; 266–268], ammonia solution [Gavrilin et al., 2009; 147–150-p.], as well as vacuum-pulse methods to increase the efficiency of separation of components [Rybalchenko et al., 2002; 55–59], is based [Pavlova et al., 2018; 229–235-p.] on the use of ultrasound [Charpe and Rathod, 2012; Pp. 37–41].

In experiments, the separation of GA from the root of licorice (Glycyrrhiza L.) was performed using a standard method [Kondratenko et al., 2001; Pp. 38–42; Matchanov et al., 2017; 1–5-p.].

In the experiments, the plant root was initially extracted in ethanol solution (70%) in a ratio of 1: 5 and kept in the dark for 5 days, stirring constantly. In the next step, the extract was filtered and the ethanol from the filtrate was evaporated by evaporation at a temperature of + 75 ° C. [Astafeva et al., 2013; 261–263-p.].

*Corresponding Author: Shakhodat Khakimboevna Ibragimova

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Page No.-863-869
The resulting mass was divided into fractions (3) by column chromatography (h = 20 cm; ø = 2.5 cm). In this case, the elution process was carried out in a 1% solution of ammonia in ethanol (75%). The obtained fractions were analyzed in a complex case by mass spectrometer using the HPLC method. The chromatographic separation process was carried out at a temperature of + 20 ° C using HPLC method using a 1200-series high-efficiency liquid chromatography device equipped with a diode-matrix DAD detector from Agilent Technologies (USA), in the column type of “Eclipse XDB C18” (150 × 4.6 mm; 5 μm), the flow rate value of the moving phase is 0.5 ml / min. In the experiments, a solution of methanol and formic acid (0.05%) was used as the mobile phase. Detection was performed in the full-length sectors of 250 nm, 275 nm, and 350 nm. In the experiments, the ammonium salt of GA (Sigma-Aldrich, Germany) isolated from the root of licorice (Glycyrrhiza glabra L.) was used as a standard sample. Experiments revealed the presence of relatively high concentrations of GA in fraction 2 (Table 1).

Table 1. The amount of GA in the ethanol (70%) extract of the root of the plant licorice (Glycyrrhiza glabra L.)

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Concentration (mg / g relative to dry matter)</th>
<th>Percentage by weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,4</td>
<td>0,24</td>
</tr>
<tr>
<td>2</td>
<td>5,8</td>
<td>0,65</td>
</tr>
<tr>
<td>3</td>
<td>3,6</td>
<td>0,38</td>
</tr>
</tbody>
</table>

In the next series of experiments, the root biomass (5 ± 0.5 g) of licorice (Glycyrrhiza glabra L.) was mixed with a solvent (distilled water + ammonia solution (3%); +150 ° C; 5 MPa) in a ratio of 1:10. The prepared extractant was boiled in a flask for 120 min and cooled and filtered. The HPLC method was used to determine the amount of GA in the extract. Detection was performed under 256 nm wavelength conditions [Tikhomirova et al., 2008; Pp. 71–74] (Table 2).

Table 2. The amount of GA in the root extract of licorice (Glycyrrhiza glabra L.) (n=3–5)

<table>
<thead>
<tr>
<th>Extraction conditions</th>
<th>Determination by gravimetric method</th>
<th>HPLC method</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>The amount of aqueous extract (%)</td>
<td>The amount of filtrate (%)</td>
</tr>
<tr>
<td>Distilled water + ammonia solution (3%); + 150 ° C; 5 MPa</td>
<td>0,074±0,005</td>
<td>0,083±0,04</td>
</tr>
</tbody>
</table>

According to the available literature, the content of GA in the aqueous extract is 13.6% [Mukhopadhyay and Panja, 2008; Pp. 539–545] of the dry plant mass, in some data - 7.3% [Tikhomirova et al., 2008; Pp. 71–74], in the ethanol extract - 0.88%, as well as was found to be equal to 3.64% under ultrasound conditions.

Synthesis and chemical identification of glycyrrhizinic acid and IAA supramolecular complex. The following figure shows glycyrrhizinic acid (GA), β-indole-3-acetic acid (IAA) recorded using the Shimadzu IR – Fure spectrophotometer (Japan) and the Perkin – Elmer Spectrum IR 10.6.1 (USA) in the absorption range of 4000–400 cm.\(^{-1}\) with IR – Fure spectra of the supramolecular complex in a 4: 1 ratio (Fig. 1; Fig. 2).
Vibration range numbers (cm⁻¹)

Figure 1. IR–Fure spectra of glycyrrhizic acid.

IR – Fure spectra were recorded in the absorption range 4000–400 cm⁻¹ using an IR – Fure spectrophotometer (Shimadzu; Japan). The spectra were determined under a tolerance value of > 4 cm⁻¹. The test specimens were pressed in the form of KBr (Merck, Germany) tablets in a spectrally pure state under vacuum (0.1–0.05 mm Hg) to adsorb moisture.

Standard IAA (Eastman Kodak, USA) was used in the experiments. IR – Fure spectra were recorded in the absorption range 4000–400 cm⁻¹ using an IR – Fure spectrophotometer (Perkin – Elmer Spectrum IR - 10.6.1; USA). The spectra were determined under a tolerance value of > 4 cm⁻¹. The test specimens were pressed in the form of KBr (Merck, Germany) tablets in a spectrally pure state under vacuum (0.1–0.05 mm Hg) to adsorb moisture.
IAA IR spectra have been analyzed in detail by a number of researchers. (I_{liquification} = 164 °C) [Kamnev et al., 2001; 565–572-6.; Shetti and Nandibewoor, 2009]. In particular, in the IR-spectrum of ISK the peak-NH group in the area of 3389 cm⁻¹; Peaks in the area 2730–3127 cm⁻¹ - N atoms located in the OH group; It is noted that the peaks in the area of 1701 cm⁻¹ represent the valence oscillations of the carboxyl groups C = O [Shetti and Nandibewoor, 2009]. It is also noted that the absorption areas in the range 3086–3310 cm⁻¹ and the areas in the area 3389 cm⁻¹ confirm the presence of the NH group and the absence of the NH2 group in the IAA structure [Shetti and Nandibewoor, 2009].

The study also analyzed the dynamics of spectral changes based on the coordination changes of functional groups in the conditions formed by the IAA complex (Fe³⁺). [Kamnev and Perfilev, 2000; 205–210-p.]

The following figure shows the IR-Fure spectra of a supramolecular compound of glycyrrhizic acid (GA) and β-indolyl-3-acetic acid (IAA) in a 4:1 ratio (Figure 3).

![Figure 3. IR-Fure spectra of supramolecular complexes of glycyrrhizic acid (GA) and β-indolyl-3-acetic acid (IAA) in a 4:1 ratio.](image)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>I_{liquification} (°C)</th>
<th>R_f (chloroform: ethanol; 3:1)</th>
<th>Productivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA:IAA (4:1)</td>
<td>+179±2</td>
<td>0,8</td>
<td>85,4%</td>
</tr>
</tbody>
</table>
CONCLUSION

Thus, in the conducted experiments, a supramolecular complex of glycyrrhizinic acid and β-indolyl-3-acetic acid in a 4:1 ratio isolated from the root of the plant licorice (Glycyrrhiza glabra L.) was synthesized. The resulting GA:IAA (4:1) complex was chemically identified based on a comparison of the IR – Fure spectra of the starting agents.

REFERENCES

