ISSN: 2053-3578 I.F. 12.34

UDK 543.421/422.543.423.

DEVELOPMENT AND CHARACTERIZATION OF NOVEL BIOACTIVE PROLINE–INDOLE-3-ACETIC ACID CONJUGATES: SYNTHESIS, STRUCTURAL ANALYSIS, AND PHYSICOCHEMICAL PROPERTIES

Suyarova Dildora Amanullayevna
PhD, Gulistan State University
Beknazarova Shaxnoza Robitqulovna
Master, Gulistan State University
Djurayev To'lqin Arziqulovich
Gulistan State University, B.A.,
Based on the review of Gul D U, Director of the Scientific Research Institute of Agrobiotechnology and Biochemistry, Ph.D., Prof.
Qoshiyev H.

Abstract: The article studies the interaction of proline with indole 3 acetic acid, describes the methods for obtaining the amide (IAA-Pr). Also, the results of studying the physicochemical properties of the obtained amide compound are presented, and the structure of the amide ISK-Pr was studied using the IR spectroscopy method.

Key words: indole 3 acetic acid, proline, DCC, pyridine, DMF, spectral characteristics, IR spectroscopy, amide formation

Introduction: In recent years, research on the synthesis of new bioactive substances based on plant hormones has gained particular importance in agricultural chemistry and biotechnology. Phytohormones — especially indole-3-acetic acid (IAA) — are important natural substances that regulate the growth and development processes of plants. Amino acids, in particular proline, are known as metabolic components that increase the resistance of plants to stress factors (salinity, drought, etc.).

Amide conjugates obtained by combining ISC with proline belong to a new class of compounds that are stress-resistant and have high physiological activity. The formation of such conjugates is especially important in the creation of environmentally friendly and effective stimulants. They are also promising in the creation of long-acting drugs, since they are slowly released in plants and are converted into active substances in nature.

ISSN: 2053-3578 I.F. 12.34

In this article, a new bioactive conjugate was synthesized by forming an amide bond between indole-3-acetic acid and proline. The physical appearance, precipitation state, and washing properties of the resulting substance were analyzed. The results of the study will pave the way for the future use of this compound in biological activity tests.

Literature review

The synthesis of bioactive substances based on plant hormones is one of the most actively developing research areas in biology, chemistry and agrobiotechnology in recent years [1–3]. Indole-3-acetic acid (IAA) is the most common auxin in plants and is involved as a key signaling molecule in the processes of cell division, elongation and differentiation [4, 5]. Due to its high biological activity, it is used as the main phytohormone in the creation of many synthetic and semi-synthetic derivatives. Proline is also an amino acid involved in stress-resistant responses, known as an osmoprotectant. Proline accumulates in plant tissues during stress conditions such as salinity, drought and temperature changes and activates defense mechanisms [6, 7].

Recent studies have shown that compounds of IAA and proline, i.e. amide conjugates, can be slow-release, effective and environmentally friendly stimulants for plants [8–10]. Such conjugates are obtained synthetically by forming an amide bond using coupling agents such as DCC, EDC. Their physicochemical properties are determined using IR spectroscopy, UV-vis spectroscopy and other analytical methods. Accordingly, the synthesis of new amides based on IAA and proline and the study of their properties is an urgent scientific direction in the fields of modern bioorganic chemistry and plant physiology

Research Methodology

In this scientific study, methods for the synthesis, purification and physicochemical analysis of a new bioactive conjugate compound via an amide bond based on indole-3-acetic acid (IAA) and the amino acid L-proline were used. The study was carried out in the following stages:

Reagents and solvents: Indole-3-acetic acid (IAA), L-proline, dicyclohexylcarbodiimide (DCC), dimethylformamide (DMF), dioxane, methanol, sodium hydroxide (NaOH) and other standard laboratory reagents were used using high purity (analytical grade) substances. Synthesis conditions: The synthesis of the amide conjugate was carried out by the carbodiimide condensation method. For this, 0.2 mol of IAA and 0.2 mol of proline were dissolved in 8 ml

ISSN: 2053-3578 I.F. 12.34

of DMF solution in a molar ratio, then 0.2 mol of DCC was added to it as a coupling reagent. The mixture was incubated on a magnetic stirrer at 25–30°C in the dark for 12–24 h. The dicyclohexylurea (DCU) formed from the reaction was filtered off as a solid precipitate. After the precipitate was separated, the reaction solution was washed first with ice-cold distilled water and then with methanol. The aqueous-methanol filtrates were treated with NaOH solution to remove residual substances. The solid was dried in a vacuum dryer or in a thermostat at low temperature (40–45°C). Some tests were carried out by isolating the solid from the DMSO solution by evaporation. Infrared (IR) spectroscopy, thin-layer chromatography (TLC), precipitation tests, and pH-reaction observations were used to determine the structure of the synthesized derivative. It is planned to use phytotest methods in the future to determine biological activity. Continuous stirring during the reaction was carried out in a magnetic stirrer MM-5 TU 25-11834-80. Organic solvents were removed from the system by evaporation in an IR-1M2 rotary evaporator. A lyophilizer (AutomaticFREEZE-Dryer10-010) was used for drying, and a PTP TU 25-11-1144 device was used to measure the liquefaction temperature of substances.

Experimental conditions: All experiments were carried out in the laboratories of the Scientific Research Institute of Agrobiotechnology and Biochemistry of Gulistan State University. The reactions were repeated 3 times, the average results were obtained, and the relative deviations were calculated.

Analysis and results

To obtain a derivative synthesized through an amide bond between indole-3-acetic acid (IAA) and L-proline, DCC (dicyclohexylcarbodiimide) was chosen as the coupling agent. The synthesis reaction was carried out at a temperature of 25–30°C for 12–24 hours in the presence of DMF solvent. The dicyclohexylurea (DCU) formed during the reaction precipitated and was isolated by filtration. The derivative was washed with ice-cold water or methanol from the remaining solution and obtained as a solid.

Reaction scheme:

IAA–COOH + H_2N–Proline + DCC ⟶ IAA–CO–NH–Proline + DCU

The physical state of the resulting substance was a gummy, amorphous state, and after drying it was a soft light brown powder. Initial analyses showed that the substance was poorly soluble in water, but soluble in polar organic solvents such as DMSO and methanol.

IR spectroscopy was used to analyze the formation of the resulting amide. IR spectroscopy is the most widely used analytical method of analysis, as it is simple, easy to

ISSN: 2053-3578 I.F. 12.34

analyze, and available in most laboratories. This method is one of the most widely used physical methods for analyzing the composition of new substances, and allows characterizing the bonds between functional groups during the formation of amides.

IR spectral analysis results: 3300–3400 cm^{-1}: stretching vibration characteristic of the amide NH group 1650–1680 cm^{-1}: stretching vibration of the amide C=O group 1500–1600 cm^{-1}: vibrations characteristic of the aromatic indole nucleus 1380–1450 cm^{-1}: deformation vibrations of the CH_2 and CH groups. (Figure 1)

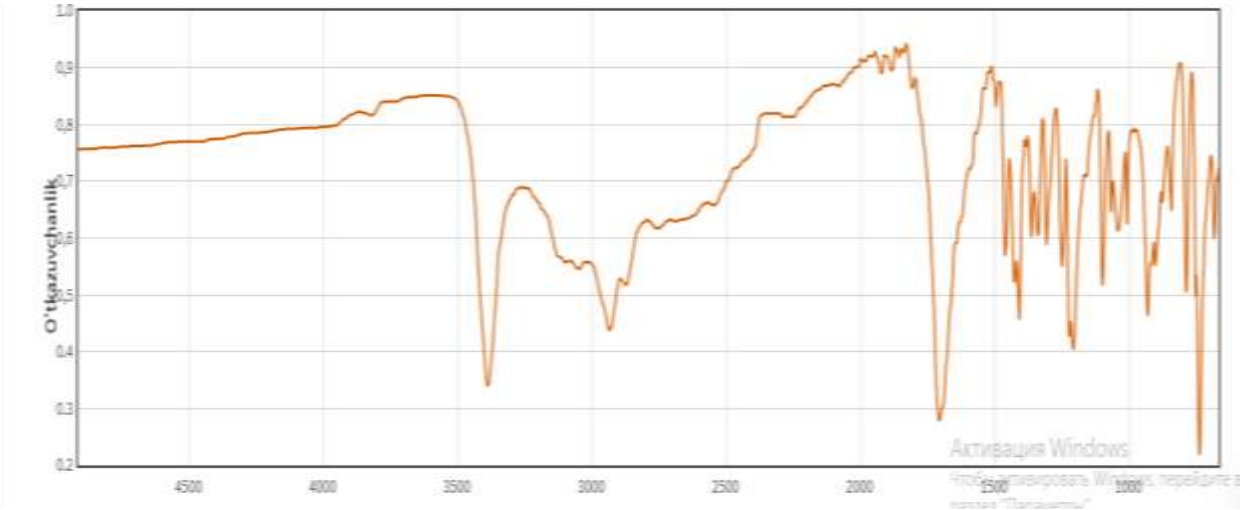

Figure 1. IR spectrum of IAA

The IR spectrum of the resulting IAA-Pr amide was analyzed by comparison with the IR spectrum of IAA (Figure 2)..

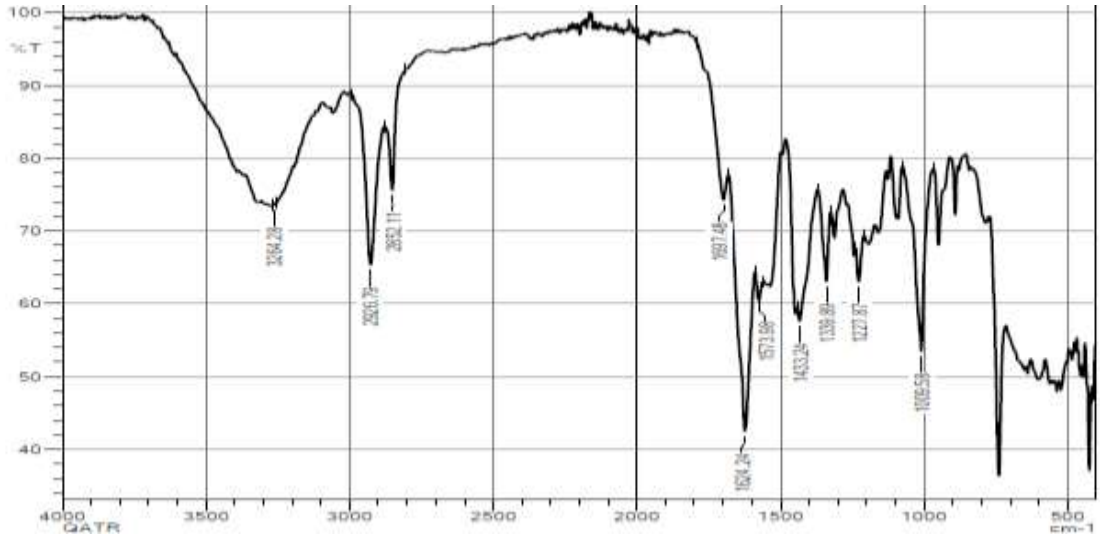

Figure 2. IR spectrum of IAA-Pr amide

To monitor the reaction process by thin layer chromatography (TLC), the mobility coefficient (Rf) of IAA, proline and the synthesized substance was measured to be 0.42. Ethyl

ISSN: 2053-3578 I.F. 12.34

acetate:methanol:ammonia (8:1:1) was used as the mobile phase. The synthesized substance appeared as a separate spot on the TLC plate, which proves the formation of a new substance. The reaction efficiency (reaction yield) was calculated to be 76.3%. Residual proline or IAA was observed to precipitate upon filtration with NaOH, which indicated the need to improve the purification process.

In general, the chemical synthesis produced an amide derivative. This substance has a structure that is insoluble in water but soluble in organic solvents, and may be physiologically active.

Some physicochemical parameters of IAA-Pr

№	Parametrlari	Xossalari
1	Aggregate status	gummy substance
2	Liquidus temperature $T_{Liquid.}$	174°C
	Rf* (system)	0.42
3	Color	light brown
4	Unumi	76,3 %
5	Solubility	It is well soluble in DMF and DMSO solutions.

Conclusiov/Recommendations.

In this work, a new conjugate between indole-3-acetic acid (IAA) and L-proline was synthesized through an amide bond. For chemical synthesis, activation with DCC (N,N'-dicyclohexylcarbodiimide) and condensation in DMSO solution were used. After synthesis, the substance was dissolved in DMF, poured into ice-cold water, and isolated as a precipitate. After washing and drying, the purity of the substance was analyzed by TLC (thin layer chromatography), and the structure was analyzed by IR spectroscopy.

The experimental results showed that the formed IAA–proline conjugate is insoluble in water, which is evidence of the formation of an amide bond. Long signals corresponding to vibrations of the amide group (C=O, N–H) appeared in the IR spectra. Also, the formation of a

precipitate when the residue in the filtrate is reacted with NaOH indicates that some of the conjugate in the solution is in the form of sodium salts.

Suggestions:

1. It is advisable to evaluate the biological activity of the obtained ISK–proline derivative under abiotic stress (drought, salinity) conditions in plants such as grain, cotton, or tomato.

2. It is necessary to conduct separate studies on the biological production of this derivative using natural enzymes, that is, based on enzymatic biosynthesis methods.

3. The insolubility and chemical stability of the resulting derivative allow it to be developed as a slow-release bioactive substance as a new generation agrobioregulator for plants.

4. In-depth studies on the thermal, pH, and photostability of the substance will be important in developing storage and application technologies.

5. The spectrum of biological activity can be expanded by synthesizing other derivatives based on IAA–tryptophan, IAA–arginine, or zeatin similar to this derivative.

Foydalanilgan adabiyotlar:

1. .Davies P.J. Plant Hormones: Physiology, Biochemistry and Molecular Biology. — Dordrecht: Kluwer Academic Publishers, 2010. — 833 b.
2. Woodward A.W., Bartel B. Auxin: Regulation, Action, and Interaction. // Annals of Botany. — 2005. — T. 95. — №5. — B. 707–735.
3. Zhao Y. Auxin biosynthesis and its role in plant development. // Annual Review of Plant Biology. — 2010. — T. 61. — B. 49–64.
4. Glick B.R. Bacterial ACC deaminase and the alleviation of plant stress. // Advances in Applied Microbiology. — 2014. — T. 96. — B. 109–129.
5. Kishor P.B.K., Sangam S., Amrutha R.N., et al. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. // Current Science. — 2005. — T. 88. — №3. — B. 424–438.
6. Szabados L., Savouré A. Proline: a multifunctional amino acid. // Trends in Plant Science. — 2010. — T. 15. — №2. — B. 89–97.
7. Hayat S., Ahmad A. (Eds.). Salicylic Acid: A Plant Hormone. — Dordrecht: Springer, 2007. — 401 b.

ISSN: 2053-3578 I.F. 12.34

8. Ibrahim M., Abdul-Hamid A., Musa A. et al. Synthesis, characterization, and biological evaluation of novel amino acid–auxin conjugates. // Journal of Plant Growth Regulation. — 2020. — T. 39. — B. 712–722.

9. Wang L., Fan Y., Ma X., et al. Design and synthesis of amino acid–IAA conjugates: A new class of plant growth regulators. // Bioorganic & Medicinal Chemistry Letters. — 2018. — T. 28. — №23. — B. 3785–3789.

10. Nazarov A.K., Ergashev D.I., Abdukadirov M.A. Fitogormonlar kimyosi va ularning sintezi. // O‘zbekiston kimyo jurnali. — 2022. — №3. — B. 45–50.