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# SYNTHESIS AND ROOT FORMING ACTIVITY OF THE INCLUSION COMPLEXES OF HETEROCYCLIC THIOANHYDRIDES WITH ARABINOGALTAN

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**Abstract.** *Introduction.* Nitrogen-containing heterocycles are structural elements of many synthetic and endogenous biologically active substances. In this regard, they have particular interest as objects of study of the relationship between the structure and biological activity. *The aim* of this work is the development of new plant growth stimulants, based on nitrogen-containing heterocyclic dithiocarbamic acids and their inclusion complexes with arabinogalactan, and the study of their root-forming activity. *Results and discussion.* Synthesis of sodium dithiocarbamate of 5-amino-1,3,4-thiadiazole-2-thiol and its carbonyl derivatives cycloprapane, 4-fluoro- and 4-methoxybenzoic thioanhydrides has been carried out. Conditions for synthesis of the inclusion complexes of thioanhydrides 2-4 with arabinogalactan (AG) have been developed. The synthesis of the bioorganic inclusion complexes of substrates with AG has been carried out in the mass ratio of the initial reagents 1:1 in dimethyl sulfoxide, the duration of the reactions has been 8 hours at the temperature of 55-60 °C. The inclusion complexes with arabinogalactan have been obtained with the yield of 62-88%. The structure of the synthesized heterocyclic thioanhydrides has been established based on IR and $^{1}H$ and $^{13}C$ NMR spectroscopy. Field tests have been carried out to identify the root-forming activity of the obtained compounds and to determine the effect of the growth stimulants on the growth of the root system of vangutta spirea cuttings. *Conclusion.* It has been found that biocomplex 4-methoxybenzoic (5-mercapto-1,3,4-thiadiazol-2-yl)carbamothioic thioanhydride with arabinogalactan 7 increases the rooting and survival of the planting material and at the concentration of 100 mg/l shows a high yield of the rooted spirea cuttings (90%), as compared with the control (13% ) and standards Kornevin (20%) and AN-16 (36%).

**Key words:** 5-amino-1,3,4-thiadiazole-2-thiol, dithiocarbamate, thioanhydrides, biocomplex, root forming activity

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## 1. Introduction

At present, an interest in arabinogalactan (AG) has been increased significantly due to the high content in plant materials and unique properties [1, 2]. AG occupies a special place among polysaccharides. In recent years, studies of biological activity of AG have intensified sharply. This is facilitated by its properties such as high solubility in water and biological activity, uniquely low viscosity of solutions and biodegradability. AG can serve as a targeted carrier for the delivery of diagnostic and therapeutic agents, as well as enzymes, nucleic acids, vitamins or hormones to specific cells [3-6]. AG is used in medicine in the treatment of various gastrointestinal diseases [7], has adhesive properties and exhibits antimicrobial [8], antimycobacterial [9], antioxidant and immunological [10], antiviral [11] and anticancer [12] activity.

If earlier AG was used mainly as an auxiliary substance in the production of various dosage forms, now due to a detailed study of the properties of AG, it is considered as an independent biologically active additive. Using AG in veterinary medicine is very promising. Feeds with the addition of AG maintain the level of bifidobacteria and lactobacilli in the gastrointestinal tract of animals, thereby improving nutritional efficiency, increasing weight gain and reducing the need for conventional antibiotics [13].

Another direction of using AG is its use as a matrix for creating bioorganic complexes of growth-stimulating action to improve solubility and improve the quality of germination, productivity and yield of plants and crops [14].

## 2. Experimental part

The progress of the reactions and the purity of the products were monitored by thin-layer chromatography on Silufol UV-254 plates with the display of spots of the compounds with iodine vapor, an eluent was ethanol. IR spectra were recorded on a Nicolet 5700 spectrometer in a thin layer. The melting points of the compounds were determined on a Hanon MP450 instrument. The $^{1}H$ and $^{13}C$ NMR spectra of the compounds were recorded on a JNM-ECA 400 spectrometer (Jeol) with the operating frequency of 400 ($^{1}H$) and 100 MHz ($^{13}C$) of the deuterated pyridine-$d_5$ solution. All reagents and solvents were received from Sigma-Aldrich and used without further purification. An elemental analysis was carried out on a Rapid Micro N Cube elemental analyzer (Elementar, Germany, 2015).

*Sodium (5-mercapto-1,3,4-thiadiazol-2-yl)carbamodithioate (1).* A solution of 2.4 g (0.075 mol) of NaOH in 5 ml of distilled water was added to a mixture of 10 g (0.075 mol) 5-amino-1,3,4-thiadiazole-2-thiol in 40 ml of ethanol. Then a

solution of 5.7 g (0.075 mol) carbon disulfide was added dropwise. The reaction mixture was stirred at r.t. at 22°C for 4 h. The solvent was distilled off in a water-jet pump vacuum. The product was purified by recrystallization from acetonitrile. The yield of the compound 1 was 13.21 g (76%), $R_f$ 0.79 (ethanol). Found, %: C 15.69; H 1.02; N 18.29; S 55.29. $C_3H_2N_3NaS_4$.Calculated, %: C 15.58; H 0.87; N 18.17; S 55.45. IR spectra, ν, $cm^{-1}$: 3309 (N−H), 1018 (C=S), 686 (C−S). NMR $^1$H spectra (pyridine–$d_5$), δ, ppm: 7.93 (broad singlet, 2H, SH; NH). NMR $^{13}$C spectra (pyridine–$d_5$), δ, м.д.: 164.7 (SH−CH=N; S−CH=N); 179.3 (C=S).

*Cyclopropanecarboxylic (5-mercapto-1,3,4-thiadiazol-2-yl)carbamothioic thioanhydride (2).* A solution of 0.9 g (0.0086 mol) of cyclopropane chloride was added dropwise to a solution of 2 g (0.0086 mol) sodium (5-mercapto-1,3,4-thiadiazol-2-yl)carbamodithioate in 25 ml of chloroform with stirring. The mixture was stirred at the room temperature of 22°C for two hours. The solvent was distilled off in a water-jet pump vacuum, the product was isolated by recrystallization from ethanol. The yield was 1.55 g (65%), m.p. 225 ºC. Found, %: C 30.45; H 2.37; N 15.27; S 46.41. $C_7H_7N_3OS_4$. Calculated, %: C 30.31; H 2.54; N 15.15; S 46.23. IR spectra, ν, $cm^{-1}$: 3236 (N−H), 1712 (C=O), 1068 (C=S), 690 (C−S). NMR $^1$H spectra (pyridine–$d_5$), δ, ppm: 0.78 (t, 2H, $CH_2$); 1.04 (t, 2H, $CH_2$); 1.82 (m, 1H, CH); 8.07 (br. s, 2H, SH; NH). NMR $^{13}$C spectra (pyridine–$d_5$), δ, м.д.: 9.1 ($2CH_2$), 14.5 (CH), 153.7 (S−CH=N); 162.7 (SH−CH=N); 173.1 (C=O); 185.7 (C=S).

*4-Fluorobenzoic (5-mercapto-1,3,4-thiadiazol-2-yl)carbamothioic thioanhydride (3)* was synthesized in a similar way. The yield was 2.52 g (88%), m.p. 223 ºC. Found, %: C 36.37; H 1.95; N 12.80; S 38.55. $C_{10}H_6FN_3OS_4$. Calculated, %: C 36.24; H 1.82; N 12.68; S 38.70. IR spectra, ν, $cm^{-1}$: 3221 (N−H), 1666 (C=O), 1060 (C=S), 671(C−S). NMR $^1$H spectra (pyridine–$d_5$), δ, ppm: 6.97 (t, 2H, Ar); 8.24 (t, 2H, Ar); 8.11 (br. s, 1H, SH; 1H, NH). NMR $^{13}$C spectra (pyridine–$d_5$), δ, ppm: 115.5, 130.9, 131.2, 163.8 (Ar); 162.8 (SH−CH=N); 163.9 (S−CH=N); 166.4 (C=O); 183.3 (C=S).

*4-Methoxybenzoic (5-mercapto-1,3,4-thiadiazol-2-yl)carbamothioic thioanhy-dride (4)* was synthesized in a similar way. The yield was 2.37 g (81%), m.p. 225 ºC. Found, %: C 38.61; H 2.83; N 12.54; S 37.55. $C_{11}H_9FN_3O_2S_4$. Calculated, %: C 38.47; H 2.64; N 12.23; S 37.34. IR spectra, ν, $cm^{-1}$: 3278 (N−H), 1651 (C=O), 1026 (C=S), 636(C−S). NMR $^1$H spectra (pyridine–$d_5$), δ, ppm: 3.61 (s, 3H, $OCH_3$); 6.89-7.11 (m, 4H, Ar); 8.16 (br. s, 1H, SH; 1H, NH). NMR $^{13}$C spectra (pyridine–$d_5$), δ, ppm: 55.4 ($OCH_3$); 114.2, 130.1, 130.7 (Ar); 162.7 (SH−CH=N), 163.6 (S−CH=N); 166.3 (C=O); 183.4 (C=S).

*Complex of cyclopropanecarboxylic (5-mercapto-1,3,4-thiadiazol-2-yl)carba-mothioic thioanhydride with arabinogalactan (5).* A mixture of 0.6 g of AG and 0.6 g of cyclopropanecarboxylic (5-mercapto-1,3,4-thiadiazol-2-yl)carbamothioic thioanhydride (2) in the mass ratio of 1:1 were dissolved in 10 ml of DMSO and stirred at the temperature of 55-60$^0$C within 8 hours. After completion of the reaction, the complex was precipitated with acetone. The

formed precipitate was filtered off and dried. An inclusion complex with AG 5 was obtained as a white powder, the yield was 1.06 g (88%).

*Complex 4-fluorobenzoic (5-mercapto-1,3,4-thiadiazol-2-yl)carbamothioic thioanhydride with arabinogalactan (6)* was synthesized in a similar way. The yield was 1.02 g (78%).

*Complex 4-methoxybenzoic (5-mercapto-1,3,4-thiadiazol-2-yl)carbamothioic thioanhydride with arabinogalactan (7)* was synthesized in a similar way. The yield was 0.86 g (62%).

Field tests were carried out in the Institute of Botany and Phytointroduction to determine the root-forming activity of the new synthesized compounds.

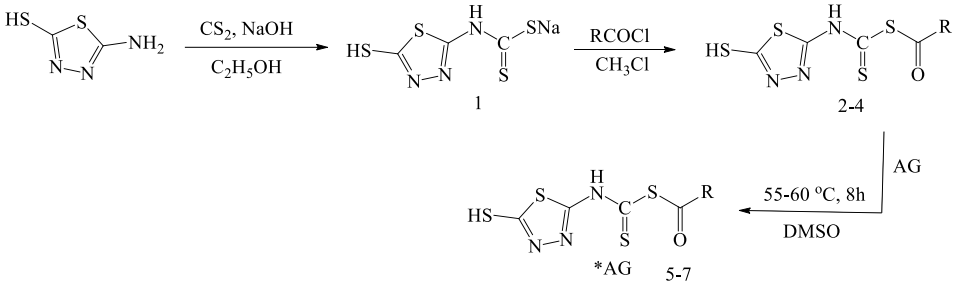
Cuttings of spirea vanhutta (Spiraaeaxvanhouttei) were used in the experiment. Semi-lignified cuttings of spirea 8-9 cm long with three buds in an amount of 30 pieces were soaked in the solutions of substances with concentrations (50 mg/l, 100 mg/l) for 6 hours. The cuttings were planted in a greenhouse with a prepared substrate (soil:sand). The duration of the experiment was 5 months. The survival rate of the planted cuttings was determined one month after planting. The following parameters: the number of rooted cuttings; the formed roots; the length of the roots were used to determine the biological activity.

The scheme of the experiments on cuttings:

1. Control (Water);
2. Kornevin (standard), (50 mg/l, 100 mg/l);
3. EN-16 (standard), (50 mg/l, 100 mg/l);
4. Compound 5 (50 mg/l, 100 mg/l);
5. Compound 6 (50 mg/l, 100 mg/l);
6. Compound 7 (50 mg/l, 100 mg/l).

**3. Results and discussion**

In order to synthesize new carbonyl dithiocarbamine derivatives, heterocyclic thioanhydrides have been synthesized from sodium (5-mercapto-1,3,4-thiadiazol-2-yl)carbamodithioate. Acylation of dithiocarbamate has been carried out by reacting sodium (5-mercapto-1,3,4-thiadiazol-2-yl)carbamodithioate 1 with acid chlorides (cyclopropane, 4-fluorobenzoic and 4-methoxybenzoic) in chloroform at the room temperature for 3 hours. Heterocyclic thioanhydrides 2-4 have been obtained in the form of crystals with the yield of 65-88%.




2, 5 R = cycle-$C_3H_5$; 3, 6 R = 2-$FC_6H_4$; 4, 7 R = 4-$CH_3OC_6H_4$.

AG is a promising synthon for the synthesis of water-soluble complexes on its basis due to its diverse biological activity and the content of hydroxyl groups in its structure. The synthesis of the supramolecular inclusion complexes of heterocyclic thioanhydrides with arabinogalactan has been carried out in dimethyl sulfoxide at the temperature of 55-60°C for 10 hours. As a result, the inclusion complexes with arabinogalactan have been obtained with the yield of 62-88%.

The structure of the synthesized compounds 2-4 has been established based on elemental analysis, IR spectroscopy and $^1H$ and $^{13}C$ NMR spectroscopy.

There is an absorption band of stretching vibrations of the C=S group in the region of ν 1026-1068 $cm^{-1}$ in the IR spectra of thioanhydrides 2-4. Stretching vibrations of the C–S bond are manifested in the region of ν 636-690 $cm^{-1}$ and absorption bands are also observed in the regions of ν 3221-3278 $cm^{-1}$, which are characteristic for the NH group. The presence of a characteristic absorption band in the region ν 1651-1712 $cm^{-1}$ confirms the introduction of the C=O group into the molecules of the new compounds.

In the $^{13}C$ NMR spectra of compounds 2-4, the signals for the carbon atoms of the C=O and C=S groups are found in the low field region δ 166.3-173.1 ppm and 183.3-185.7 ppm, respectively.

The study of the effect of root-forming activity of the synthesized compounds on cuttings of spirea vanhutte (Spiraaeaxvanhouttei) has been carried out in the field.

The treatment of cuttings with the compounds has led to an increase in the survival rate of the rooted cuttings. A more stable positive trend of root formation has been noted in variants 6 and 7, taking into account the applied concentration and the degree of development of the root system.

A high percentage of rooting of semi-lignified cuttings has been recorded at the concentration of 100 mg/l for the compound 7-90%, which exceeds the control by 6.9 times, and the reference standard (Kornevin) by 4.5 times and (AN-16) by 2.5 times (Figure 1).

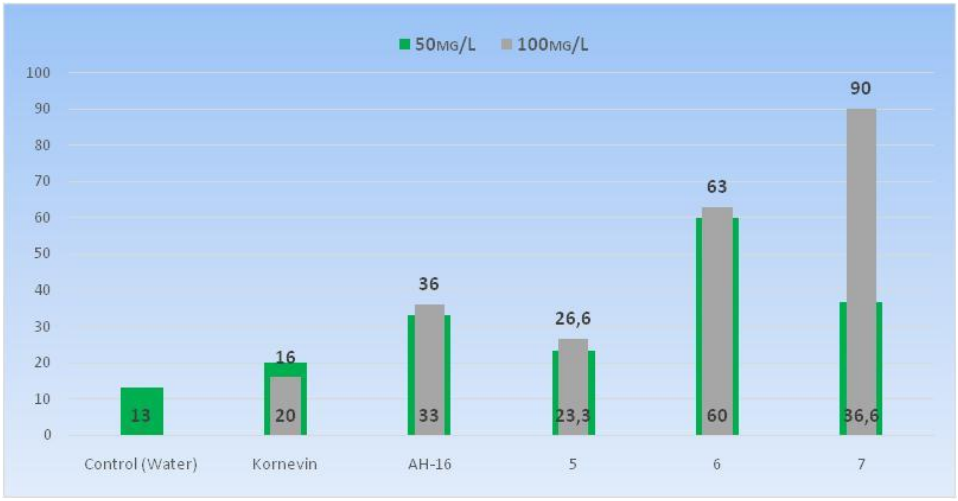



**Figure 1** – Influence of the compounds 5-7 on the number of rooted cuttings of spirea vanhuttei (Spiraaeaxvanhouttei) at the concentrations of 50 and 100 mg/l.

An analysis of the test results has shown that the compound 7 at the concentration of 50 mg/l exhibits a high root-forming activity on the cuttings as compared with the control and standards (Kornevin and AN-16). The number of roots and the length of the formed roots at the concentration of 50 mg/l have been: for the control 2.75 pcs and 4.01 cm, respectively; for Kornevin standard 3.67 pieces and 3.4 cm; for AN-16 4 pcs and 2.42 cm and using the compound 7, 4.62 pcs and 2.29 cm.

The compounds 5, 6 have shown the same root-forming activity at the level of the reference standard AN-16 (Figure 2).

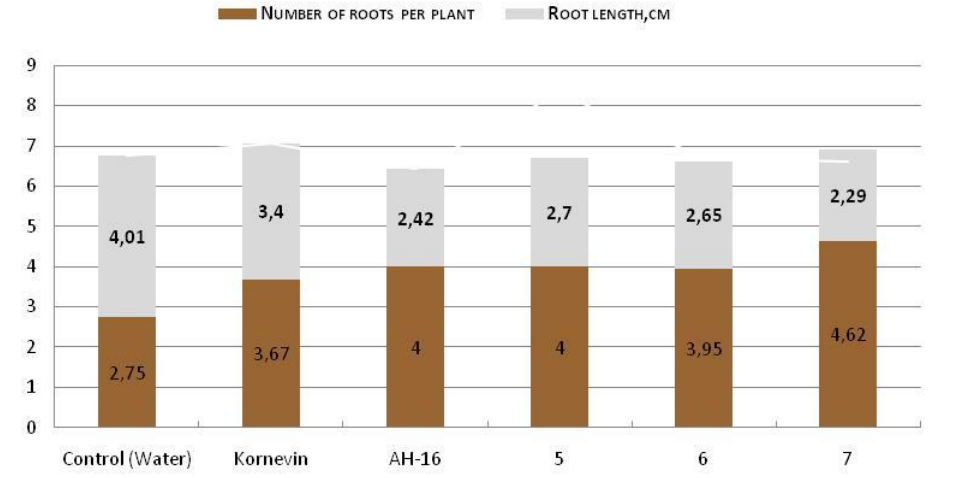



**Figure 2** – The effect of the compounds 5-7 on the number and length of roots of spirea vanhuttei (Spiraaeaxvanhouttei) at a concentration of 50 mg/l.

The compound 7 has shown a high number of root-forming centers (4.88 pcs) at the concentration of 100 mg/l as compared with the control (2.75 pcs), Kornevin standard (3.2 pcs) and AN-16 (3.5 pcs) (Figure 3).

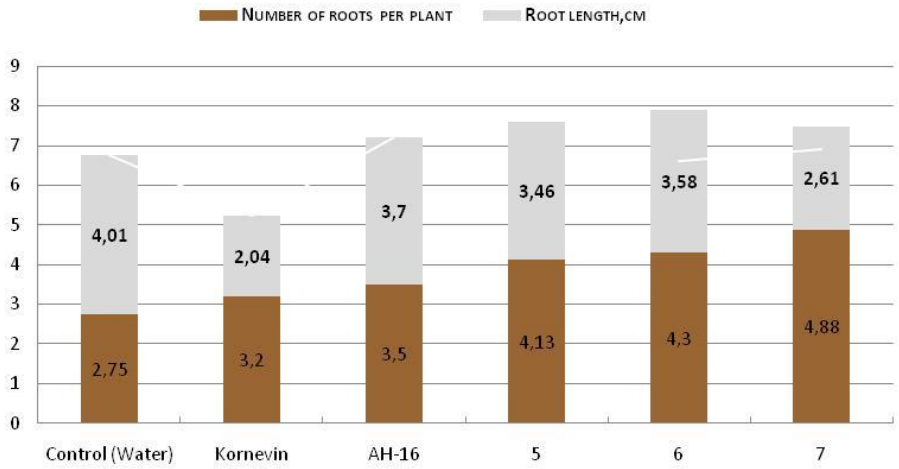



**Figure 3** – An influence of the compounds 5-7 on the number and length of roots of spirea vanhuttei (Spiraaeaxvanhouttei) at a concentration of 100 mg/l.

## 4. Conclusion

New heterocyclic thioanhydrides of dithiocarbamic acids, based on sodium (5-mercapto-1,3,4-thiadiazol-2-yl)carbamodithioate, and their supramolecular inclusion complexes with the natural polysaccharide arabinogalactan have been synthesized as a result of the performed study.

It has been established that using the inclusion complexes of heterocyclic thioanhydrides with arabinogalactan increases the rooting and survival of the planting material. The complex of 4-methoxybenzoic (5-mercapto-1,3,4-thiadiazol-2-yl)carbamothioic thioanhydride with arabinogalactan at the concentration of 50 mg/l has provided up to 90% survival of semi-lignified spirea cuttings, and contributed to the increased root formation in the initial period of growth.

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**Conflict of Interest:** All authors declare that they have no conflict of interest.

## АРАБИНОГАЛАКТАНМЕН ГЕТЕРОЦИКЛДІ ТИОАНГИДРИДТЕРДІҢ КЕШЕНДІ ҚОСЫЛЫСТАРЫНЫҢ СИНТЕЗІ ЖӘНЕ ТАМЫРТҮЗГІШ БЕЛСЕНДІЛІГІ

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**Түйіндеме.** *Кіріспе.* Құрамында азот бар гетероциклдер көптеген синтетикалық және эндогендік биологиялық белсенді заттардың құрылымдық элементтері болып табылады. Осыған байланысты олар құрылым мен биологиялық белсенділіктің байланысын зерттеу объектілері ретінде ерекше қызығушылық тудырады. *Жұмыстың мақсаты* құрамында азот бар гетероциклді дитиокарбамин қышқылдары, олардың арабиногалактанмен кешенді қосылыстары негізінде жаңа өсімдіктің өсуін ынталандырғыштар алу және олардың тамыртүзгіш белсенділігін зерттеу болып табылады. *Нәтижелер және талқылау*. 5-амин-1,3,4-тиадиазол-2-тиол натрий дитиокарбаматының және оның карбонилді циклопропан, 4-фтор- және 4-метоксибензой тиоангидридтері туындыларының синтезі жүзеге асырылды. 2-4 тиоангидридтердің арабиногалактанмен (АГ) кешенді қосылыстарын синтездеу жағдайлары жасалынды. Арабиногалактанмен субстраттардың биоорганикалық кешенді қосылыстарының синтезі диметилсульфоксидтегі бастапқы реагенттердің 1:1 массалық қатынасында жүргізілді, реакциялардың ұзақтығы 55-60 °С температурада 8 сағатты құрады. Синтезделген гетероциклді тиоангидридтердің құрылымы ИҚ спектроскопиясы және $^{1}$Н және $^{13}$С ЯМР спектроскопиясы мәліметтері негізінде анықталды. Алынған қосылыстардың тамыртүзгіш белсенділігін анықтау және вангутта спиреясы кесінділерінің тамыр жүйесіне өсуін ынталандыратын әсерін анықтау үшін далалық сынақтар жүргізілді. *Қорытынды.* Карбамотио қышқылы 4-метоксибензой (5-меркапто-1,3,4-тиадиазол-2-ил) тиоангидридінің арабиногалактанмен 7 биокешені отырғызу материалының тамырлануы мен жерсінуін арттырады және 100 мг/л концентрацияда бақылаумен (13%), Корневин (20%) және АН-16 (36%) стандарттарымен салыстырғанда спиреяның тамырлы кесінділерінің жоғары шығымдылығын (90%) көрсетті.

**Түйін сөздер:** 5-амино-1,3,4-тиадиазол-2-тиол, дитиокарбамат, тиоангидридтер, биокешендер, тамыртүзгіш белсенділік

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## СИНТЕЗ И КОРНЕОБРАЗУЮЩАЯ АКТИВНОСТЬ КОМПЛЕКСОВ ВКЛЮЧЕНИЯ ГЕТЕРОЦИКЛИЧЕСКИХ ТИОАНГИДРИДОВ С АРАБИНОГАЛАКТАНОМ

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**Резюме.** *Введение.* Азотсодержащие гетероциклы являются структурными элементами многих синтетических и эндогенных биологически активных веществ. В связи с этим, они представляют особый интерес как объекты изучения взаимосвязи структуры и биологической активности. *Целью* данной работы является разработка новых стимуляторов роста растений на основе азотсодержащих гетероциклических дитиокарбаминовых кислот, их комплексов включения с арабиногалактаном и изучение их корнеобразующей активности. *Результаты и обсуждение.* Осуществлен синтез дитиокарбамата натрия 5-амино-1,3,4-тиадиазол-2-тиола и его карбонильных производных циклопропанового, 4-фтор- и 4-метоксибензойных тиоангидридов. Разработаны условия синтеза комплексов включения тиоангидридов 2-4 с арабиногалактаном (АГ). Синтез биоорганических комплексов включения субстратов с АГ проводили при массовом соотношении исходных реагентов 1:1 в диметилсульфоксиде, продолжительность реакций составила 8 часов при температуре 55-60 °С. Комплексы включения с арабиногалактаном получены с выходами 62–88%. Строение синтезированных гетероциклических тиоангидридов установлено на основании данных ИК спектроскопии и спектроскопии ЯМР $^{1}$H и $^{13}$C. Проведены полевые испытания по выявлению корнеобразовательной активности полученных соединений и определению действия стимуляторов роста на рост корневой системы черенков спиреи вангутта. *Заключение.* Установлено, что биокомплекс 4-метоксибензойного (5-меркапто-1,3,4-тиадиазол-2-ил) тиоангидрида карбамотиевой кислоты с арабиногалактаном 7 повышает укореняемость и приживаемость посадочного материала и в концентрации 100 мг/л показывает высокий выход укорененных черенков спиреи (90%) по сравнению с контролем (13%), стандартами Корневин (20%) и АН-16 (36%).

**Ключевые слова:** 5-амино-1,3,4-тиадиазол-2-тиол, дитиокарбамат, тиоангидриды, биокомплексы, корнеобразующая активность

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