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SUPRAMOLECULAR 3-d METAL 1,10-PHENANTHROLINE TARTRATOSTANNATES(IV) AS MODIFIERS OF α-L-RHAMNOSIDASE ACTIVITY OF *CRYPTOCOCCUS ALBIDUS*, *EUPENICILLIUM ERUBESCENS*, AND α-GALACTOSIDASE ACTIVITY OF *PENICILLIUM RESTRICTUM*

In recent years, the particular interest of researchers is focused on such enzymes as α-L-rhamnosidase and α-galactosidase. These enzymes are considered useful for various applications. α-L Rhamnosidases may be applied for debittering of citrus fruit juices, due to the less bitter taste of the derhamnosylated flavonones, for rhamnose production, and for the determination of the anomeric configuration in polysaccharides, glycosides and glycolipids. These enzymes may enhance wine aroma and flavonoid bioavailability, or assist in the synthesis of pharmaceuticals. α-Galactosidase finds application in many areas. It is widely used in the food industry to improve the quality of soy products by hydrolyzing indigestible galactosides such as raffinose and stachyose, in the processing of raw materials in order to increase the yield of sugar from molasses, and for the biotransformation of human blood erythrocytes of group B (III) in universal donor erythrocytes, as well as in enzyme therapy of some congenital disorders of sphingolipid metabolism. Earlier, as a result of screening microorganisms of different taxonomic groups, we has selected active α-L-rhamnosidase and α-galactosidase producers. One way to increase their activity is using various effector compounds capable of modifying the enzyme activity. The study of the influence of various effectors is one of the priority areas of modern research in biochemistry, biocoordination chemistry, and biotechnology. Recent advantages in the area of biocoordination chemistry revealed high activating properties of double heterometallic mixed-ligand coor-

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dination compounds with germanium(IV)/tin(IV) tartaric complex anions and 1,10-phenanthroline/2,2`-bipyridine d-metallic cations. ***The aim*** *is to estimate the enzyme-effector activity of five similar tartratostannates for the* α*-L-rhamnosidases of Cryptococcus albidus, Eupenicillium erubescens, and α-galactosidase of Penicillium restrictum.* ***Methods.*** The *activity of* α-Galactosidase *was determined using p-nitrophenyl-α-D-galactopyranoside («Sigma», USA) as a substrate. The activity of α-L-rhamnosidase was determined using the Davis method. As modifiers of enzyme activity,* $[Fe(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 2H_2O$ *(1),* $[Co(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 8H_2O$ *(2),* $[Ni(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 2H_2O$ *(3),* $[Cu(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 2H_2O$ *(4), and* $[Zn(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 6H_2O$ *(5) were used.* ***Results.*** *The study of the effect of complexes 1—5, which are supramolecular salts consisting of the same tartrate stannate anion (electrophilic agent) and a 1,10-phenanthroline d-metal cation (nucleophilic agent), on the Cryptococcus albidus, Eupenicillium erubescens* α-L-rhamnosidases*, and Penicillium restrictum α-galactosidase showed that the compounds tested had a different influence on the enzymes' activity. The catalytic activity of* α*-L-rhamnosidase is significantly influenced by all complexes. The effectiveness of compounds 1—5 for P. restrictum* α*-galactosidase was less pronounced in comparison with C. albidus and E. erubescens* α*-L-rhamnosidases. It was mostly at the control level. There was observed a certain pattern in the influence of complexes on* α*-L-rhamnosidases of Cryptococcus albidus and Eupenicillium erubescens. Compounds 2 and 5 turned out to be the most effective and activated enzymes by 500-900%.* ***Conclusions.*** *Compound 2* $[Co(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 8H_2O$ *is promising for further use as an effector of the α-L-rhamnosidase activity.*

Keywords: *Cryptococcus albidus, Eupenicillium erubescens, Penicillium restrictum,* α*-L-rhamnosidase, α-galactosidase activity, tartratostannates coordination compounds.*

In recent years, the particular interest of researchers is focused on glycosidases, enzymes of the class of hydrolases (O-glycoside hydrolases), capable to catalyze the hydrolysis of O-glycosidic bonds in glycosides, oligo-, polysaccharides, glycolipids, and other glycoconjugates. Such enzymes are α-L-rhamnosidase (E.C. 3.2.1.40), which hydrolytically cleaves the terminal unreduced α-1,2, α-1,4 and α-1,6 linked L-rhamnose residues in natural products such as naringin, rutin, quercitrin, hesperidin, and other rhamnose-containing glycosides [1], as well as α-galactosidase (E.C. 3.2.1.22) capable to split the terminal nonreducing D-galactose residues from α-D-galactosides including galactooligosaccharides, galactolipids, and galactoproteins. These enzymes are considered to be useful for various applications. α-L Rhamnosidases may be applied for debittering of citrus fruit juices, due to the less bitter taste of the derhamnosylated flavonones, for rhamnose production, and for the determination of the anomeric configuration in polysaccharides, glycosides, and glycolipids. These enzymes may enhance wine aroma and flavonoid bioavailability, or assist in the synthesis of pharmaceuticals. α-Galactosidase finds application in many areas. It is widely used in the food industry to improve the quality of soy products by hydrolyzing indigestible galactosides such as raffinose and stachyose [2], in the processing of raw materials, in order to increase the yield of sugar from molasses for the biotransformation of human blood erythrocytes of group B (III) in universal donor erythrocytes and enzyme therapy of some congenital disorders in sphingolipid metabolism [3].

Earlier [4], as a result of screening microorganisms of different taxonomic groups, we have selected active α-L-rhamnosidase and α-galactosidase producers. One way to increase their activity is using various effector compounds capable of modifying the enzyme activity. The study of the influence of various effectors is one of the priority areas of modern research in biochemistry, biocoordination chemistry, and biotechnology. Recent advantages in the area of biocoordination chemistry revealed high activating properties of double heterometallic mixed-ligand coordination compounds with germanium(IV)/tin(IV) tartaric complex anions and 1,10-phenanthroline/2,2`-bipyridine d-metallic cations. The significant influence on the α-L-rhamnosidases *Penicillium tardum* IMV F-100074 and *Penicillium restrictum* IMV F-100139 has been already established for the raw of isostructural tartratostannates

$[M(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]$ (M(II) = Fe, Co, Ni, Cu, Zn) [5]. Computation studies of them, insights of the intermolecular interactions and crystal voids calculations have allowed interpreting the specific patterns in the biological activity. The present study extends the research and reports the enzyme-effector activity of the five similar tartratostannates on the α-L-rhamnosidases of *Cryptococcus albidus, Eupenicillium erubescens,* and also *α-galactosidase* of *P. restrictum*.

Materials and methods. α-L-Rhamnosidases of *C. albidus, E. erubescens* and *α-galactosidase* of *P. restrictum* are investigated in the present study. The processes of culture growing and enzymes production and purification have been described by us earlier [6, 7].

As modifiers of enzyme activity, the complexes $[Fe(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 2H_2O$ (1), $[Co(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 8H_2O$ (2), $[Ni(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 2H_2O$ (3), $[Cu(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 2H_2O$ (4), and $[Zn(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 6H_2O$ (5) were used. Synthesis of the complexes, study of their properties by the X-Ray, IR- and MS-analyzes have been described by us earlier [5]. Structural data were specified at the CCDC Cambridge Crystallographic Data Center (CCDC) under the numbers 1995154 (1), 1985185 (2), 1985186 (3), 1995153 (4), 1985187 (5). Complexes 1—5 were dissolved in 0.1% DMSO and used in concentrations 0.1 and 0.01%; the duration of interaction with enzymes was 1 and 24 hr.

α-L-Rhamnosidases of *C. albidus, E. erubescens* and *α-galactosidase* of *P. restrictum* were obtained by precipitation with ammonium sulfate at a concentration of 90% in the culture liquid supernatant. Further purification on columns Toyopearl HW-55 («Toyo Soda», Japan) and Fractogel DEAE-650-м («Merck», Germany) was carried out as described by us earlier [6, 7].

The activity of α-L-rhamnosidases was measured by the Davis method [8], using naringin as a substrate.

The α-Galactosidase activity was determined using *p*-nitrophenyl-α-D-galactopyranoside (Sigma, USA) as a substrate [9]. For this, 0.1 mL of the enzyme solution was mixed with 0.2 mL 0.1 M phosphate-citrate buffer (PCB) pH 5.2, and 0.1 mL 0.01 M substrate solution in PCB. The reaction mixture was incubated for 10 min at 37 °C. The reaction was stopped by adding 2 mL of 1 M sodium bicarbonate. The amount of released nitrophenol after hydrolysis was determined colorimetrically by the absorption at 400 nm. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μMol of *p*-nitrophenol per min at 37 °C in 0.1 M PCB, pH 5.2. When studying the effect of various germanium-containing compounds on the activity of enzymes, we used concentrations of 0.1 and 0.01% and time of exposure of 0.5 hr and 24 hr. The studied compounds were dissolved in 0.1% DMSO.

All experiments were performed in no less than 3—5 replications. Statistical processing of the results of the experimental series was carried out by standard methods using Student's t-test at the 5% significance level. The results presented in graphs were processed using Microsoft Excel 2007.

Results. The study of the effect of complexes 1—5, which are supramolecular salts consisting of the same tartrate stannate anion (electrophilic agent) and a 1,10-phenanthroline d-metal cation (nucleophilic agent), showed that, depending on the concentration and exposure time, they affect the activity of the studied enzymes differently. So, at a concentration of 0.01% and an exposure time of 1 hr, compound 2 showed the greatest activating influence (43%) on the α-L-rhamnosidase activity of *C. albidus*, somewhat less — compounds 1 (36.6%), 3 (20%), 4 (16%), and 5 (13%) (Fig. 1, *a, b*). Similar results were obtained with an increase in exposure time to 24 hours: at a concentration of 0.01%, compounds 2 (36%), 5 (32%), 4 (29%), as well as 1 and 3 (27%) show the activating effect.

An increase in the concentration of the studied compounds leads to a more significant effect on the activity of *C. albidus* α-L-rhamnosidase

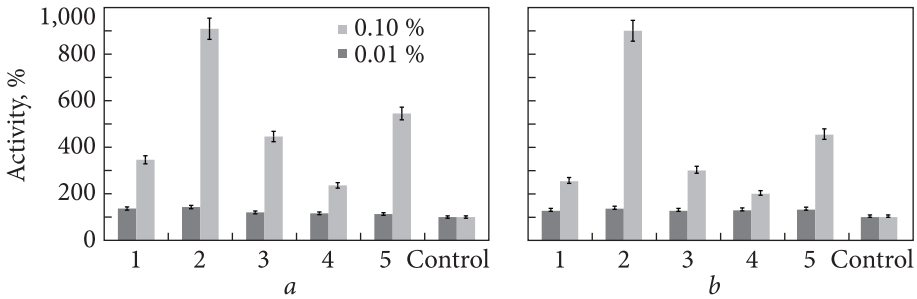

Fig. 1. Influence of compounds 1—5 on the activity of *C. albidus* α-L-rhamnosidase: exposure time 1 hr (*a*), exposure time 24 h (*b*)

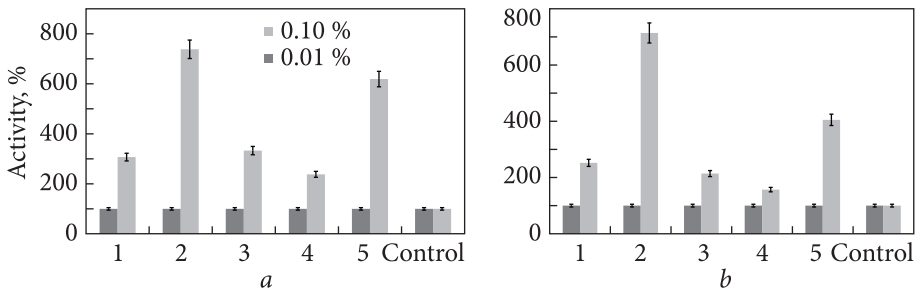

Fig. 2. Influence of compounds 1—5 on the activity of E. erubescens α-L-rhamnosidase: exposure time 1 hr (*a*), exposure time 24 hr (*b*)

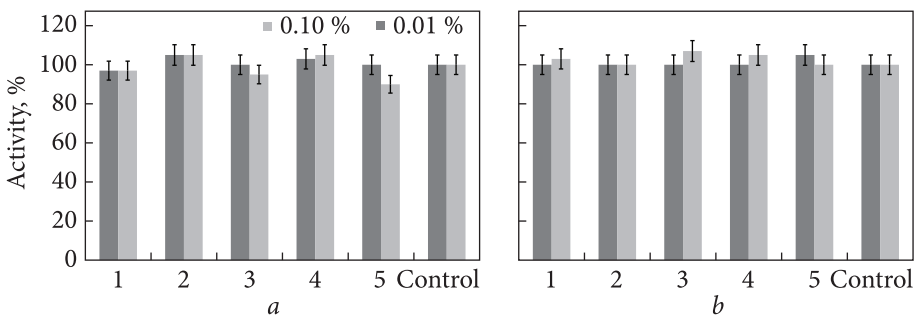

Fig. 3. Influence of compounds 1—5 on the activity of *P. restrictum α-galactosidase:* exposure time 1 hr (*a*), exposure time 24 hr (*b*)

(Fig. 1, *a, b*). At an exposure time of 1 hour, compound 2 showed the greatest activating effect (by 9 times), compounds 5 (by 5.45 times), 3 (by 4.4 times), 1 (by 3.5 times) and 4 (more than 2 times). A similar pattern was also found with an increase in the exposure time to 24 hours: the greatest activation of enzyme activity occurred under the influence of compound 2 (by 9 times), somewhat less — by compounds 5 (by 4.5 times), 3 (by 3 times), 1 (by 2.54 times), and 4 (2 times). In this case, changing the exposure time slightly affects the enzyme activity.

In general, it can be noted that the α-L-rhamnosidase activity of *C. albidus* is most affected by the compounds at a concentration of 0.1% and an exposure time of 1 hr.

The study of the influence of coordination compounds on the activity of *E. erubescens* α-L-

rhamnosidase (Fig. 2, *a, b*) showed that their effect was different depending on the concentration used. So, at a concentration of 0.01%, the activity indexes did not differ from the control ones, regardless of the exposure time. The effect of the compounds on the activity of *E. erubescens* α-L-rhamnosidase at a concentration of 0.1% was more diverse. With an exposure time of 1 hr (Fig. 2, *a*), the activating effect of all the studied compounds was noticeable: the largest (by 7.38 and 6.19 times) was for 2 and 5, respectively, and somewhat less — for 3, 1, and 4 (3.3, 3.07, and 6.19, respectively). With an increase in the exposure time to 24 hours, a decrease in the activating effect of all compounds tested was observed at a given concentration.

The effect of compounds 1—5 on *P. restrictum* α-galactosidase was less pronounced in comparison with *C. albidus* and *E. erubescens* α-L-rhamnosidases

Activity was mostly at the control level. Only compounds 1, 3 and 5 at a concentration of 0.1% with an exposure time of 1 hr inhibited the activity of *P. restrictum* α-galactosidase by 3—10%, while compounds 2 and 4 in both concentrations increased activity by only 3—5%. With an increase in the exposure time to 24 hr, the activity was increased for compounds 1 (3%), 3 (7%), and 4 (5%) at a concentration of 0.1% and compound 5 (5%) at a concentration of 0.01%.

Discussion. In the last few years, enzymes of the microbial origin have been widely studied as catalysts in the various biotechnological processes such as food preservation, development of medicines, diagnostics and other environmental processes. Their advantages over plant and animal ones (easy, cost-effective, and consistent production) stimulate growing request for the discovery of novel highly-productive strains of microorganisms and obtaining enzymes from them. Today, preparations of α-L-rhamnosidase and α-galactosidase are not available in Ukraine, and the high price of foreign (USA) commercial enzyme products significantly impedes their use in industrial technologies in Ukraine. With this in mind, we searched for effective producers of highly specific α-L-rhamnosidases and α-galactosidases among the strains of microorganisms of various taxonomic groups – bacteria, micromycetes, and yeast from the depositary of IMV, NAS of Ukraine. We selected the most active strains as producers of α-L-rhamnosidase (*P. tardum*, *E. erubescens* [7] and *C. albidus* [6]) and α-L-galactosidase (*P. restrictum).* At the same time, one more important research strategy to increase enzyme activity is the use of cheap, biocompatible and effective enzyme modifiers. According to the current hypothesis, the high conformation mobility of a specific enzyme allows formation of temporary bonds with an effector that stabilize the structure and make reaction thermodynamically more profitable. Earlier [5], we have established that complexes $[M(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]$ (M=Fe(II), Co(II), Ni(II), Cu(II), Zn(II)) are perspective enzyme effectors for *P. tardum* and *P. restrictum* α-L-rhamnosidase. Since the effect of various effectors differ from the strain and type of microorganism producing the enzyme, in this work we studied the effect of substances 1—5 on the activity of *E. erubescens*, *C. albidus* α-L-rhamnosidases, and *P. restrictum* α-L-galactosidase.

The catalytic activity of α-L-rhamnosidase is significantly influenced by all complex compounds 1—5 owing to their unique structure. The complex cation $[M(phen)_3]^{2+}$ and anion $[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]^{4+}$ in their composition contain different charges and therefore can bind to the corresponding sites of enzyme and increase the rate of enzymatic reactions. Furthermore, according to the mechanism of multimetallic catalysis, two metal centers can act in a synergy to activate and transform the substrate when one of them performs as an «assisting» metal and reinforces the other «active» one [10—12]. Due to this mechanism, the higher activity of compounds 1—5 rather for

α-L-rhamnosidase than for α-galactosidase is explained by the synergetic action, complementarity of components, and specific steric, compositional properties of the substrate. Nevertheless, there is observed a certain pattern in the influence of complexes on α-L-rhamnosidases of *C. albidus* and *E. erubescens*. Compounds 2 $[Co(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 8H_2O$ and 5 $[Zn(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 6H_2O$ turned out to be the most effective and activated enzymes by 500—900%. Previous studies of their crystal structures [5] have revealed that all of them have low percent of crystal voids and big number of water molecules. The presence of 8/6 H_2O in their composition together with the special activity of Co(2)/Zn(5) becomes crucial for the activation properties and leads to the formation of stronger electrostatic interactions with the active sites of the substrate making it more conformationally stable.

Conclusions. Thereby a significant influence of the metallic cation $[M(phen)_3]^{2+}$ in the isostructural compounds on the enzymatic activity of α-L-rhamnosidase and α-*galactosidase* is observed. It is established that the presence of Co and Zn, as well as the bigger number of water molecules in the crystal hydrates of compounds 1—5 leads to the intensification of their properties. Nevertheless, namely the nature of the studied enzyme (α-L-rhamnosidases or *α-galactosidase*) plays *a* crucial role in the catalytic activity. Compound 2 $[Co(phen)_3]_2[Sn_2(\mu\text{-}Tart)_2(H_2Tart)_2]\cdot 8H_2O$ is promising for further use as an effector of the α-L-rhamnosidase activity.

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НАДМОЛЕКУЛЯРНІ 3-d МЕТАЛІЧНІ 1,10-ФЕНАНТРОЛІН ТАРТРАТОСТАНАТИ (IV) ЯК МОДИФІКАТОРИ α-L-РАМНОЗИДАЗНОЇ АКТИВНОСТІ *CRYPTOCOCCUS ALBIDUS, EUPENICILLIUM ERUBESCENS* ТА α-ГАЛАКТОЗИДАЗНОЇ АКТИВНОСТІ *PENICILLIUM RESTRICTUM*

В останні роки особливий інтерес дослідників зосереджений на таких ферментах, як α-L-рамнозидаза та α-галактозидаза. Ці ферменти вважаються корисними для різних застосувань. α-L Рамнозидази можуть бути використані для зменшення гіркоти цитрусових соків (через менш гіркий смак дерамнозильованих флавононів), для виробництва рамнози, а також для визначення аномерної конфігурації в полісахаридах, глікозидах і гліколіпідах. Ці ферменти можуть посилити аромат вина та біодоступність флавоноїдів або сприяти синтезу фармацевтичних препаратів. α-Галактозидаза знаходить застосування в багатьох областях. Фермент широко використовується в харчовій промисловості для підвищення якості соєвих продуктів шляхом гідролізу неперетравлюваних галактозидів, таких як рафіноза і стахіоза, при переробці сировини з метою збільшення виходу цукру з меляси, для біотрансформації еритроцитів крові групи В (III) в універсальні донорські еритроцити та в ензимотерапії деяких вроджених порушень сфінголіпідного обміну. Раніше в результаті скринінгу мікроорганізмів різних таксономічних груп ми відібрали активні продуценти α-L-рамнозидази та α-галактозидази. Одним із способів підвищення їх активності є використання різноманітних ефекторних сполук, здатних модифікувати активність ферменту. Вивчення впливу різних ефекторів є одним із пріоритетних напрямків сучасних досліджень у галузі біохімії, біокоординаційної хімії та біотехнології. Останні дослідження в галузі біокоординаційної хімії виявили високі активуючі властивості подвійних гетерометалічних змішано лігандних координаційних сполук катіонів Fe(II)/Co(II)/Ni(II)/Cu(II)/Zn(II)1,10-фенантроліну та подібних комплексних аніонів тартратостаннату(IV). **Мета** — оцінити ферментативну активність п'яти подібних тартратостаннатів на α-L-рамнозидази *Cryptococcus albidus*, *Eupenicillium erubescens* та α-галактозидазу *Penicillium restrictum*. **Методи.** Активність α-галактозидази визначали, використовуючи як субстрат п-нітрофеніл-α-D-галактопіранозид («Sigma» США). Активність α-L-рамнозидази визначали за допомогою методу Девіса. Як модифікатори ферментативної активності були використані комплекси [Fe(phen)3]2[Sn2(μ-Tart)2(H2Tart)2]·2H2O (1), [Co(phen)3]2[Sn2(μ-Tart)2(H2Tart))2]××8H2O (2), [Ni(phen)3]2[Sn2(μ-Tart)2(H2Tart)2]·2H2O (3), [Cu(phen)3]2[Sn2(μ-Tart) 2(H2Tart)2]·2H2O (4), [Zn(phen)3]2[Sn2(μ-Tart)2(H2Tart)2]·6H2O (5). **Результати.** Вивчення впливу комплексів 1—5, які являють собою надмолекулярні солі, що складаються з того ж аніона тартратостаннату (електрофільний агент) та катіона d-металу 1,10-фенантролін (нуклеофільний агент), на α-L-рамнозидази *Cryptococcus albidus* і *Eupenicillium erubescens* та α-галактозидазу *Penicillium restrictu,* показало, що випробувані сполуки мали різний вплив на їх активність. Усі комплекси суттєво впливають на каталітичну активність α-L-рамнозидази. Ефективність сполук 1—5 щодо α-галактозидази *P. restrictum* була менш вираженою порівняно з α-L-рамнозидазами *C. albidus* та *E. erubescens.* Вона була переважно на рівні контролю. Спостерігається певна закономірність впливу комплексів на α-L-рамнозидази *Cryptococcus albidus* та *Eupenicillium erubescens.* Сполуки 2 та 5 виявилися найбільш ефективними і підвищували активність ферментів на 500—900%. **Висновки.** Сполука 2 [Co(phen)3]2[Sn2(μ-Tart)2(H2Tart)2]·8H2O є перспективною для подальшого використання як ефектора активності α-L-рамнозидази.

Ключові слова: *Cryptococcus albidus, Eupenicillium erubescens, Penicillium restrictum, α-L-рамнозидаза, α-галактозидазна активність, координаційні сполуки тартратостаннатів.*