

РОЗДІЛ V. БІООРГАНІЧНА ХІМІЯ ТА БАР

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SYNTHESIS AND TOXICITY OF DI-NA-SALT OF 2-(PYRIDINE-4-ILTIO)SUCCINIC ACID

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Search of new bioactive substances containing in the molecule a heterocycle and a deputy with high antioxidant properties such as succinic acid is perspective. Di-Na-salt of 2-(pyridine-4-iltio)succinic acid was synthesized, its physical and chemical properties were studied. It is found out that this substance id is relatively safe at intra-abdominal introduction to the wide range of doses (her LD₅₀ = 4960 mg/kg). Research of its antibacterial and cytotoxic action confirmed absence of toxic effects. Methods of di-Na-salt of 2-(pyridine-4-iltio)succinic acid receipt are technologically accessible and economic, substance is un toxic and can come forward as a substance for further researches to create new effective pharmaceutical preparations on its basis.

Key words: synthesis, di-Na-salt of 2-(pyridine-4-iltio)succinic acid, toxicity.

СИНТЕЗ ТА ТОКСИЧНІСТЬ ДИНАТРІЄВОЇ СОЛІ 2-(ПРИДИН-4-ІЛТІО)БУРШТИНОВОЇ КИСЛОТИ

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Показано перспективність пошуку нових біологічно активних сполук, які містять у своїй молекулі, крім гетероциклу, замісник з високими антиоксидантними властивостями, зокрема бурштинову кислоту. Синтезовано динатрієву сіль 2-(піридин-4-ілтїо)бурштинової кислоти, вивчено її фізико-хімічні властивості. Встановлено, що ця сполука є відносно безпечною при внутрішньочеревному введенні в широкому діапазоні доз (її ЛД₅₀ = 4960 мг/кг). Дослідження антибактеріальної та цитотоксичної дії підтвердили відсутність токсичних ефектів. Методи одержання динатрієвої солі 2-(піридин-4-ілтїо)бурштинової кислоти технологічно доступні та економічні, сполука нетоксична і може виступати як субстанція для подальших досліджень на предмет створення на її основі нових ефективних лікарських засобів.

Ключові слова: синтез, динатрієва сіль 2-(піридин-4-ілтїо)бурштинової кислоти, токсичність.

СИНТЕЗ И ТОКСИЧНОСТЬ ДИНАТРИЕВОЙ СОЛИ 2-(ПИРИДИН-4-ИЛТІО) ЯНТАРНОЙ КИСЛОТЫ

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Показана перспективность поиска новых биологически активных веществ, содержащих в своей молекуле, кроме гетероцикла, заместитель с высокими антиоксидантными свойствами, в частности янтарную кислоту. Синтезирована динатриевая соль 2-(пиридин-4-илтио)янтарной кислоты, изучены ее физико-химические свойства. Установлено, что это соединение является относительно безопасным при внутрибрюшинном введении в широком диапазоне доз (его ЛД₅₀ = 4960 мг/кг). Исследование антибактериального и цитотоксического действия подтвердили отсутствие токсических эффектов. Методы получения динатриевой соли 2-(пиридин-4-илтио)янтарной кислоты технологически доступны и экономичны, соединение нетоксично и

может выступать как субстанция для последующих исследований на предмет создания на его основе новых эффективных лекарственных средств.

Ключевые слова: синтез, динатриевая соль 2-(пиридин-4-илтио)янтарной кислоты, токсичность.

INTRODUCTION

Pyridine chemistry for the last decades grew into one of the widest division of chemistry of heterocyclic substances. The heterocyclic system of pyridine is basis of many medicinal facilities that have a wide spectrum of pharmacological action. Substances, that show an antioxidant, antimicrobial, fungicide and other types of activity, were found among them. The possibility of using pyridinethioles to receive the preparations with a cardioactive, analgetic, neurotropic action is especially valuable. Well-known facilities are on the basis of nicotinic and isonicotinic acids.

Clearly the practical value of substances of the indicated row does not finish on that examples and is not exposed yet. That's why the pyridine system nowadays pretends on an intent attention of researchers. So the purposeful organic synthesis is the most perspective way to develop the pyridine chemistry. The research of biologically active substances in the rows of insufficiently known S-pyridinederivates is important and has the theoretical and practical value [1, 2].

Succinic acid (butanedioic acid, ethane-1,2-dicarboxic acid) that is an universal energy source in an organism causes an interest the last time. This natural substance participates in the cycle of Krebs and is present in all organisms. Adding of succinic acid from outside activates the cycle of Krebs, that allows to accelerate the process of leading out of not completely oxidized exchange products. Succinic acid acts as a power substratum inside the cells and take part in different biochemical reactions. With the time ability of cells to produce energy is getting weaker, some functions get violated and that's why organism gets old. The intake of succinic acid can prevent to this process. Succinic acid has antioxidant action, renders harmless free radicals, participates in the process of the cellular breathing and assists the cells to assimilate oxygen.

The physiology action of this acid on an organism is many-sided. Such effects of succinic acid are today known: organstimulating, cytoprotective, oncopreventive, immunomodulating, antistress, antiinflammatory, detoxication, vasoprotective, neuromodulating, cardioprotective [3, 4].

Results of researches of the last years [5, 6] show that combination of nitrogen-containing heterocycle and mercaptocarbonic acids influence on strengthening of biological action or appearance of new effects, that conditioned, in particular, by influence on the processes of free-radical oxidation in tissues. Therefore a search of new bioactive substances containing in the molecule a heterocycle and a deputy with high antioxidant properties such as succinic acid is perspective. That's why on the basis of those studies [7] the di-Na-salt of 2-(pyridine-4-iltio)succinic acid was selected for the deep research.

Purpose of the work was a study of sharp toxicity, antibacterial activity and cytotoxicity of new synthesized substance – di-Na-salt of 2-(pyridine-4-iltio)succinic acid.

RESEARCH MATERIALS AND METHODS

Structure of the synthesized substance is confirmed by data of PMR-, IR-spectroscopy and element analysis, and the cleanness – by the method of thin-layer chromatography (TLC).

The spectrum of PMR are taken off on the device of "Bruker AC-300" (300 Mhz) in DMSO-d₆ CCl₄ (1:1), internal standard – TMS, the sizes of chemical displacements (δ) measured within 0,01 m.s. The element analysis of substance is conducted by means of

element analyzer of ELEMENTAR vario EL cube. IR-spectrum are written in on the spectrometer Bruker ALPHA FT-IR on prefix of ATR.

TLC is conducted on the plates of "Silufol UV-254" the Slovakia production. The display of chromatogram is carried out by means of UV- beams.

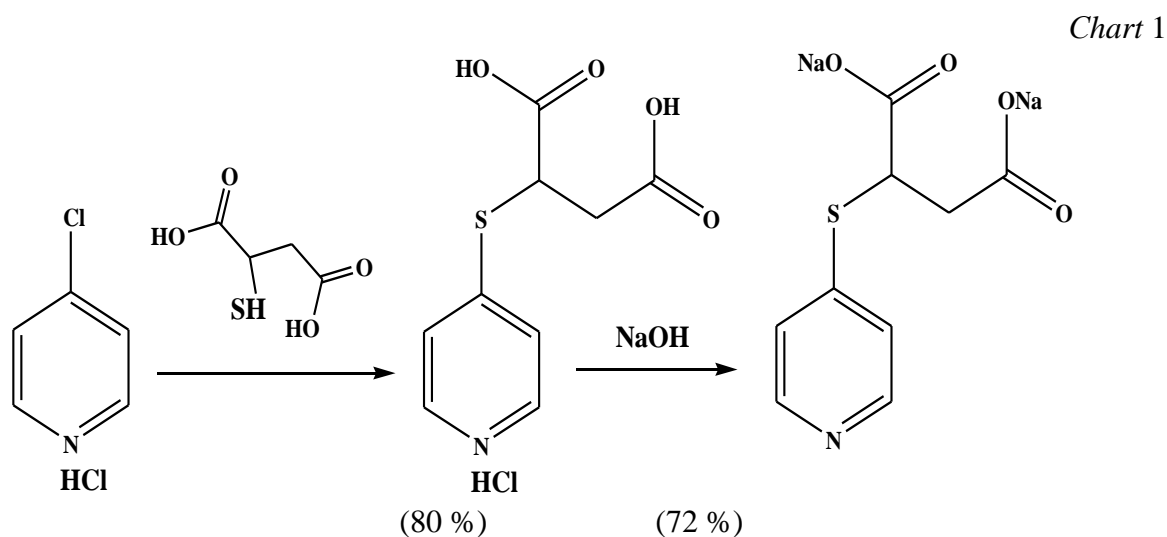
The sharp toxicity was studied on white intact adults bisexual mouse weighing $20 \pm 3,0$ g by means of Prozorovsky V. B. tabular express-method to determinate middle effective measures of influence on biological objects [8].

Researches of antibacterial activity of substance were conducted *in vitro* on methodology of the serial double breeding in a liquid nourishing environment (broth of Hottinger) [9]. Experiments executed on the 4 strain of bacteria, from that 2 cultures were gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*), and other 2 – gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*). The estimation of results was carried out depending on intensity by oppressions of height of that or other culture of bacteria by a substance with a maximal concentration 500 mcg/ml.

The investigation of synthesized substance influence on a fission and cell growth (cytotoxicity) was done on a root test on the sprouts of *Cucumis sp.* (during experience used the cucumbers of sort "Competitor") [10]. Cytotoxicity of substance was estimated after reduction of the marked parameters in an experiment comparatively with control. Control (water) indexes are taken for a zero.

RESULTS AND DISCUSSION

Substance of di-Na-salt of 2-(pyridine-4-iltio)succinic acid was synthesized in the conditions of the extended laboratory synthesis on the following chart 1.



Dioxane was added to 4-hlorpyridine hydrochloride solution with minimal quantity of distilled water. 2-mercaptosuccinic acid was dissolved in a dioxane. The received solutions were mixed up and heated on sandy heater 4,5 hours, and then were cooled. A solvent was poured out and hydrochloride 2-(pyridine-4-iltio)succinic acid, that fell out, dissolved in methanol. The saturated solution of NaOH was prepared. Both solutions were cooled and NaOH was driply added to methanol solution to pH=8.

The received reactionary mixture was outpoured in a cup for evaporation and a solvent was half evaporated. Then isopropyl alcohol was added and boiled 3 times in succession a few minutes, pouring out an old solvent and adding new each time. Sediment that fell out was

filtered, washed with diethyl ether, dried out and crystallized di-Na-salt of 2-(pyridine-4-ylthio)succinic acid from methanol.

Di-Na-salt of 2-(pyridine-4-ylthio)succinic acid is the crystalline substance of lilac color with the temperature of melting of 238-240 °C, water and methanol soluble. The cleanness of connection was controlled and confirmed by the method of TLC in the system of solvents: methanol –chloroform (1:4). Value of R_f 100 = 73.

In the IR-spectrum of di-Na-salt of 2-(pyridine-4-ylthio)succinic acid (figure 1) the presence of conjugating connection of C=C in the aromatic ring of pyridine is shown by the stripe of absorption of 830-810 cm^{-1} . Stripe of middle intensity in the range of 635-675 cm^{-1} answers to the valency oscillation of S-CH₂- group, that is common for sulfur-containing substances.

Two intensive stripes within the limits of 1640-1610 cm^{-1} and 1405-1380 cm^{-1} appear instead of stripes absorption of $\nu_{\text{C=O}}$ when the salt is forming. These stripes answer to antisymmetric and symmetric oscillation of groups of -COO-.

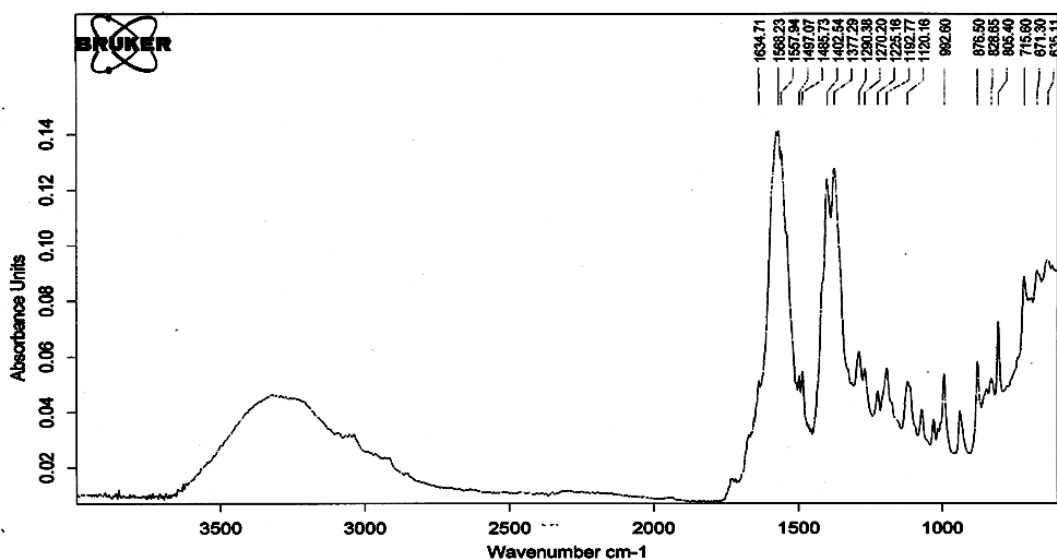


Figure 1. IR-spectrum of di-Na-salt of 2-(pyridine-4-ylthio)succinic acid

The studies of sharp toxicity proved that di-Na-salt of 2-(pyridine-4-ylthio)succinic acid is more or less safe according to the classification of Sidorov at intra-abdominal introduction to the wide range of doses. Animals were put the dose 4000 mg/kg and they were alive and active through 12, 24 hours and on a 14 day. Reflex activity, breathing depth and frequency of mice was not damaged. The reception of water, meal, egestion were not changed. Weight was not changed in comparison with the control group of animals. LD₅₀ of di-Na-salt of 2-(pyridine-4-ylthio)succinic acid presents 4960±66 mg/kg.

Research of antibacterial and cytotoxic action of di-Na-salt of 2-(pyridine-4-ylthio)succinic acid confirmed the absence of toxic effects. It is found out that investigated substance does not have antibacterial activity at concentrations 500 mcg/ml. Cytotoxic action of di-Na-salt of 2-(pyridine-4-ylthio)succinic acid was not marked, but even for concentrations 500 mcg/ml this substance stimulates fission and cell growth of sprouts of *Cucumis sativus* (figure 2).

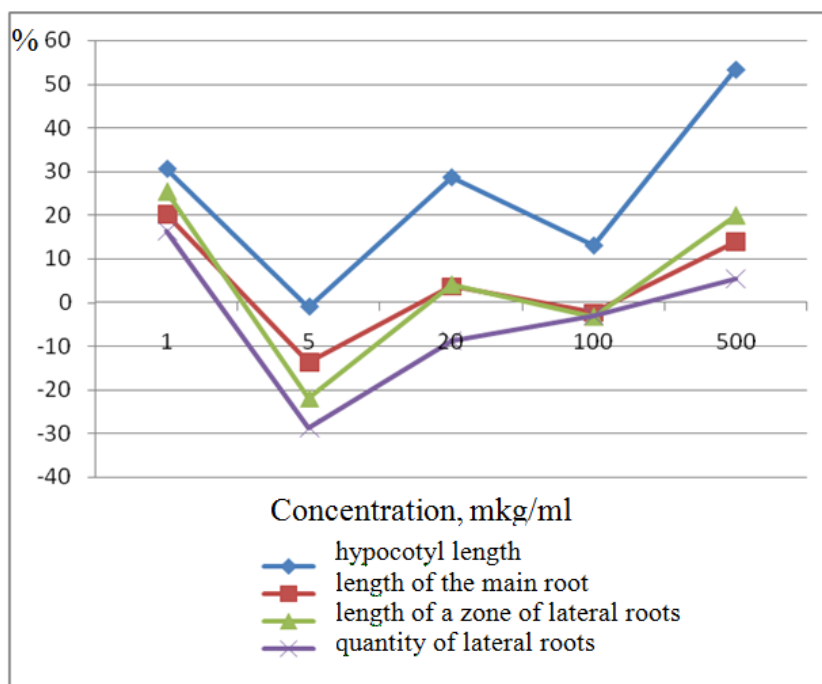


Figure 2. Influence of di-Na-salt of 2-(pyridine-4-ylthio)succinic acid on a fission and cell growth of sprouts of *Cucumis sp.*

The prospect of further study will be a study of other types of biological activity of di-Na-salt of 2-(pyridine-4-ylthio)succinic acid and creation new effective pharmaceutical preparations on its basis.

CONCLUSIONS

1. The preparation synthesis method of di-Na-salt of 2-(pyridine-4-ylthio)succinic acid was developed, its physical and chemical properties were studied.
2. It is found out that di-Na-salt of 2-(pyridine-4-ylthio)succinic acid is relatively safe at intra-abdominal introduction to the wide range of doses (her $LD_{50} = 4960$ mg/kg). Research of its antibacterial and cytotoxic action confirmed absence of toxic effects.
1. Methods of di-Na-salt of 2-(pyridine-4-ylthio)succinic acid receipt are technologically accessible and economic, substance is un toxic and can come forward as a substance for further researches to create new effective pharmaceutical preparations on its basis.

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