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CYCLOALKANECARBALDEHYDES IN SYNTHESIS OF NOVEL 1,2-BENZOXATHIIN-4(3*H*)-ON 2,2-DIOXIDE DERIVATIVES AND STUDY OF THE ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS

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Мета: *Метою даної роботи було дослідження взаємодії циклоалканкарбальдегідів, 1,2-бензоксатіїн-4(3H)-он 2,2-діоксиду та метиленактивних нітрилів та вивчення антимікробних властивостей одержаних сполук.*

Методи: *Як вихідні речовини були використані циклоалканкарбальдегіди, 1,2-бензоксатіїн-4(3H)-он 2,2-діоксид та метиленактивні нітрили. В ході дослідження застосовувались методи органічного синтезу. Структуру синтезованих сполук було підтверджено елементним аналізом та ^{1}H ЯМР-спектроскопією. Антимікробну активність вимірювали методом дифузії в агар.*

Результати: *Нові 2-аміно-4H-пірани синтезували шляхом трикомпонентної взаємодії циклоалканкарбальдегідів, 1,2-бензоксатіїн-4(3H)-он 2,2-діоксиду та малонодинітрилу. Заміна останнього на етилціаноацетат у випадку циклогексанкарбальдегіду призвела до виділення відповідної триетиламонієвої солі. Виходячи з цього результату та з огляду на його новизну було одержано ряд амонієвих солей циклогексанкарбальдегіду з іншими вторинними та третинними амінами. Синтезовані сполуки виявили більш високу антимікробну активність, ніж препарати порівняння щодо грампозитивних штамів.*

Висновки: *Дані дослідження показали перспективний шлях розширення існуючої різноманітності 2-аміно-4H-піранів з використанням в їх синтезі такого енолнуклеофілу та карбонільних сполук як 1,2-бензоксатіїн-4(3H)-он 2,2-діоксид та циклоалканкарбальдегіди відповідно. Виявлена антимікробна активність отриманих сполук проти грампозитивних мікроорганізмів дає можливість для подальших досліджень щодо створення антибіотиків вузького спектру дії*

Ключові слова: *1,2-бензоксатіїн-4(3H)-он 2,2-діоксид, 2-аміно-4H-піран,багатокомпонентні реакції, циклоалканкарбальдегіди, амонієві солі, антимікробна активність*

1. Introduction

Nowadays the variety of organic compounds is absolutely tremendous. In 2015 the world's largest database of unique chemical substances CAS registered the 100 millionth chemical substance [1], and thousands of new molecules are still generated by scientists all over the world each year. Among this number the compounds with prospective biological properties are of the greatest interest, because they usually give rise to the development of novel medicines. 2-Amino-4*H*-pyrans, that were for the first time synthesized nearly 60 years ago, can be considered as such group, because of the known antimicrobial [2] and antitumor [3] activities for this class of compounds on the one hand and convenient chemical pathways to their synthesis on the other hand. According to this, the extension of the condensed 2-amino-4*H*-pyranseries is justified, especially by means of utilization of novel unexplored molecules in order to investigate the influence of this one on the biological activity. In this regard 1,2-benzoxathiin-3(4*H*)-on 2,2-dioxide is one of insufficiently studied compounds, that may be used for construction of novel condensed 2-amino-4*H*-pyrans as prospective antimicrobial agents.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Multicomponent reactions are an effective and convenient tool in organic synthesis, because they provide rapid way to the creation of large novel series of compounds through applying of wide range of initial compounds. The most common and attractive route towards 2-amino-4*H*-pyrans **E** represents the three-component reaction of active methylene nitriles **A**, carbonyl compounds **B** and enolnucleophiles **C** (Fig. 1) and includes the *hetero*-Thorpe-Ziegler cyclization of adducts **D** [4]. According to the recent literature data such interaction proceeds perfectly as multicomponent reaction, when adducts **D** are generated in situ from the mixture of starting compounds. Formation of **D** and heterocyclization processes occurs sequentially and is considered as Knoevenagel-Michael-*hetero*-Thorpe-Ziegler domino type reaction.

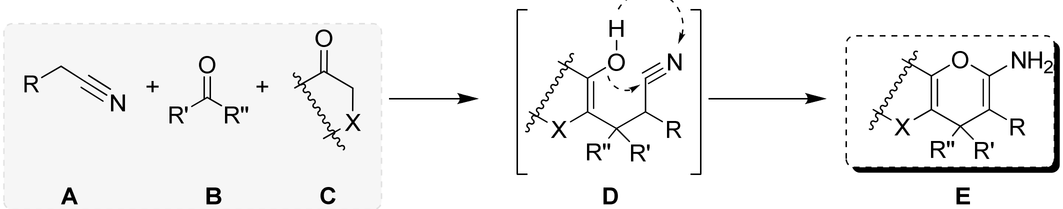


Fig. 1. The general route towards 2-amino-4*H*-pyrans synthesis

Among carbonyl compounds cycloalkanecarbaldehydes were not previously widely used in such interactions type. Utilization of latter along with 1,2-benzoxathiin-3(4*H*)-on 2,2-dioxide as enolnucleophile gives the possibility to obtain novel condensed 2-amino-4*H*-pyrans, the evaluation of the antimicrobial activity of which are of the greatest interest due to the current necessity of the search of new core-structures with such properties.

3. Analysis of recent studies and publications in which a solution of the problem is foundand which draws on the author

The structural diversity of 2-amino-4*H*-pyrans depends on the initial compounds that are used in their synthesis: active methylene nitriles, carbonyl compounds and enolnucleophiles.

The most well-known active methylene nitriles applied in the interaction described above are malononitrile **A1** and esters of cyanoacetic acid **A2**. Cyanacetamide **A3** and thiocyanacetamide **A4** are not so widely used. As for carbonyl compounds they are represented with different types of aldehydes, the most common of which are aromatic **B1** and hetarenecarbaldehydes **B2**. In the case of ketones (isatins **B5** and ningidrin **B6**) the products of the interaction are spirocondensed compounds. Enolnucleophiles is the widest group in the 2-amino-4*H*-pyrans synthesis and is represented with diketones **C1**, ketoesters **C2-C4**, cyanoketones **C5-C6**, nitroketones **C7**and many other related compounds that are shown on Fig. 2.

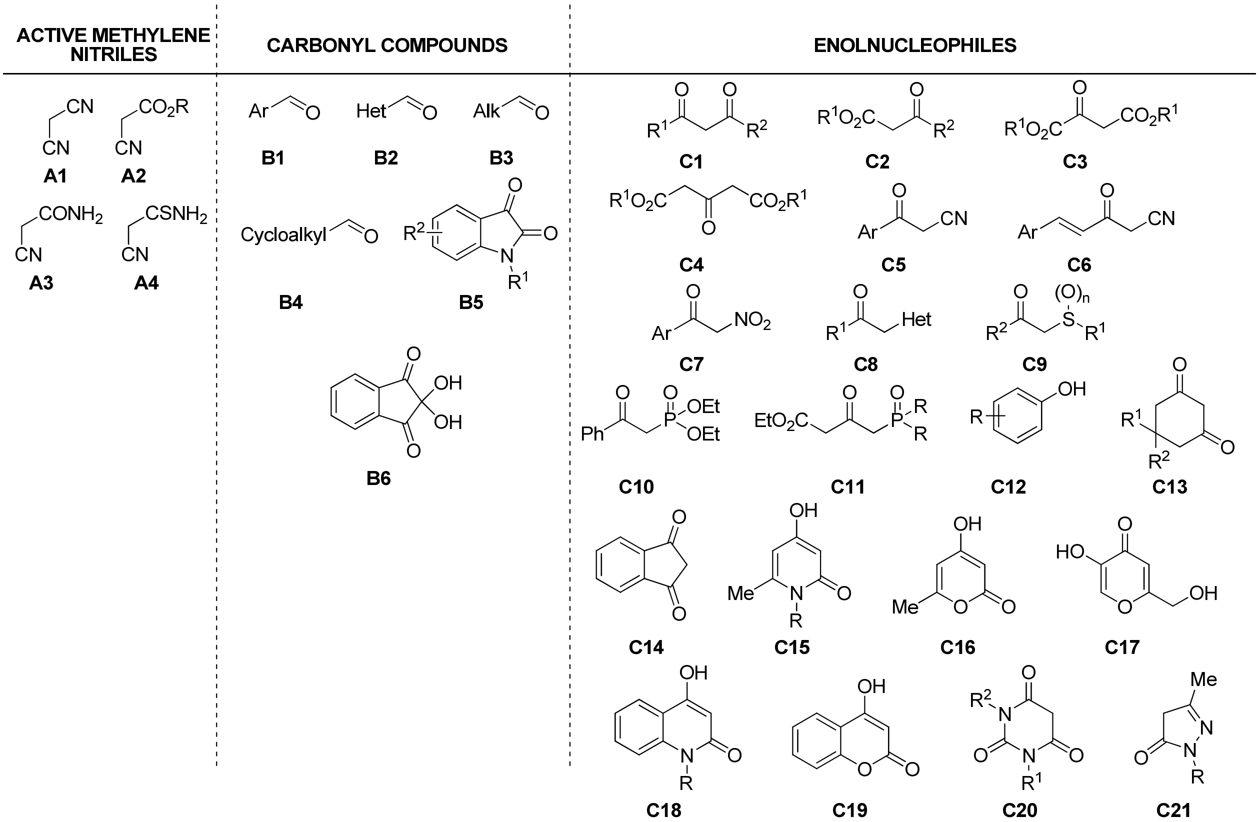


Fig. 2. The variety of components in 2-amino-4*H*-pyrans synthesis

Corresponding multicomponent interactions generally proceed under reflux in polar solvents medium in the presence of basic catalysts, such as triethylamine, morpholine, piperidine [4].

Current investigations in this synthetic area are mainly focused on the selection of new reaction conditions and catalysts, and to search of some novel suitable components, that could replace well-known ones in order to expand the existing 2-amino-4*H*-pyrans diversity.

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

As it was mentioned above, the huge variety of enolnucleophiles have been already used in multicomponent 2-amino-4*H*-pyrans synthesis. That is why searching of some new compounds of this group is challenging and interesting at the same time.

Previously we reported the utilization of 1*H*-2,1-benzothiazin-4-on 2,2-dioxide in 2-amino-4*H*-pyrans synthesis [5, 6]. This “newcomer” turned out to be the prospective core structure for such interactions due to the presence of SO_2CH_2CO moiety. Moreover the series of obtained compounds were screened for antibacterial, antifungal, analgesic and anti-inflammatory activities [7], and among them some promising substances with moderate to high levels of them were found.

As the next consequential step of the research we drew our attention to the other almost unknown enolnucleophile – 1,2-benzoxathiin-4(3*H*)-on 2,2-dioxide, that is an isostere of 1*H*-2,1-benzothiazin-4-on 2,2-dioxide.This approach gave us the opportunity not only to compare the chemical properties of such close related compounds, but also to investigate the structure/bioactivity relationships for the obtained substances.

We also decided to use cycloalkanecarbaldehydes as a carbonyl component for the 2-amino-4*H*-pyrans synthesis, because this group was not broadly employed in such interactions before.

5. Formulation of goals (tasks) of the article

According to the information given above we aimed to synthesize 2-amino-4*H*-pyrans with the use of cycloalkanecarbaldehydes, 1,2-benzoxathiin-4(3*H*)-on 2,2-dioxide and active methylene nitriles three-component interaction, to determine the most suitable reaction conditions and to evaluate the antimicrobial properties of the obtained compounds.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

Considering the previous research of the interaction of 1*H*-2,1-benzothiazin-4-on 2,2-dioxide with active methylene nitriles and cycloalkanecarbaldehydes [6] we began our investigation from the studying of the reaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** with malononitrile **2** and cyclopropanecarbaldehyde **3a**.It was discovered, that as in the previous case, the reaction proceeded under room temperature in ethanol with the presence of catalytic amount of triethylamine and resulted into the formation of 2-amino-4-cyclopropyl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide **4a** in 55 % yield (Scheme 2). The same reaction conditions were successfully applied for cyclopentane- and cyclohexanecarbaldehydes **3b** and **3c** respectively.

Inspired by such encouraging results we continued the research and replaced malononitrile with ethyl cyanoacetate, which is also often used in such interactions type. The reaction was carried out with equimolar quantities of all reagents in the same conditions as previous one, but unfortunately gave no rise to the desired products. We did not succeed in isolation of any product at all in this case. Simultaneously the performance of the reaction at 60–70 °C in the case of aldehyde **3c** allowed us to isolate a crystalline powder that appeared to be the triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(cyclohexane)methyl]-2,1-benzoxathiin-5-olat 2,2-dioxide **5c**.

This unexpected result can be explained by two ways (Fig. 3). The first one comprises the formation of Michael adduct **F** which looses the molecule of ethyl cyanoacetate and is converted into enone **G**, that gives triethylammonium salt according to what was reported previously [6]. The second possible way involves the formation of enone **G** by direct interaction of 1,2-benzoxathiin-4(3*H*)-on 2,2-dioxide **1** with aldehyde **3c**, that next reacts with the second molecule of **1** forming symmetrical bis-derivative isolated as triethylammonium salt **5c**. On the current stage of the research these two ways can be considered as equiprobable and further investigations are needed to disclose finally the mechanism of salts **5** formation via applying of multicomponent format of the reaction.

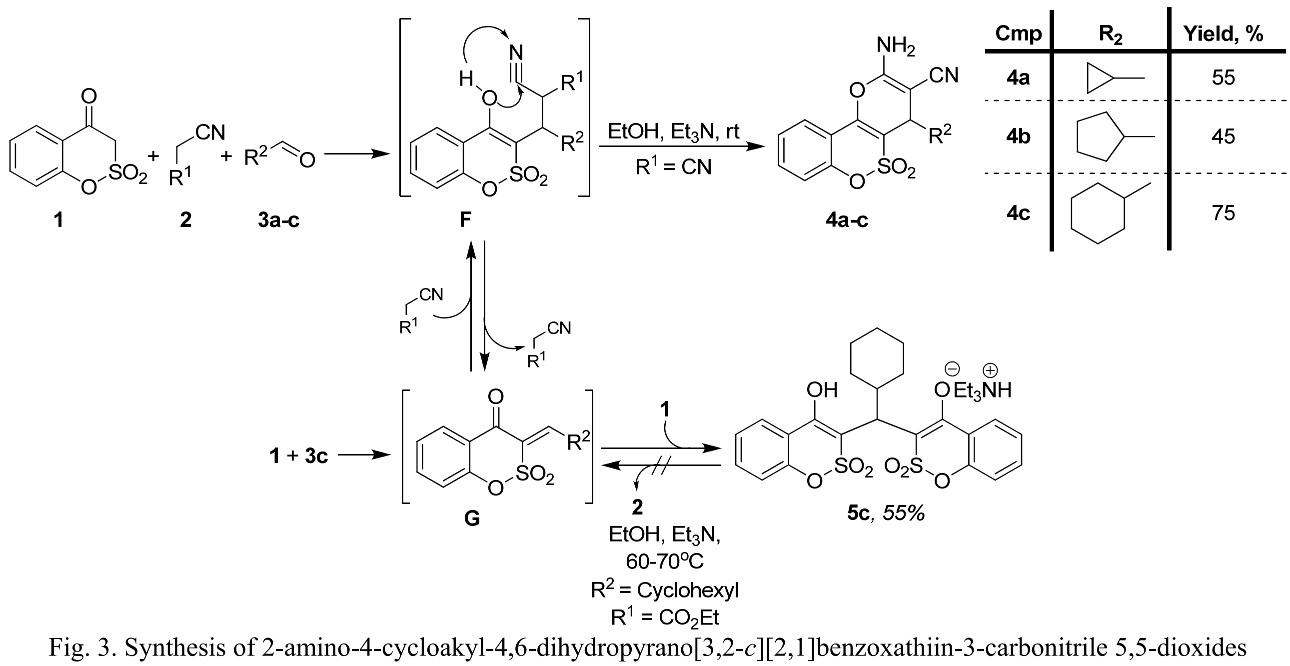


Fig. 3. Synthesis of 2-amino-4-cycloakyl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides

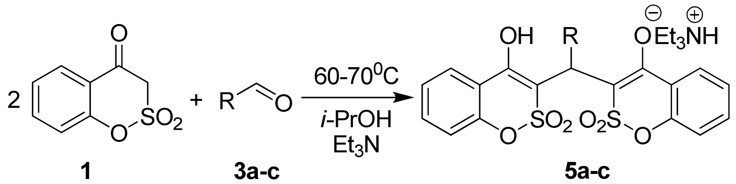
Since no traces of triethylammonium salts were found for three-component reaction of 1,2-benzoxathiin-4(3*H*)-on 2,2-dioxide with malononitrile and cycloalkanecarbaldehydes, we can assume that 2-amino-4*H*-pyran-3-carbonitriles **4** are more stable in the applied conditions than the corresponding salts **5**. Considering the possibility of the enones **G** to be formed into the reaction mixture we tried to convert triethylammonium salt **5c** into 2-amino-3-carbonitrile-4*H*-pyran **4c** by treating the former with malononitrile. In spite of this the attempt appeared to be unsuccessful and only initial material was recovered after the reaction. In this way, we can suppose that such triethylammonium salts **5** are rather stable compounds and the *retro*-Michael cleavage reaction with formation of the enones **G** is not typical for them.

Formation of such pharmacologically interesting compounds type as triethylammonium salts and previously reported results devoted to the two-component reaction of 1*H*-2,1-benzothiazin-4-on 2,2-dioxide with cycloalkanecarbaldehydes [6] encouraged us to continue these investigations and to study similar interaction for 1,2-benzoxathiin-4(3*H*)-on 2,2-dioxide.

So the reaction between 1,2-benzoxathiin-4(3*H*)-on 2,2-dioxide **1** and appropriate cycloalkanecarbaldehydes **3a-c** was carried out applying conditions found before [6] and the solution of starting compounds in *i*-PrOH was mixed for 1 h at 60-70°C in the presence of equimolar quantity of triethylamine. The obtained white crystalline substances appeared to be the expected triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(cycloalkyl) methyl]-2,1-benzoxathiin-5-olate 2,2-dioxides **5a-c**. It is interesting to mention that the poorest yield was again detected for the cyclopentanecarbaldehyde **3b**, as it was described for the corresponding 2-amino-4*H*-pyrans **4** synthesis (Fig. 4).

In regard to expand the range of such ammonium salts we also examined the possibility of secondary amines utilization in this reaction. The desired products **6c, 7c, 8c, 9c** were isolated for aldehyde **3c** in the case of piperidine, morpholine, 1,2,3,4-tetrahydroisoquinoline and dimethylamine (Fig. 5).

The reaction proceeded under gently heating and mixing for 1 h in *i*-PrOH with equimolar quantity of the corresponding amine.



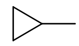
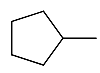
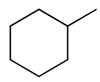
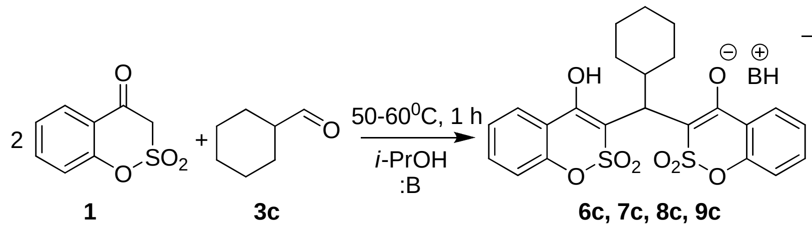
Compound	R	Yield, %
5a		52
5b		42
5c		70

Fig. 4. The formation of triethylammonium salts **5a-c**



Comp.	Base	Yield, %
6c	piperidine	73
7c	morpholine	70
8c	1,2,3,4-tetra-hydroisoquinoline	42
9c	dimethylamine	35

Fig. 5. Synthesis of the ammonium salts **6c, 7c, 8c, 9c**

The antimicrobial properties of the obtained compounds was studied according to the international standards [8, 9] by the agar "well" diffusion method against the standard test-strains of gram-positive and gram-negative bacteria and fungi. The results revealed higher antimicrobial activity than for the reference drugs against *S. aureus* and *E.coli*. The compound **4c** appeared to be the most active among all tested samples. The diameters of its growth inhibition zones were 21 mm for *S. aureus,* 19 mm for *E. coli,* 17 mm for *C. albicans* and 16 mm for *B. subtilis, P. aeruginosa and P. vulgaris.* The results for the reference drugs Synthomycine and Metronidazole did not exceed 17 mm.

There was no significant distinction of antimicrobial properties of triethylammonium salts corresponding to 2-amino-4*H*-pyrans. The average diameter of growth inhibition zones was 17 mm against all tested strains.

Most of the similar derivatives of 1*H*-2,1-benzothiazin-4(3*H*)-on 2,2-dioxide did not possess antibacterial activity against gram-positive and gram-negative microorganisms and revealed moderate antifungal activity [6]. Thereby the isosteric replacement of 1-N-R-group to O-atom appeared to be the effective tool allowing to find more active antimicrobial agents.

7. Findings from the research and prospects of further development of this area

Series of 2-amino-4-cycloalkyl-4,6-dihydropyrano [3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides and ammonium salts were synthesized based on cycloalkanecarbaldehydes and 1,2-benzoxathiin-3(4*H*)-on 2,2-dioxide. The screening of antimicrobial properties of the obtained substances revealed generally higher activity than for the reference drugs against the gram-positive strains. The 2-amino-4-cyclohexyl-4,6-dihydropyrano [3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide as the lead compound may be proposed for further investigations in this area.

Experimental chemical part

Initial aldehydes and active methylene nitriles were obtained from commercial sources and used without further purification. Melting points were determined on a Gallenkamp melting point apparatus, Model MFB-595 in open capillary tubes. ^1H NMR spectra were recorded on Varian WXR-400 spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. Elemental analyses were carried out using Carlo Erba CHNS-O EA 1108 analyzer.

General procedure for the synthesis of 2-amino-4-cycloalkyl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides (4a-c)

To a solution of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** (0.198 g, 0.001 mol), malononitrile **2** (0.066 g, 0.001 mol) and appropriate alicyclic aldehyde **3a-c** (0.001 mol) in ethanol (5-10 mL) catalytic amount of triethylamine was added. The mixture stayed for 24 h at room temperature. In the cases of **3a**, **c** the products **4a**, **4c** were formed as crystalline precipitates which were filtered off, washed with ethanol and dried on air. For **3b** the solvent was evaporated under reduced pressure and the residue was treated with methanol producing light yellow crystalline powder of **4b**.

2-Amino-4-cyclopropyl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4a)

Yellow powder; mp 255-258°C (from EtOH); Anal.Calcd for $C_{15}H_{12}N_2O_4S$, %: C 56.95; H 3.82; N 8.86. Found, %: C 56.91; H 3.79; N 8.84; ^{1}H NMR (400 MHz, DMSO-d_6): δ (m.n.) 7.79 (d, *J*=7.83 Hz, 1H, Ar); 7.60-7.70 (t, 1H, Ar); 7.44-7.55 (m, 2H, Ar); 7.32 (s, 2H, N<u>H</u>$_2$); 2.89 (d, *J*=9.00 Hz, 1H, C<u>H</u>); 0.97-1.11 (m, 1H, C<u>H</u>); 0.36-0.62 (m, 4H, C<u>H</u>$_2$cyclopropyl).

2-Amino-4-cyclopentyl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4b)

Light yellow crystalline powder; mp>250°C (from EtOH); Anal. Calcd for $C_{17}H_{16}N_2O_4S$, %: C 59.29; H 4.68; N 8.13. Found, %: C 59.25; H 4.63; N 8.09; ^{1}H NMR (400 MHz, DMSO-d_6): δ (m.n.) 7.81 (d, *J*=7.63 Hz, 1H, Ar); 7.62-7.72 (m, 1H, Ar); 7.49-7.57 (m, 2H, Ar); 7.43 (s, 2H, N<u>H</u>$_2$); 3.63 (d, *J*=3.66 Hz, 1H, C<u>H</u>); 2.27 (br. s., 1H, C<u>H</u>); 1.36-1.77 (m, 8H, C<u>H</u>$_2$cyclopentyl).

2-Amino-4-cyclohexyl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4c)

White crystalline powder; mp>250°C (from EtOH); Anal.Calcd for $C_{18}H_{18}N_2O_4S$, %: C 60.32; H 5.06; N 7.82. Found, %: C 60.29; H 5.01; N 7.78; ^{1}H NMR (400 MHz, DMSO-d_6): δ(m.n.) 7.80 (d, *J*=7.83 Hz, 1H, Ar); 7.66 (d, *J*=8.22 Hz, 1H, Ar); 7.47-7.57 (m, 2H, Ar); 7.43 (s, 2H, N<u>H</u>$_2$); 3.46 (s, 1H, C<u>H</u>); 0.77-1.80 (m, 11H, cyclohexyl).

General procedure for the synthesis of triethylammonium 3-[(4-hydroxy-2,2-dioxido-1,2-benzoxathiin-3-yl)(cycloalkyl)methyl]-1,2-benzoxathiin-5-olat 2,2-dioxides (5a-c)

To a solution of 1,2-benzoxathiin-4(3*H*)-on 2,2-dioxide **1** (0.198 g, 0.001 mol) and appropriate alicyclic aldehyde **3a-c** (0.0005 mol) in propanol-2 (10 mL), triethylamine (0.13 mL, 0.001 mol) was added. The solution was mixed for 1 h at 60-70°C. The obtained precipitates of **5a-c** were filtered off, washed with propanol-2 and dried on air.

Triethylammonium3-[(4-hydroxy-2,2-dioxido-1,2-benzoxathiin-3-yl)(cyclopropyl)methyl]-1,2-benzoxathiin-5-olat 2,2-dioxide (5a)

White crystalline powder; mp 135-137 °C (from EtOH); Anal.Calcd for $C_{26}H_{31}NO_8S_2$, %: C 56.81; H 5.68; N 2.55. Found, %: C 56.78; H 5.62; N 2.51; ^{1}H NMR (400 MHz, DMSO-d_6): δ(m.n.) 17.84 (br. s., 1H, O<u>H</u>); 7.88 (d, *J*=7.63 Hz, 2H, Ar); 7.42-7.51 (m, 2H, Ar); 7.31 (t, *J*=7.48 Hz, 2H, Ar); 7.22 (d, *J*=8.24 Hz, 2H, Ar); 3.16 (d, *J*=10.07 Hz, 2H, C<u>H</u>, C<u>H</u>cyclopropyl); 3.03 (q, *J*=7.22 Hz, 6H, N(C<u>H</u>$_2$CH$_3$)$_3$); 1.12 (t, *J*=7.17 Hz, 9H, N(CH$_2$C<u>H</u>$_3$)$_3$); 0.44 (d, *J*=7.32 Hz, 2H, C<u>H</u>$_2$cyclopropyl); 0.27 (d, *J*=4.58 Hz, 2H, C<u>H</u>$_2$cyclopropyl).

Triethylammonium 3-[(4-hydroxy-2,2-dioxido-1,2-benzoxathiin-3-yl)(cyclopentyl)methyl]-1,2-benzoxathiin-5-olat 2,2-dioxide (5b)

White crystalline powder; mp 158-160 °C (from EtOH); Anal.Calcd for $C_{28}H_{35}NO_8S_2$, %: C 58.21; H 6.11; N 2.42. Found, %: C 58.18; H 6.07; N 2.38; ^{1}H NMR (400 MHz, DMSO-d_6): δ(m.n.) 17.75 (s, 1H, O<u>H</u>); 7.84 (d, *J*=7.63 Hz, 2H, Ar); 7.39-7.49 (m, 2H, Ar); 7.29 (t, *J*=7.48 Hz, 2H, Ar); 7.20 (d, *J*=8.24 Hz, 2H, Ar); 3.78 (d, *J*=11.29 Hz, 1H, C<u>H</u>); 3.44 (br. s., 1H, C<u>H</u>cyclopentyl); 2.98-3.11 (m, 6H, N(C<u>H</u>$_2$CH$_3$)$_3$); 1.22-1.64 (m, 8H, C<u>H</u>$_2$cyclopentyl); 1.14 (t, *J*=6.41 Hz, 9H, N(CH$_2$C<u>H</u>$_3$)$_3$).

Triethylammonium 3-[(4-hydroxy-2,2-dioxido-1,2-benzoxathiin-3-yl)(cyclohexyl)methyl]-1,2-benzoxathiin-5-olat 2,2-dioxide (5c)

White crystalline powder; mp 165-167 °C (from EtOH); Anal.Calcd for $C_{29}H_{37}NO_8S_2$, %: C 58.86; H 6.30; N 2.37. Found, %: C 58.82; H 6.26; N 2.33; ^{1}H NMR (400 MHz, DMSO-d_6): δ(m.n.) 17.65 (s, 1H, O<u>H</u>); 7.84 (d, *J*=7.63 Hz, 2H, Ar); 7.39-7.52 (m, 2H, Ar); 7.24-7.34 (m, 2H, Ar); 7.20 (d, *J*=7.93 Hz, 2H, Ar); 3.74 (d, *J*=10.68 Hz, 1H, C<u>H</u>); 3.04 (q, *J*=7.32 Hz, 6H, N(C<u>H</u>$_2$CH$_3$)$_3$); 2.71 (d, *J*=10.99 Hz, 1H, C<u>H</u>) 1.73-1.53 (m, 5H, C<u>H</u>$_2$cyclohexyl) ;1.13 (t, *J*=7.17 Hz, 9H, N(CH$_2$C<u>H</u>$_3$)$_3$); 1.07 (br. s., 2H, C<u>H</u>$_2$cyclohexyl); 0.86 (m, 2H, C<u>H</u>$_2$cyclohexyl).

General procedure for the synthesis of ammonium 3-[(4-hydroxy-2,2-dioxido-1,2-benzoxathiin-3-yl)(cyclohexyl)methyl]-1,2-benzoxathiin-5-olat 2,2-dioxides (6c, 7c, 8c, 9c)

To a solution of 1,2-benzoxathiin-4(3*H*)-on 2,2-dioxide **1** (0.198 g, 0.001 mol) and cyclohexanecarbaldehyde **3c** (0.0005 mol) in propanol-2 (10 mL) the equimolar amount of corresponding amine was added. The solution was mixed for 1 h at 60-70°C. The obtained precipitates of **6c, 7c, 8c, 9c** were filtered off, washed with propanol-2 and dried on air.

Piperidinium 3-[(4-hydroxy-2,2-dioxido-1*H*-1,2-benzoxathiin-3-yl)(cyclohexyl)methyl]-1,2-benzoxathiin-5-olate 2,2-dioxide (6c)

White crystalline powder; mp 138-140 °C (from EtOH); Anal.Calcd for $C_{28}H_{33}NO_8S_2$, %: C 58.42; H 5.78; N 2.43. Found, %: C 58.40; H 5.75; N 2.41; ^{1}H NMR (400 MHz, DMSO-d_6): δ(m.n.) 17.63-17.69 (m, 1H, O<u>H</u>); 7.79-7.85 (m, 2H, Ar); 7.41-7.49 (m, 2H, Ar); 7.25-7.34 (m, 2H, Ar); 7.18-7.23 (m, 2H, Ar); 4.33 (d, *J*=1.00 Hz, 1H, C<u>H</u>); 3.67-3.78 (m, 6H, C<u>H</u>$_2$piperidine); 3.02-3.09 (m, 4H, C<u>H</u>$_2$piperidine); 1.00 (d, *J*=6.10 Hz, 11H, C<u>H</u>, C<u>H</u>$_2$cyclohexyl).

Morpholinium 3-[(4-hydroxy-2,2-dioxido-1*H*-1,2-benzoxathiin-3-yl)(cyclohexyl)methyl]-1,2-benzoxathiin-5-olate 2,2-dioxide (7c)

White crystalline powder; mp 128-130 °C (from EtOH); Anal.Calcd for $C_{27}H_{31}NO_9S_2$, %: C 56.14; H 5.41; N 2.42. Found, %: C 56.12; H 5.38; N 2.41; ^{1}H NMR (400 MHz, DMSO-d_6): δ(m.n.) 17.63-17.70 (m, 1H, O<u>H</u>); 7.82 (d, *J*=6.41 Hz, 2H, Ar); 7.40-7.49 (m, 2H, Ar); 7.29 (s, 2H, Ar); 7.21 (d, *J*=7.93 Hz, 2H, Ar); 4.33 (d, *J*=1.00 Hz, 1H, C<u>H</u>); 3.66-3.77 (m, 4H, CH2morpholine); 3.02-3.08 (m, 4H, CH2morpholine); 1.00 (d, *J*=6.10 Hz, 11H, CH, CH2cyclohexyl).

1,2,3,4-Tetrahydroisoquinolinium3-[(4-hydroxy-2,2-dioxido-1*H*-1,2-benzoxathiin-3-yl)(cyclohexyl)methyl]-1,2-benzoxathiin-5-olate 2,2-dioxide (8c)

Light yellow crystalline powder; mp 135-138 °C (from EtOH); Anal. Calcd for $C_{32}H_{33}NO_8S_2$, %: C 61.62; H 5.33; N 2.25. Found, %: C 61.59; H 5.31; N 2.22; ^{1}H NMR (400 MHz, DMSO-d_6): δ (m.n.)17.67 (br. s., 1H, OH); 7.83 (d, *J*=7.93 Hz, 2H, Ar); 7.39-7.49 (m, 2H, Ar); 7.29 (t, *J*=7.32 Hz, 2H, Ar); 7.21 (d, *J*=8.24 Hz, 6H, Ar); 4.23 (s, 2 H, CH2isoquinoline); 3.73 (d, *J*=9.16 Hz, 1H, CH); 3.31-3.38 (m, 2H, CH2isoquinoline), ; 2.95 (br. s., 2H, CH2isoquinoline); 2.69 (br. s., 1H, CHcyclohexyl); 1.49-1.76 (m, 5H, CH2cyclohexyl); 0.94-1.12 (m, 3H, CH2cyclohexyl); 0.86 (br. s., 2, CH2cyclohexyl).

Dimethylammonium3-[(4-hydroxy-2,2-dioxido-1*H*-1,2-benzoxathiin-3-yl)(cyclohexyl)methyl]-1,2-benzoxathiin-5-olate 2,2-dioxide (9c)

White crystalline powder; mp 175-178 °C (from EtOH); Anal.Calcd for $C_{25}H_{29}NO_8S_2$, %: C 56.06; H 5.46; N 2.61. Found, %: C 56.04; H 5.43; N 2.59; ^{1}H NMR (400 MHz, DMSO-d_6): δ(m.n.) 17.67 (s, 1H, OH); 8.01-8.18 (m, 1H, NH); 7.83 (d, *J*=6.41 Hz, 2H, Ar); 7.40-7.50 (m, 2H, Ar); 7.26-7.34 (m, 2H, Ar); 7.21 (d, *J*=7.93 Hz, 2H, Ar); 3.72 (d, *J*=10.99 Hz, 2H, CH); 1.46-1.80 (m, 6H, CH2cyclohexyl); 1.06 (br. s., 2H, CH2cyclohexyl); 1.00 (d, J=6.10 Hz, 6H, N(CH3)2); 0.86 (br. s., 2H, CH2cyclohexyl).

Experimental microbiological part

According to the WHO recommendations [8] the following test-strains were used: Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633, Proteus vulgaris ATCC 4636, Candida albicans ATCC 653/885. The inoculum suspension was prepared using a Densi-La-Meter apparatus (made by PLIVA-Lachema, Czech Republic; 540-nm wavelength).

The suspension was prepared according to the manual for the device and the information sheet No. 163-2006 "Standardization for preparation of microbial suspensions" (Kyiv) about innovations in the healthcare system. The inoculum density was 10^7 cells in 1 ml of the medium, and it was determined by comparing with McFarland standard [10]. The 18 to 24-hour old culture of the microorganism was employed for the test. For the antimicrobial evaluation the Mueller-Hinton agar was used, for the Candida albicans strain the Sabouraud agar was taken.

The compounds were introduced into agar by the "wells" method [8]. The antibacterial activity was evaluated by measuring zones of inhibition of the corresponding microorganism and was compared with those for reference antimicrobial drugs.

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IDENTIFICATION AND QUANTITATIVE DETERMINATION OF STEROIDAL COMPOUNDS IN THE PLANT MATERIAL OF CABBAGE

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Овочеві культури є невичерпним джерелом біологічно активних речовин. Капуста городня (Brassica oleracea L.) – рослина, яка здавна застосовується в народній медицині багатьох країнах світу для лікування різноманітних захворювань, та проявляє протизапальну, відхаркувальну, бронхолітичну, сечогінну, загальнозміцнювальну дії. В Україні сировина капусти городньої є неофіцінальною і тому потребує комплексного фармакогностичного дослідження.
Мета. *Ідентифікація та визначення кількісного вмісту стероїдних сполук у листі, насінні та качанах капусти городньої.*
Методи дослідження. *Ідентифікацію стероїдних сполук та визначення їх кількісного вмісту у сировині капусти городньої проводили методом газової хроматографії/мас-спектрометрії (ГХ/МС).*
Результати дослідження. *В результаті проведеного дослідження були ідентифіковані: у листі капусти городньої сорту «Білосніжка» - 3 сполуки стероїдної природи , сортів «Українська осінь» та «Ярославна» – 4, у насінні сорту «Білосніжка» - 4, сортів «Українська осінь» та «Ярославна» – по 3 сполуки, у качанах всіх сортів виявлено по 3 стероїдних речовини. За кількісним вмістом в усіх досліджуваних зразках переважав β-ситостерол. Його найбільший вміст складав у листі (2499 мг/кг) та насінні (1728 мг/кг) сорту «Ярославна», а також у качанах (1148 мг/кг) сорту «Українська осінь».*
Висновки. *Результати досліджень можуть бути використані при розробці методів контролю якості на сировину капусти городньої та при одержанні перспективних біологічно активних субстанцій з досліджуваної сировини*
Ключові слова*: капуста городня, листя, насіння, качани, стероїдні сполуки, газова хроматографія, мас/спектрометрія*

1. Introduction

The study of crops as sources of medicinal preparations obtaining on their basis is actual for pharmacy nowadays. Cabbage (*Brassica oleracea L.*) is a member of the family *Brassicaceae* (or *Cruciferae*). In Ukraine it is grown as a vegetable culture, which has a sufficient raw material base and a big number of varieties. The complex of compounds contained in cabbage gives it many pharmacotherapeutic properties.

In folk medicine of the West and the East cabbage has long been used against various diseases. The leaves, roots, stumps, and seeds of the plant are use [1]. Cabbage juice is prescribed for gastritis and peptic ulcer of the stomach and duodenum, in ulcerative colitis, as well as in a mixture with honey in lung tuberculosis, in liver disorders. Roots and stumps are considered as an antitumor agent [2, 3]. The decoction of the seeds is used for gout, pain in the joints, as an antiseptic and a diuretic. Folk medicine recommends that fresh leaves of cabbage can be applied to purulent wounds and ulcers, to the mammary glands during mastopathy. Cabbage leaves are also used for abscess and other inflammatory diseases of the skin, including burns [3]. Fiber contained in cabbage improves the motor function of the intestines, shows positive influence on gut microflora. The presence of group B vitamins in the fiber normalizes fat metabolism, promotes the removal of excess cholesterol, and inhibits the development of atherosclerotic plaques on the walls of the aorta and vessels of the heart, that is, helps in the treatment and prevention of atherosclerosis [4]. The