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SEPARATION AND STRUCTURE OF 5-METHYLIMIDAZOLIDINE-2,4-DIONE FROM THE FUNGUS AGARICUS BISPORUS GROWN IN NATURAL CONDITIONS

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Abtract. The reason why the white button fungus, namely Agaricus bisporus strain, is widely used and used on large scales around the world is that, in addition to its excellent flavor and nutritional composition, it is very diverse and synthesizes a large number of primary and secondary metabolites. As part of this study, the aim was to chemically profile the secondary metabolites contained in the naturally grown A. bisporus (brown) fungus and to determine the chemical structure of some of them. From biomass obtained from ethanol extract of Agaricus bisporus fungus, by column chromatography by sequential washing in a solvent system, namely chloroform: methanol 19:1 (v/v) fractionation step, results in 2,4-imidazolidinedione,5-methyl was determined by GC-MS chromatography and the chemical formula of the compound 2,4-imidazolidinedione,5-methyl determined on the basis of IR and NMR spectroscopic analyses. The biological activity of the obtained secondary metabolites was commented on.

Keywords: A. bisporus, secondary metabolite, 5-methylimidazolidine-2,4-dione, GC-MS, NMR spectroscopy, biologic activity.

Аннотация. Причина широкого использования белого шампиньона, а именно штамма Agaricus bisporus, в больших масштабах по всему миру заключается в том, что, помимо превосходного вкуса и питательного состава, он очень разнообразен и синтезирует большое количество первичных и вторичных метаболитов. В рамках данного исследования целью было провести химический анализ вторичных метаболитов, содержащихся в естественно произрастающем грибе A. bisporus (коричневом), и определить химическую структуру некоторых из них. Из биомассы, полученной из этанольного экстракта гриба Agaricus bisporus, методом колоночной хроматографии с последовательной промывкой в системе растворителей, а именно хлороформ:метанол 19:1 (об)/об), методом

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фракционирования был определен 2,4-имидазолидиндион,5-метил, а химическая формула соединения 2,4-имидазолидиндион,5-метил была определена на основе ИК- и ЯМР-спектроскопического анализа. Была прокомментирована биологическая активность полученных вторичных метаболитов.

Ключевые слова: A. bisporus, вторичный метаболит, 5-метилимидазолидин-2,4-дион, ГХ-МС, ЯМР-спектроскопия, биологическая активность.

Annotatsiya. Dunyo bo‘ylab oq tugmachali qo‘ziqorin ya’ni Agaricus bisporus shtammlari keng tarqalgani va katta miqiyosida foydalanilishining sababi uning ajoyib mazasi va ozuqaviy tarkibidan tashqari juda xilma-xil va ko‘p miqdordagi birlamchi va ikkilamchi metabolitlarni o‘zida sintezlashidir. Ushbu tadqiqot doirasida tabiiy sharoitda o‘sgan A. bisporus (jigarrang) qo‘ziqorini tarkibidagi ikkilamchi metabolitlarni kimyoviy profillash va ba’zilarini kimyoviy tuzilishi aniqlashga qaratildi. Agaricus bisporus qo‘ziqorinining etanolli ekstraktidan olingan biomassasidan kalonkali xromatografiya usuli yordamida erituvchilar sistemasida keta-ket yuvish natijasida, aynan xloroform: methanol 19:1 (v/v) fraksiyalash 2,4-imidazolidinedione,5-metil, birikmasi GC-MS xromatografiyasi yordamida aniqlandi hamda shu fraksiyalash bosqichida ajratib olingan 2,4-imidazolidinedione,5-metilga o‘xshash deb taxmin qilingan birikmasining IQ va NMR spektroskopik tahlillari asosida kimyoviy tuzulishi aniqlandi. Olingan ikkilamchi metabolitning biologik faolliklariga izoh keltirildi.

Kalit so‘zlar: A. bisporus, ikkilamchi metabolit, 2,4-imidazolidinedione,5-metil, biologik faollik, YaMR spektri, GX-MS

INTRODACTION.

Agaricus bisporus is one of the most important fungi in terms of its worldwide cultivation and consumption, it is a species distinguished not only by its valuable nutritional composition, but also by the presence of various secondary metabolites contained in it. For this reason, attention has been paid to the sources on secondary metabolites in A. bisporus. In A. bisporus species, phenolic, flavonoid, and saturated fatty acid compounds were found to be 28.74 mg g-1, 9.56 mg g-1 and 45.64%, respectively [1]. In the wild edible species of this species found in the Zarnagh region, metabolites such as quercetin and kaempferol, rutin, myriccetin, have been detected by HPLC-UV analysis [2]. Based on infrared spectroscopy examination, the bioactive chemical components, especially β-(1→3)-glucan, are known to be very similar [3]. The resulting findings indicate that (a) ethanolamines and cholines are the bulk of diacylphospholipids, (b) sterols are relatively large in the presence of mainly ergosterols, and (c) the phospholipidic fatty acid content consists of almost linoleic acid [4]. Two fatty

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acids, namely methyl esters (the relative amount of more than 40%, are known): methyl (E, E)-9,12-octadecadienoate (methyl linoleate) and methyl (Z)-9-octadecanoate (methyl oleate) [5]. Agaricus blazei is known to contain unique agaricol compounds with anti-cancer activity as used against liver cancer [6]. A. bisporus linoleic acid, phenolic, indole, sterol, and vitamins D2, B1, B2, B6, B7, and C [7]. In the wild strain CCMJ1363 of A. bisporus, large amounts of phenolic compounds such as -7,840 mg gallic acid as well as metabolites such as 1,1-diphenyl-2-picrylhydrazyl have been identified [8]. A large amount of ergothionine, phenolic compounds, and antioxidant properties were also present in the fungal strain A-SPA15 (brown color) when the phenolic compound analysis was performed with the Folin-Ciocalteau method [9]. The reconstituted crude polysaccharides contained 11.57% α-glucan and 16.37% β-glucan. Carbohydrates are known to make up 44.18% [10]. The major antioxidant components found in ethanol extracts were total phenols (9.44 to 10.02 μg/g). Common antioxidant components (ascorbic acid, total tocopherols, and total phenols) have been studied [11]. A total of 19 phenolic compounds have been found in the ABE substrate: polyphenols (caffeine, chlorogen, catechin, gallic and ferulic acids, resorcinol, propyl quercchin, rutin, didroxacetine, l-didroxyphenylalanine, gallate) and monophenols (phenol, p- nitrophenol, o-cresol, m- cresol l-tyrosine, o-nitrophenol, guaacol, p- cresol) [12]. A significantly higher amount of uridine levels has been found in culture strains of A. bisporus [13]. The fact that A. brasiliensis is rich in large amounts of unsaturated fatty acids, phenolic acids, tocopherols, and ergosterols has been identified in ethanol and methanol extracts [14].

Traces of carbohydrates such as mannose and xylose in Agaricus bisporus have been detected using gas chromatography [15]. Agaricus bisporus is known to contain gallic, trans-cinnamon, and chlorogenic acids [16]. In GC-MS analysis of hexane and ethyl acetate extracts of this fungus, derivatives of butyl ester, behenic alcohol, S, S-dioxide were found to contain some effective microbial resistance active ingredients [17]. Linoleic acid and 1-octen-3-ol are known to be major fatty acid and flavor compounds, respectively [18]. The soluble sugars in A. bisporus arabitol, glucose, and trehalose were considered to be higher than 10% [19]. Ergosterol, brassicasterol, trilinolein, and linoleic acid have been isolated from dichloromethane extracts of A. bisporus [20]. This type of pleasant almond flavor has been found to be complementary flavoring compounds benzyl alcohol, methyl benzoate, and 4-hydroxybenzaldehyde, along with the main volatile campounds benzaldehyde and benzoic acid [21]. Higher amounts of benzyl alcohol, furancarboxaldehyde, benzaldehyde, sedrol volatile compounds than several varieties of this species from Tibet have been identified [22].

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MATERIAL AND METHODS.

Under natural conditions, the fungus A. bisporus (brown) was harvested as a biomass with its fruit and cap in field fields in Kashkadarya region, Kasbi, Mirishkor and Koson districts, and the resulting mass was completely dried at room temperature and ground until it became a powder. The finished mass was dissolved (3:1), extracted and filtered several 10 times in an ethyl alcohol (96%). The resulting filtration is extracted by driving 78.5°C in a vacuum rotor evaprotor to extract biomass 12,4 gr. Biommasa was dried, placed in a chromatographic column and fractionated in a system of different solvents. The fraction obtained in the chloroform: methanol (19:1) system of washing in solvents was analyzed by GX-MS [23].

Chromatec 5000 gas chromatographide (GC) equipped with HP-5 column (30 m × 0.32 mm × 0.25 μm) was performed. Helium was used as the carrier gas. Temperature of the GX evaporator was initially kept at 80°C for 5 min, then temperature was raised to 300°C at a speed of 4°C/min and held for 30min. The components were identified from the mass spectra of each component after comparing it with the available spectral data from the MS library NIST 2017.

NMR spectra were acquired on a JNM-ECZ600R spectrometer (600 MHz for 1H and 150 MHz for 13C, JEOL, Japan) in CD3OD. TMS (δ 0.00 ppm) was used as an internal standard for 1H NMR shifts, and solvent signals (CD3OD, 49.00 ppm vs. TMS) were used as a reference for 13C NMR shifts.

RESULTS AND DISCUSSION.

By fractionation in the chloroform: methanol (19:1) system of the fungus Agaricus bisporus, 5-methylimidazolidin-2,4-dione was obtained, and its structure was determined using physical survey methods (Fig. 1).

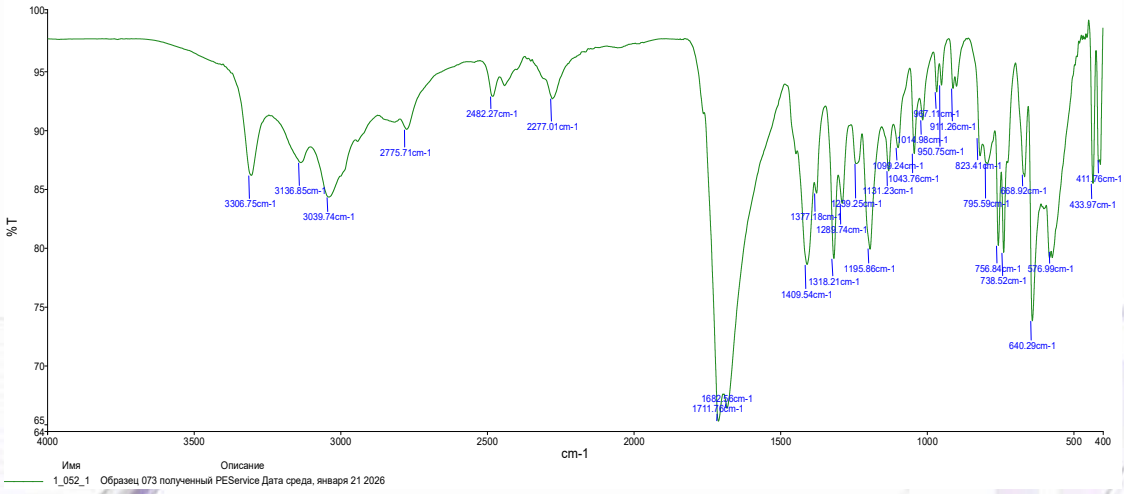
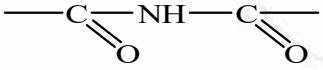


Figure 1. IR spectrum of 5-methylimidazolidin-2,4-dione substance

In the IR spectrum of the substance, the presence of the stretching vibration of the signal characteristic of the =N-H group in the group CH_3-CH-NH-C=O at

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3306.75 cm^{-1} and the presence of the stretching vibration of the signal characteristic of the =N-H group in the group  at 3136.85 cm^{-1} are in the region for active hydrogen bonds in the secondary amine groups in the molecule. The presence of the stretching vibrations characteristic of the carbonyl group at 1711.76 cm^{-1} and 1682.56 cm^{-1} indicate the presence of two carbonyl –C=O groups attached to the secondary amine group. In the IR spectrum of 5-methylimidazolidine-2,4-dione, the presence of signals with asymmetric stretching vibrations characteristic of the -CH3 group in the region of 2775.71 cm^{-1} and symmetric stretching vibrations characteristic of the -CH3 group in the region of 2482.27 cm^{-1}, and the presence of asymmetric deformation vibrations characteristic of the -CH3 group in the region of 738.52 cm^{-1} and symmetric deformation vibrations characteristic of the -CH3 group in the region of 640.29 cm^{-1} indicates the presence of a -CH3 group in the molecule. The presence of deformation vibrations characteristic of the group  in the molecule in the region of 1409.54 cm^{-1} indicates the presence of a group  attached to the methyl group in the molecule.

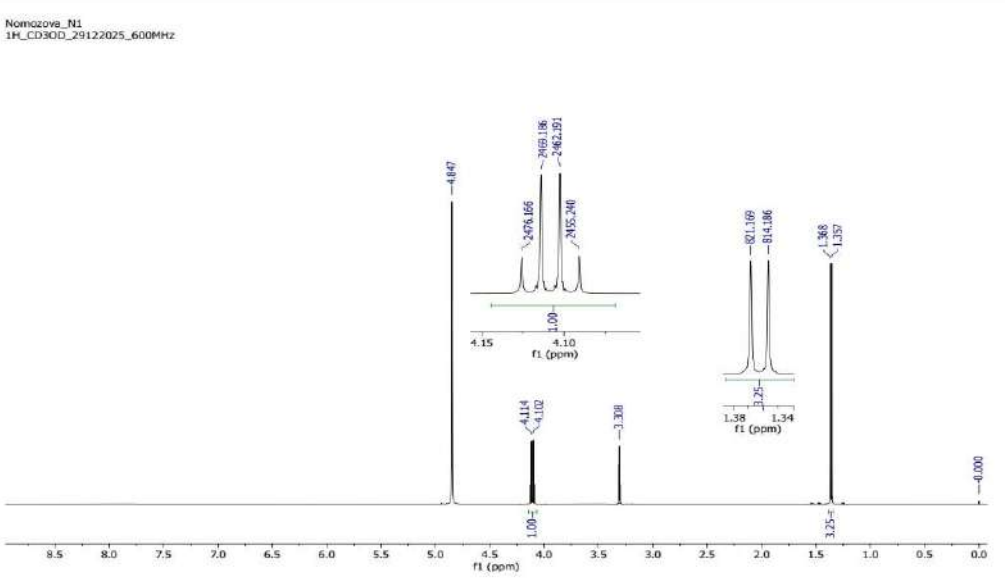
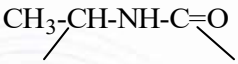
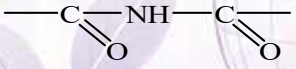


Figure 2. H-NMR spectrum of 5-methylimidazolidin-2,4-dione substance

The presence of 3 proton-specific duplicate signals at 1.3 ppm in the H-NMR spectrum (fig. 2) of the 5-methylidazolidin-2,4-dione substance indicates that the molecule has a group bound to the methyl group,  and the presence  of a single proton-specific quartet signal at 4.10 ppm of the group-specific signal indicates that it is bound to the CH3 group.

The fact  that the secondary amine group located in the molecule has a singlet signal at 3.3 ppm, and that the 

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secondary amine specific to the group in the group has a singlet signal at 4.8 ppm, indicates that there are two secondary amine groups in the molecule.

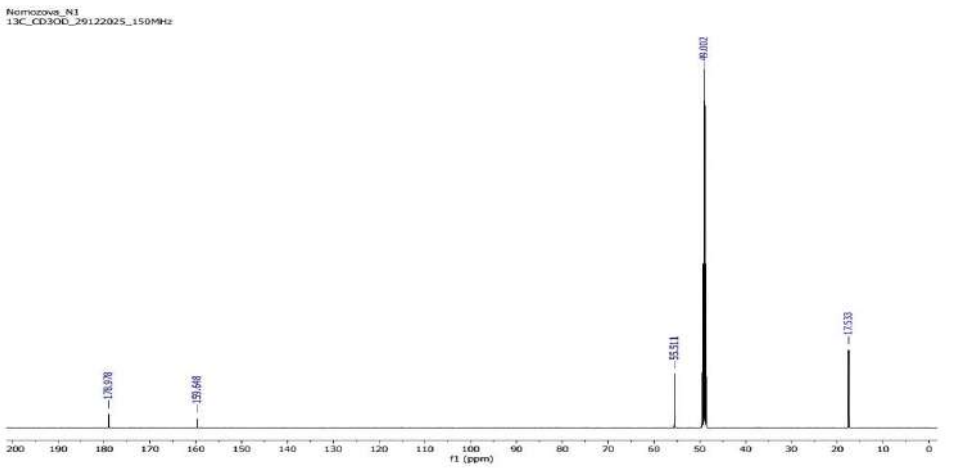


Figure. 3. Spectrum of 5-methylimidazolidine-2,4-dione substance ^{13}C

Since the molecule contains 4 carbons in the 13C spectrum of 5-methylimidazoline-2,4-dione, it has been shown that at 17.53 ppm, the carbon located in the CH3 group (fig.3), at 55.51 ppm $\rangle CH$, the carbon located in the carbonyl group at 159.64 ppm, CH_3-CH-NH-C=O and the carbonyl group in the carbonyl group at —C(=O)—NH—C(=O)— 178.97 ppm

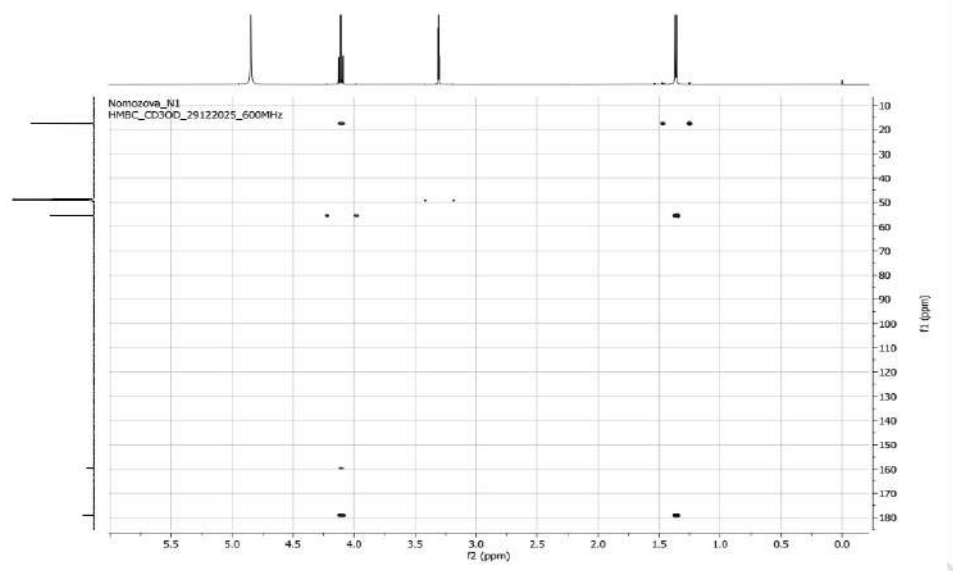


Figure 4. HMBC spectrum of 5-methylimidazolidin-2,4-dione.

HMBC divides signals in the spectrum into two dimensions (fig. 4). We see the H-NMR spectrum along the X and the Y NMR spectrum along ^{13}C. At 17.53 ppm in the ^{13}C spectrum, the spin-spin interaction of the C-6 carbon located in the H-NMR spectrum at 1.3 ppm with the protons located in the C-5 carbon spectrum, and at 4.10 ppm with the protons located in the C-5 carbon, indicate their positioning. $\rangle CH$ Spin-spin interactions of C-5 carbon located in the H-NMR

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spectrum at 4.10 ppm with protons located in the C-5 carbon in the heminal state and at 1.3 ppm with protons located in the –CH3 region indicate that C-5, C-6 carbons are located in the geminal and vitsinal state.

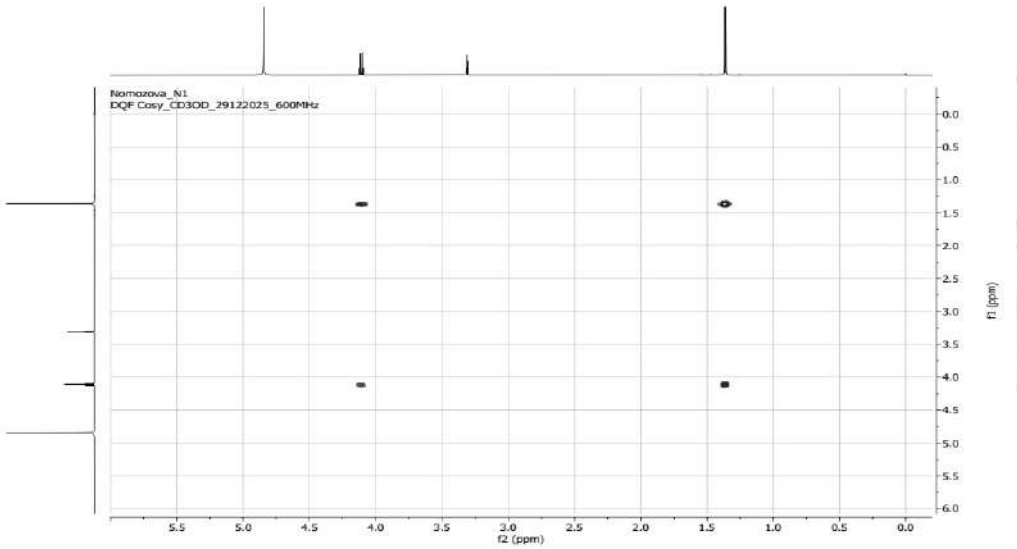


Figure 5. COSY spectrum of 5-methylimidazolidin-2,4-dione substance

COSY divides signals in the spectrum into two dimensions (fig 5). We see the H-NMR spectrum along the X-axis and the H-NMR spectrum along the Y-axis. The C-6 carbon proton in the H-NMR spectrum signals at 1.3 ppm. The C-5 carbon-located protons signal at 4.10 ppm the spin-spin interaction without geminal and vitsinal with the signal of the C-5 carbon at 4.10 ppm indicates their location location. Spin-spin interactions with protons located in the C-5 carbon at 4.10 ppm in the H-NMR spectrum, and in the vitsinal state with protons located in the C-6 carbon–CH3 region at 1.3 ppm, indicate that the protons are located in the C-5, C-6 carbons in the geminal and vitsinal states relative to each other. Based on the above spectrum data, it was determined that the structure of the extracted substance was as follows.

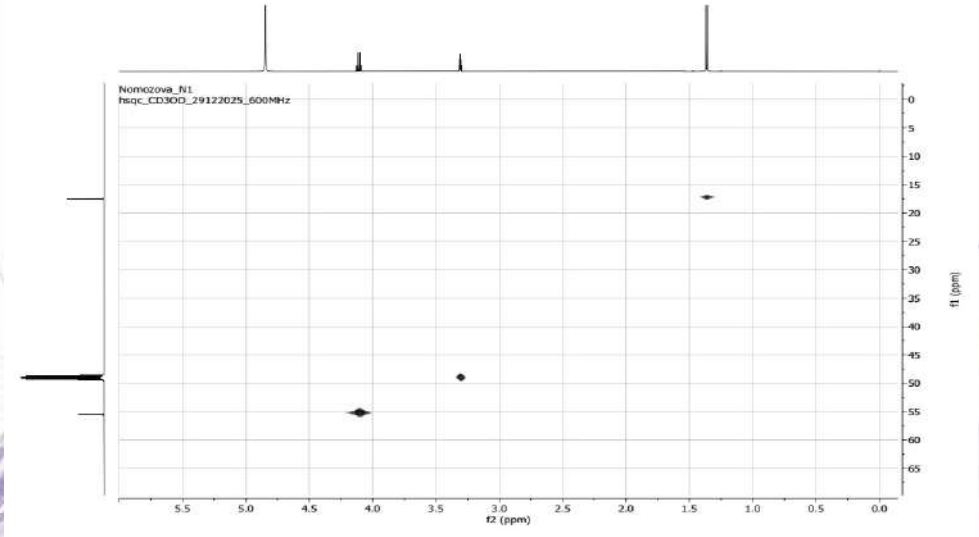
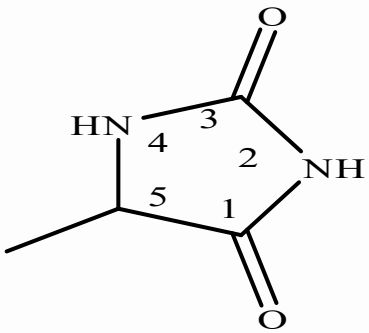


Figure 6. HSQC spectrum of 5-methylimidazolidin-2,4-dione substance

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The HSQC divides signals in the spectrum into two dimensions. We see the H-NMR spectrum along the X-axis and the ^{13}C NMR spectrum along the Y-axis. At 17.53 ppm in the –CH3 spectrum, the spin-spin interaction of C-6 carbon located in the PMR spectrum with protons located in the C-5 carbon–CH3 group at 1.3 ppm –CH3 indicates their location in the heminally. At 55.51 ppm, the $\rangle CH$ spin-spin interaction of the C-5 carbon located in the H-NMR spectrum with the protons located in the C-5 carbon spectrum at 4.10 ppm indicates that the C-5 carbon belongs to the proton located in the C-5 carbon.



5-methylimidazolidin- 2,4-dion

CONCLUSION. From the obtained results and spectral analysis, it turned out that one of the decomposing substances in this solvent system is 5-methylmidazolidin-2,4-dione. This compound occupies an important place in medicine and pharmaceuticals, mainly due to its participation as an intermediate in the preparation of antimicrobial and anti-inflammatory drugs.

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