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ISOLATION OF PYROLE-2-CARBOXYLIC ACID METHYL ESTER FROM *A. BISPORUS* MUSHROOM GROWING IN NATURAL CONDITIONS AND EXPLANATION OF ITS STRUCTURE USING SPECTROSCOPIC METHODS

Annotation

A. bisporus mushroom is considered one of the leading mushrooms in the world in terms of consumption and chemical composition, as well as its richness in various components. In the framework of this study, the substances obtained from *A. bisporus* mushroom during the chloroform: methanol (5:1) system fractionation stage by column chromatography method were analyzed by GC-MS: secondary metabolites belonging to the following classes were formed: complex esters, various heterocyclic compounds, organic acids, etc. Among them, the substance pyrrole-2-carboxylic acid methyl ester was also found, and its chemical structure was determined using physical research methods (IR, H-NMR, NMR).
Keywords: *A. bisporus*, metabolite, Pyrrole-2-carboxylic acid methyl, chromatography, fraction, chloroform.

ВЫДЕЛЕНИЕ МЕТИЛОВОГО ЭФИРА ПИРРОЛ-2-КАРБОНОВОЙ КИСЛОТЫ ИЗ ГРИБА *A. BISPORUS*, РАСТУЩЕГО В ЕСТЕСТВЕННЫХ УСЛОВИЯХ, И ОБЪЯСНЕНИЕ ЕГО СТРУКТУРЫ С ПОМОЩЬЮ СПЕКТРОСКОПИЧЕСКИХ МЕТОДОВ

Аннотация

Гриб *A. bisporus* считается одним из ведущих грибов в мире по объему потребления и химическому составу, а также по богатству различных компонентов. В рамках данного исследования вещества, полученные из гриба *A. bisporus* на стадии хлороформного фракционирования методом колоночной хроматографии, были проанализированы методом ГХ-МС: образовались вторичные метаболиты, относящиеся к следующим классам: сложные эфиры, различные гетероциклические соединения, органические кислоты и др. Среди них было обнаружено присутствие метилового эфира пиррол-2-карбоновой кислоты, химическая структура которого была определена с помощью физических методов исследования (ИК, ПМР, ЯМР).
Ключевые слова: *A. bisporus*, метаболит, метиловый эфир пиррол-2-карбоновой кислоты, хроматография, фракция, хлороформ.

TABIIY SHAROITDA O‘SGAN *A. BISPORUS* QO‘ZIQORINIDAN PIROL-2-KARBOKSILIK KISLOTA METIL EFIRI AJRATIB OLISH VA TUZILISHINI SPEKTROSKOPIK USULLAR YORDAMIDA IZOHLASH

Annotatsiya

Dunyo bo‘ylab *A. bisporus* qo‘ziqorini iste’moli va kimyoviy tarakibi bo‘yicha turli komponentlarga boyligi tomondan ham eng yetakchi o‘rinni egallab kelayotgan qo‘ziqorinlardan biri hisoblandi. Ushbu tadqiqot doirasida esa A. bisporus qo‘ziqorinidan etil spirtli ekstraksion biomassani kalonkali xromatografiya usulida xloroformli fraksiyalash bosqichida olingan moddalar GX-MS tahlilidan o‘tkazilganda: murakkab efirlar, turli xil geterosiklik birikmalar, organic kislotalar, kabi sinflarga mansub bo’lgan ikkilamchi metabolitlar hosil bo‘ldi. Ular orasida pirol-2-karboksilik kislota metil efirining moddasi ham borligi ma’lum bo‘ldi va uning kimyoviy tuzilishi fizik tadqiqot usullari (IQ, PMR, YaMR) yordamida aniqlandi.
Kalit so‘zlar: *A. bisporus*, metabolit, Pirol-2-karboksilik kislota metil, xromatografiya, fraksiya, xloroform

Introduction. The *Agaricus* genus is one of the most widespread fungi in nature, which, along with the production of secondary metabolites of new structures, exhibit significant biological activity.

Literature review. *A. bisporus* mainly containing terpenoids, nitrogen compounds, steroids, sphingoids, aliphatic compounds and other structural types, some of which have antitumor, anti-neurolysin, antibacterial, anti-angiotensin-converting enzyme effects [1]. Polyols, mainly fungal sugars such as trehalose and mannitol, were detected in five test strains of *A. bisporus* [2]. When non-volatile flavor components were determined in dried commercial mushrooms from Taiwan, such as *A. blazei* (Brazilian mushroom), *A. cylindracea* (black bolete), and Boletus edulis (king bolete), all three species were found to have high carbohydrate, crude fiber, and protein contents, and low crude ash and fat contents. Arabitol, myo-inositol, mannitol, and trehalose were detected in the three mushrooms [3]. GC and GC/MS analysis of *A. bisporus* revealed 40 components (totaling approximately 150 μg/g), and a small amount of benzyl isothiocyanate was detected, indicating the presence of benzyl glucosinolates in *A. bisporus* [4]. When a vaporized extract with a strong mushroom-like odor was extracted from the white mushroom *Agaricus bisporus*, volatile compounds such as 3-methylbutanal, 3-octanone, oct-1-en-3-one, 3-octanol, oct-1-en-3-ol, furfural, benzaldehyde, phenylacetaldehyde, and benzyl alcohol were identified by gas chromatography-mass spectrometry [5]. It was found that compounds such as 1-octen-3-ol, benzyl alcohol, and 3-octanone were the most abundant flavor compounds in *A. bisporus*. It was found that the increase in the amount of isovaleraldehyde and 3-octanone during the final storage period of the mushroom may cause its quality deterioration [6]. When the compositional analysis of *A. bisporus* was performed to investigate the fungitoxic properties, 1-octanol was found to exhibit the highest activity. The activity of this compound can be explained by the following: the high hydrophobicity of the alkyl group, the primary structure of the alcohol group, and the unbranched structure of the alkyl group have been found to exhibit the high toxicity of 1-octanol [7]. Secondary metabolites in *A. bisporus*: dodecane, benzo-2,3-pyrrole, tetradecane, and volatile components such as 2,4-bis(1,1-dimethylethyl) phenol have been identified [8]. The composition of *A. bisporus* sample No. 0107 obtained from Guangzhou was 27.24% with benzyl alcohol, furancarboxaldehyde, benzaldehyde, 2-(1,1-dimethylethyl)-3-methyl-Oxirane, 5,6-dihydro-6-pentyl-2H-Pyran-2-one, and 2-Propen-1-ol. The results showed that the source of the mushroom, the climate of origin, and the cultivation methods were found to affect the taste of *A. bisporus* [9]. MALDI TOF/TOF MS analysis of *A. bisporus* extract revealed sugar compounds such as GH27 alpha-glucosidases, GH47 alpha-mannosidases, GH20 hexosaminidase and alkaline phosphatases [10]. Ergosterol, trilinoleic acid, brassicasterol and linoleic acid were found in dichloromethane extract of *A. bisporus* [11]. Sugars such as urea, ergosterol, mannitol, ergosterol peroxide, nitrogenous compounds and steroids were found in 95% ethanol extracts of *Agaricus silvaticus* in Mongolia [12]. *A. blazei* (A. blazei) is a mushroom that has the ability to produce metabolites with a wide range of biological effects. It has been found to contain steroids such as ergosterol, which is a tumor killer [13]. Compounds such as benzoylergostane, N-benzoyl-L-leucine methyl ester, 2 identified ergostanes, and a large amount of insterol, along with 2 phenylhexane derivatives, were detected from the fruit body of *A. blazei* [14]. The most common components in the volatile and butanol content were found to be phenol and urea compounds, respectively [15].

Research Methodology. Biomass was prepared from the fruit body of A. bisporus mushroom collected from the fields of some districts of the Kashkadarya region [16]. The obtained extraction mass was placed in a glass chromatographic column equipped with silica gel, washed in a hexane, hexane, and chloroform system, and then fractionated in pure chloroform. The fraction sum obtained at this stage was evaporated in a vacuum rotor evaporator at 61.5 ℃ at a speed of 40-50 rpm in a water bath. The composition of the obtained samples was examined by thin-layer chromatography, similar samples were separated and examined by GX-MS analysis.

Analysis and results. As a result of fractionation of Agaricus bisporus mushroom in chloroform:methanol (5:1) system, a white crystalline substance pyrrole-2-carboxylic acid methyl ester was obtained, and its structure was determined using physical research methods and its mass spectrum was obtained (Fig 1).

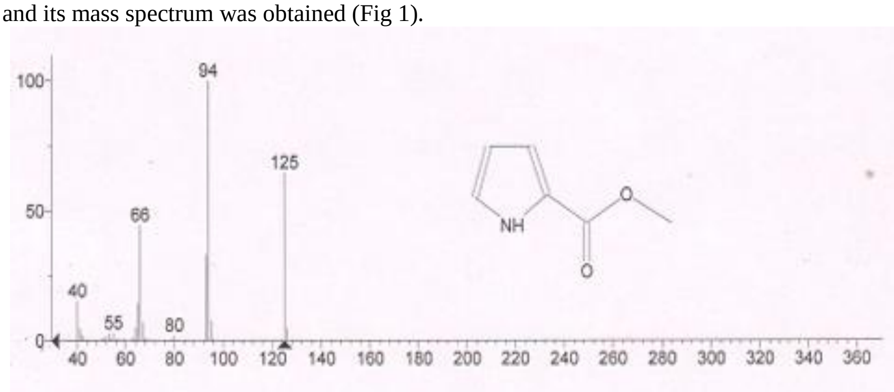
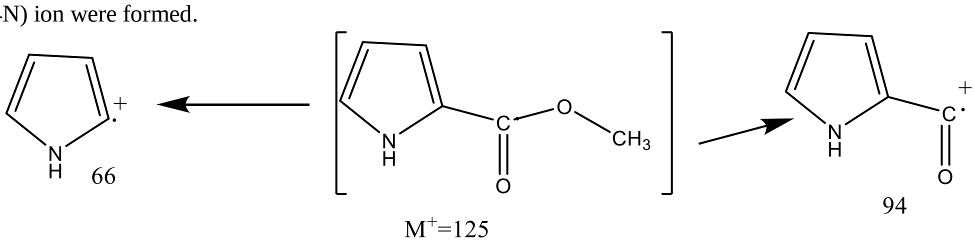


Figure 1. Mass spectrum of pyrrole-2-carboxylic acid methyl ester

In the mass spectrum of pyrrole-2-carboxylic acid methyl ester, it was determined that the M+ molecular ion m/e 125 (C6H7NO2) was equal to. It shows that during the fragmentation of the molecule, a high-intensity m/e 94 (C5H4NO) ion and a m/e 66 (C4H4N) ion were formed.



In the IR spectrum of pyrrole-2-carboxylic acid methyl ester, high-intensity signals were observed in the following regions (Fig 2).

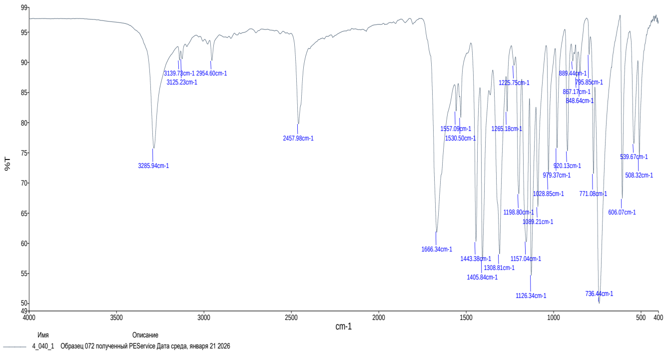


Figure 2. IR spectrum of pyrrole-2-carboxylic acid methyl ester

The absence of the stretching vibration of the signal characteristic of the =N-H bond in the pyrrole group in the IR spectrum of the substance at ν=N-H =3285.94 cm-1 indicates the presence of an active hydrogen bond in the secondary amine group in the molecule. The stretching vibration characteristic of the –CH bond in the heterocyclic group of pyrrole-2-carboxylic acid methyl ester is 2457.98 cm^{-1}, the symmetrical and asymmetrical stretching vibration characteristic of the –CH bond in the group is 3139.73 cm^{-1}, 3125.23 cm^{-1} and the stretching vibration characteristic of the - bond is 2954.60 cm^{-1}, indicating the presence of two double bonds in the five-membered heterocyclic compound. The presence of a very strong stretching vibration characteristic of the -C=O carbonyl group in the molecule at 1666.34 cm^{-1} indicates that the carbonyl group is bound to the oxygen atom in the molecule. In the IR spectrum of pyrrole-2-carboxylic acid methyl ester, the symmetric and asymmetric stretching vibrations characteristic of the -C=C- bond in the molecule are visible in the 1557.09-1530.50 cm^{-1} regions. The symmetric stretching vibrations characteristic of the ester -O-CH3 group in the molecule are visible in the 1308.81 cm^{-1} region and the –CH3 deformation vibrations are visible in the 1443.38-1405.84 cm^{-1} regions. The deformation vibrations of the =C-H bonds located in the double bond in the pyrrole heterocycle in the molecule are visible in the 771.08; 736.44, and 848.64 cm^{-1} regions.

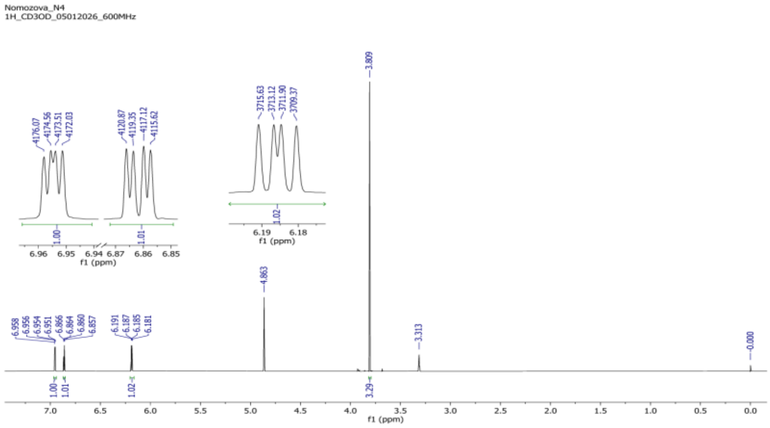


Figure 3. H-NMR spectrum of pyrrole-2-carboxylic acid methyl ester spectrum

The presence of a singlet signal of 3 protons specific to the -O–CH3 group at 3.809 m.u. in the PMR spectrum (Fig 3) of pyrrole-2-carboxylic acid methyl ester indicates the presence of –CH3 in the molecule, and the presence of a singlet signal at 4.863 m.u. specific to the NH bond in the pyrrole ring, and the presence of a singlet-doublet signal of one proton specific to the -N-C-H group in the pyrrole ring at 6.18 m.u. indicates that the C-H group attached to the secondary nitrogen is located in the pyrrole ring. The presence of a triplet-doublet signal at 6.86-6.95 m.u. of the C-H bond of the - group attached to the secondary amine of the pyrrole ring in the molecule indicates the presence of a pyrrole ring.

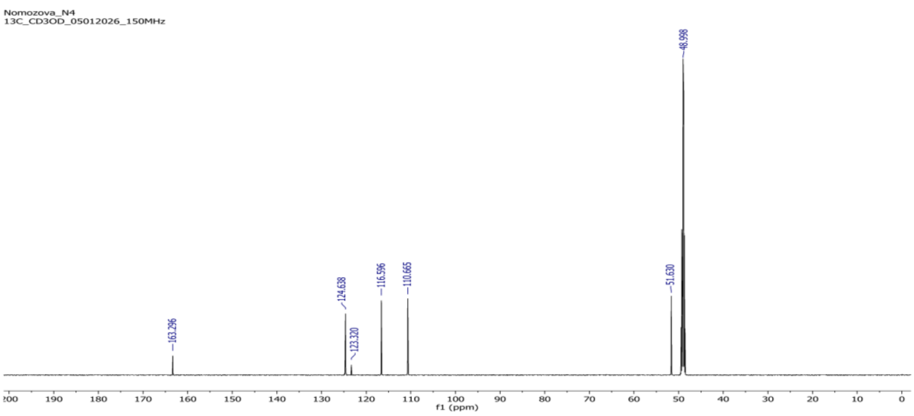
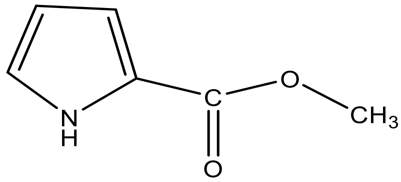


Figure 4. ^{13}C -NMR spectrum of pyrrole-2-carboxylic acid methyl ester spectrum

In the ^{13}C spectrum of pyrrole-2-carboxylic acid methyl ester, the singlet signal of the carbon located in the –O-CH3 group in the molecule at 51.63 m.u., the singlet signal of the carbon located in the carbonyl group at 163.29 m.u., and the signals of the

C-2 carbons bound to the carbonyl group of the 4 carbons located in the pyrrole ring at 124.63 m.u., the signals of the carbon bound to the electron donor –NH group with a positive mesomeric effect +M at 110.66 m.u., and the singlet signals characteristic of the carbons holding a double bond in the pyrrole ring that bind one hydrogen each at 123.32 m.u. and 116.59 m.u. are visible (Fig 4).



Pirol-2-karboksilik kislota metil efiri

Conclusion. Pyrrole-2-carboxylic acid methyl ester was found to be a white crystalline substance with a density of 1.184 g / cm3, melting point: 74-78 ℃, molar mass: 125 g / mol.

REFERENCES

1. Cao Jinfeng, Chen Xuhui, Liu Shiwei, Ding Jianhai. Research Advances in Secondary Metabolites and Biological Activities of Mushroom Genus Fungi // Academic Journal of Agriculture & Life Sciences, 2023, 4(1); doi: 10.25236/AJALS.2023.040109.
2. Wen Li, Zhen Gu, Yan Yang, Shuai Zhou, Yanfang Liu, Jingsong Zhang. Non-volatile taste components of several cultivated mushroomsm // Food Chemistry,2014, 143, 427-431, https://doi.org/10.1016/j.foodchem.2013.08.006
3. Shu-Yao Tsai, Hui-Li Tsai, Jeng-Leun Mau. Non-volatile taste components of Agaricus blazei, Agrocybe cylindracea and Boletus edulis // Food Chemistry, 2008, 107(3), 977-983, https://doi.org/10.1016/j.foodchem.2007.07.080
4. Alexander J. MacLeod, Shnehlata D. Panchasara. Volatile aroma components, particularly glucosinolate products, of cooked edible mushroom (Agaricus bisporus) and cooked dried mushroom // Phytochemistry,Volume 22, Issue 3,1983, Pages 705-709
5. Cronin D. A., Margaret K. Ward. The characterisation of some mushroom volatiles // Food and Agriculture Science Journal, Volume22, Issue9, 1971 477-479
6. Shao,Yang-yang and Gao,Hai-yan and Liu,Rui-ling and Fang,Xiang-jun and Chen,Hang-jun, ., Effect of harvesting method on the quality and volatile flavor compounds of Agaricus bisporus // China Food Publishing, 2022, 43(5), 218–226.
7. Eugene Sebastian J. Nidiry. Structure–fungitoxicity relationships of some volatile flavour constituents of the edible mushrooms Agaricus bisporus and Pleurotus florida. "Flavour and Fragrance Journal", 2001, 16(4), 245-248.
8. Bartkiene E, Zarovaite P, Starkute V, Mockus E, Zokaityte E, Zokaityte G, Rocha JM, Ruibys R, Klupsaite D. Changes in Lacto-Fermented Agaricus bisporus (White and Brown Varieties) Mushroom Characteristics, including Biogenic Amine and Volatile Compound Formation // Foods. 2023; 12(13):2441. https://doi.org/10.3390/foods12132441
9. Yan, S. J., Xie, X. M., Lin, S. Z., Xu, X. G., Jiang, S. P., Chen, B., ... & Liu, L. (2015). Study on volatile flavor components from Agaricus bisporus of Tibet. Acta Scientiarum Naturalium Universitatis Sunyatseni, 54, 70-78.
10. Morosanova, M. A., Fedorova, T. V., Polyakova, A. S., & Morosanova, E. I. (2020). Agaricus bisporus crude extract: Characterization and analytical application // Molecules, 25(24), 5996.
11. Consolacion Y Ragasa, Jo Madeleine Ann Reyes, Maria Carmen S Tan, Robert Brkljača, Sylvia Urban. 2016. Research Article Sterols and Lipids from Agaricus bisporus // International Journal of Pharmaceutical and Clinical Research 2016; 8(10): 1451-1453.
12. Munkhgerel, L., Erdenechimeg, N., Dumaa, M., Zhang, G., Odonmajig, P., & Regdel, D. (2014). Chemical and biological investigation of the Agaricus silvaticus Schaeff ex. Secr // Mongolian Journal of Chemistry, 12, 92–97. https://doi.org/10.5564/mjc.v12i0.180
13. Shimizu T, Kawai J, Ouchi K, Kikuchi H, Osima Y, Hidemi R. Agarol, an ergosterol derivative from Agaricus blazei, induces caspase-independent apoptosis in human cancer cells // Int J Oncol. 2016 Apr;48(4):1670-8. doi: 10.3892/ijo.2016.3391. Epub 2016 Feb 15. PMID: 26893131.
14. Ueguchi Y, Matsunami K, Otsuka H, Kondo K. Constituents of cultivated Agaricus blazei // Journal of Natural Medicines, 2011 65(2):307-12. DOI:10.1007/s11418-010-0495-5
15. Petrova A, Alipieva K, Kostadinova E, Antonova D, Lacheva M, Gjosheva M, Popov S, Bankova V. GC-MS studies of the chemical composition of two inedible mushrooms of the genus Agaricus // Chem Cent J. 2007 Dec 20;1(33). doi: 10.1186/1752-153X-1-33. PMID: 18096035; PMCID: PMC2228291
16. Nomozova, M.Z., Kamolov, L. S., Nakhatov I., Nuraliyeva D.I. Extraction of secondary metabolites from agaricus bisporus fungus // Universum: химия и биология. 2026, fevral, 2(140), pp 26-30