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# ACYLATION REACTION OF THE ALKALOID 13,22-DIMETHOXYSTACHYBOTRYNE ISOLATED FROM THE MICROFUNGUS STACHYBOTRYS CHARTARUM

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## Abstract

The structure of the isolated alkaloids and terpenoids was determined by conducting acylation reactions with acetic anhydride based on the alkaloids and terpenoids isolated from the *Stachybotrys chartarum* fungus, and biologically active compounds were obtained.13,22-Dimethoxystachybotryn is one of the important alkaloids of the *Stachybotrys chartarum* microfungus grown in laboratory conditions, and was isolated using column chromatography and an acylation reaction was carried out on it. In the process of forming O-Monoacetate, the reagents were taken in a 1:1 molar ratio and the reactions were carried out at room temperature. When 13,22- Dimethoxystachybotryn was taken with acetic anhydride in a 1:2 molar ratio, O-diacetate compounds were formed.

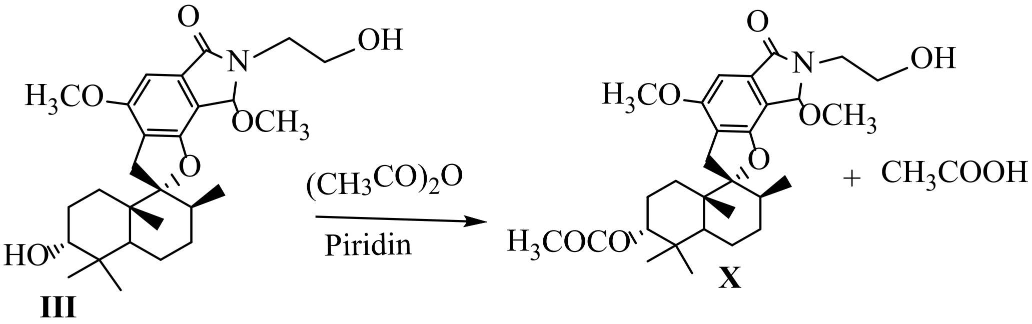
**Keywords:** *Stachybotrys chartarum, acetic anhydride, pyridine, terpenoid, benzene ring, geminal, correlation, chromic anhydride, acetic anhydride, nucleophilic reagent, carbon atom*

## Introduction

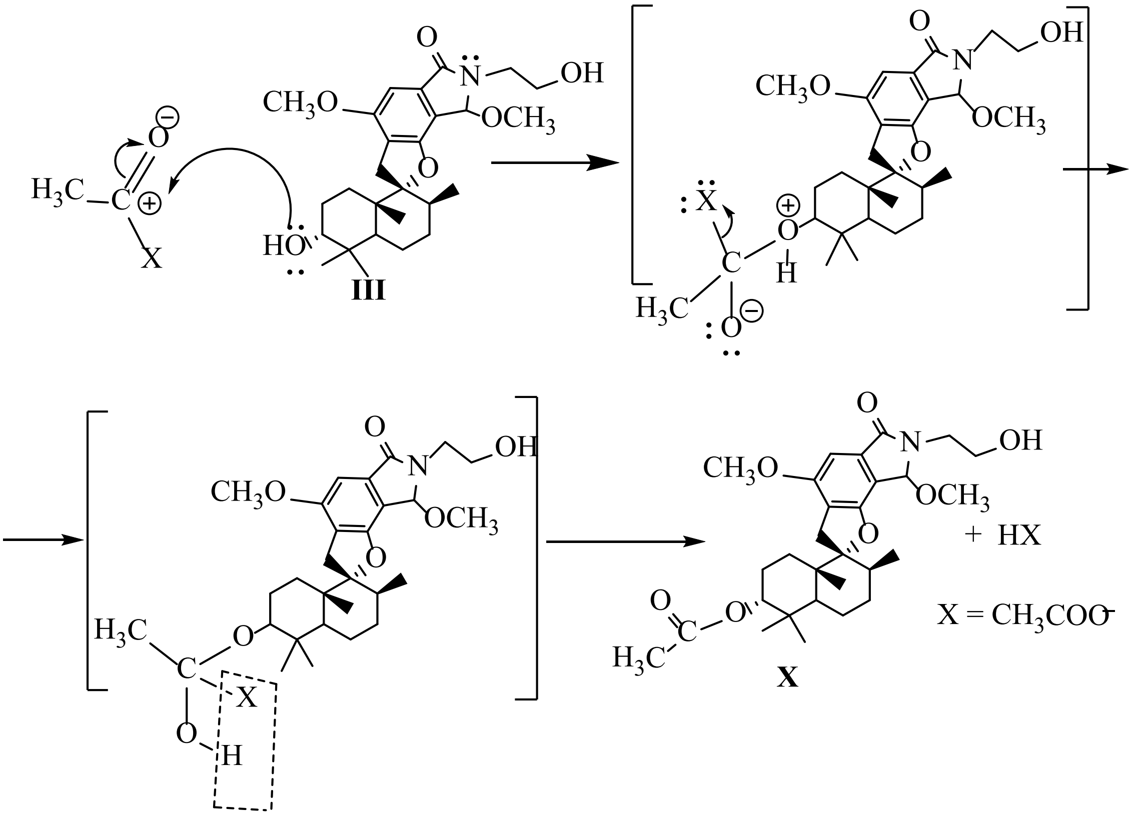
S. chartarum fungi are hydrophilic organisms that require moist conditions for their growth and development. They can also live on gypsum, cellulose-based building boards, fiberglass coatings, wallpaper, natural fiber carpets, insulated pipes, wood and wood chip-based panels, and organic residues. They are also found in soil, plant grains, and garbage (Hodgson, M.J. et al., 1998; Kuhn, D.M. et al., 2003). S.chartarum is one of the most common indoor fungi that produces pathogenic mycotoxins. The areas where this fungus is present are dangerous for human life (Kuhn, D.M. et al., 2003). Indoor air and surfaces contaminated with the fungus or its mycotoxins cause serious pathological diseases and even death in humans (Hodgson, M.J. et al., 1998; Castlebury, L.A., 2004). Common symptoms of such a disease are fatigue, chest tightness, inflammation of the mucous membranes, headache, etc. (Hodgson, M. J. et al., 1998). It can also cause more serious respiratory diseases, ranging from cough and hiccups to damage to the bronchi and alveoli or pulmonary fibrosis (Johanning, E. et al., 1996). Even more dangerous, cases of pulmonary hemorrhage in infants have been reported as a result of exposure to this fungus (Dearborn, D. G. et al., 2002).

## Research methodology

Determination of the structure of the isolated alkaloids and terpenoids and obtaining biologically active compounds by conducting acylation reactions with acetic anhydride based on the alkaloids and terpenoids of the fungus Stachybotrys chartarum.The alkaloid 13,22- Dimethoxystachybotrine (III) was isolated by growing the Stachybotrys chartarum fungus strain on Mandels nutrient medium and its physicochemical constants were determined as $C_{27}H_{39}NO_6$, liquid. tem. 225° (MeOH), $R_f = 0.58$.(YUQX, silufol, 11 system), $[\alpha]_D^{24} -13.4 \pm 2°$(c 0.8; $CHCl_3$-MeOH, 1:1).13,22- Dimethoxystachybotryne (III) is one of the important alkaloids of the laboratory-grown microfungus *Stachybotrys chartarum*, which was isolated using column chromatography and subjected to an acylation reaction. In the process of forming O-Monoacetate, the reagents were taken in a 1:1 molar ratio and the reactions were carried out at room temperature. The reaction was carried out as follows.



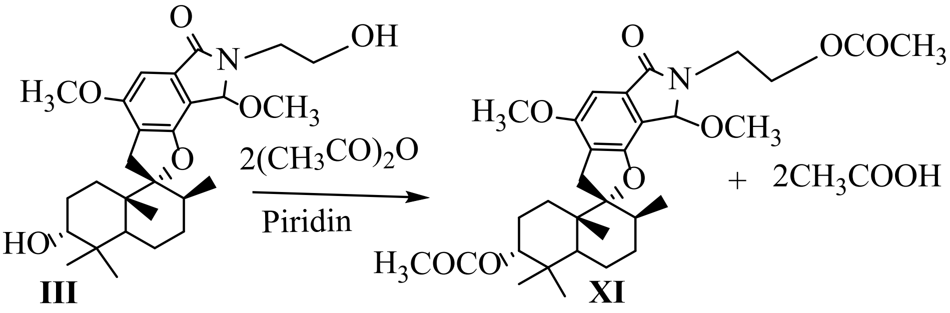
The reactions proceed according to the nucleophilic substitution mechanism.



During the reaction of the -OH group in the 13,22-dimethoxystaxybothrin molecule and acetic anhydride, the oxygen in the acetic anhydride molecule acquires a partial negative charge due to the electrons of the double bond in the carbonyl group moving towards the electronegative oxygen. As a result of the action of the electronegative oxygen atoms, the carbon atom becomes partially positively charged. The pair of electrons of the oxygen atom in the hydroxyl group located in the terpenoid ring in the 13,22-dimethoxystaxybothrin molecule interacts with the carbon atom in the acetic anhydride molecule, forming an intermediate compound. During the reaction, a new intermediate compound is formed due to the covalent bond between oxygen and carbon. Acetic acid is released due to the transfer of the hydrogen atom in the hydroxyl group. The resulting acetic acid forms a salt when taken with pyridine in a 1:2 molar ratio and does not affect the course of the reaction. 3O-Monoacetate-13,22-dimethoxystaxybothrin (X) was obtained by acylation of 13,22-dimethoxystaxybothrin (III) with acetic anhydride under dry pyridine conditions.

In order to study the reactions that are carried out due to the second reaction center group in the molecule of 13,22- Dimethoxysatibotrin (III), the reactions of compound (III) with acetic anhydride in a 1:2 molar ratio were studied in the presence of various solvents.

During the experiments, when 13,22-Dimethoxysatibotrin is taken with acetic anhydride in a 1:2 molar ratio, O-diacetate compounds are formed. The effect of the nature of the solvents on the reaction processes was studied and it was found that pyridine is a relatively favorable solvent for these reactions. The reactions proceed according to the following equation.



Theobtained3O,24O-diacetate–13,22-dimethoxystaxybotrin (XI) compound was found to have the elemental composition $C_{31}H_{42}NO_8$ and $R_f$=0,33.Based on the studies conducted, it was found that O-diacetate products are formed in the reactions of 13,22-dimethoxystaxybotrin with acetic anhydride in a 1:2 molar ratio.

The appearance of signals at 156.72; 150.24; 135.75; 118.12; 115.32; 97.43 m.u. in the$^{13}$C NMR spectrum of the investigated substance III (Table 1), indicates the presence of a benzene nucleus bound to five carbon atoms in the 13,22-Dimethoxystaxybotrin molecule. Accordingly, in the$^{1}$H NMR spectrum of III at 7.35 m.u. The presence of a singlet signal indicates the presence of one aromatic proton.

The formation of diacetate indicates the presence of two hydroxyl groups in the 13,22-dimethoxystaxybothrin (III) molecule. The occurrence of resonance frequencies at 3.79 m.u., characteristic of the methoxyl group in the 1H NMR spectrum of diacetate XI, indicates that the methoxyl group in this region is a methoxyl group attached to the benzene ring. Indeed, the appearance of a signal at 154.97 m.u., which belongs to the carbon atom attached to the methoxyl group attached to the benzene ring, in the 13C NMR spectrum, once again proves the presence of a methoxyl group attached to the benzene ring in 13,22-dimethoxystaxybothrin (III).

A comparative analysis of the$^{1}$H NMR spectra of compounds I and XI shows that the appearance of a single proton triplet signal at 4.56 m.u. in the$^{1}$H NMR spectrum of diacetate XI indicates that the proton in the acetoxyl group is geminally bound. Therefore, the corresponding hydroxyl group is located on the secondary carbon, and the appearance of a signal at 74.82 m.u. in the $^{13}C$ NMR spectrum of 13,22-dimethoxystaxybothrin confirms the presence of a secondary carbonyl carbon atom.

**Figure 2.** *$^{1}H$-$^{13}C$ NMR correlation spectrum of 13,22-Dimethoxystaxybothrin diacetate(XI) showing the effect of chemical shift*

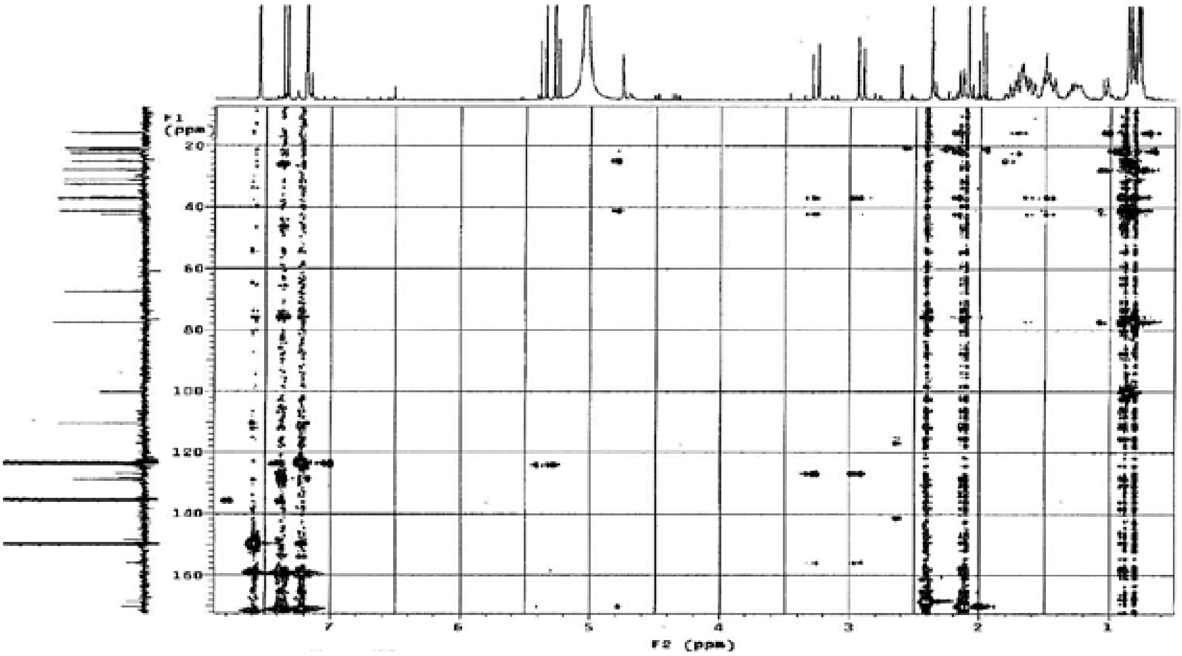
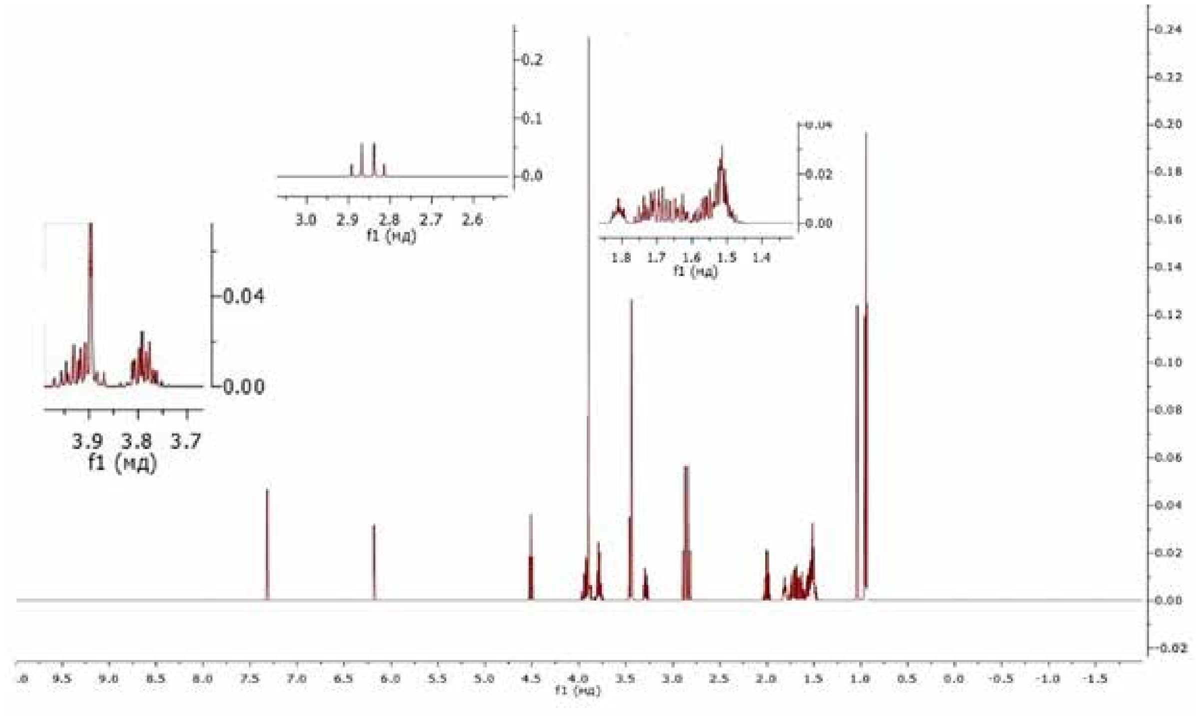


**Figure 1.** *H NMR spectrum of 13,22-Dimethoxysatibotrin*



The formation of a signal in the $^{13}C$ NMR spectrum of 13,22-Dimethoxysatibotrin (III) at 168.85 m.u. indicates the presence of a functional group in the substance, either urea or an isomerization group with an azomethineoxy rearrangement. The choice between them will be made later.

**Table 1.**

| Atom C | Substances | | | |
|---|---|---|---|---|
| | III | | XI | |
| | Δc | δн(J, Hz) | Δc | δн(J, Hz) |
| 1 | 24.71 | α 2.27 td (13; 3.5)<br>β 1.10 dt (13; 3.4) | 24.62 | α 1.71 td (13; 3.5)<br>β 1.56 dt (13; 3.4) |
| 2 | 26.07 | α 1.70; β 1.98 tt (13; 3.4) | 30.68 | α 1.65; β 1.80 tt (13; 3.4) |
| 3 | 74.82 | 3.60 | 80.32 | 4.56 |
| 4 | 38.25 | – | 38.07 | – |
| 5 | 40.39 | 2.57 dd (13; 2.5) | 46.09 | 1.91 dd (13; 2.5) |
| 6 | 21.32 | β 1.43 kd (13; 3.5) | 21.38 | β 1.53 kd (13; 3.5) |
| 7 | 31.60 | | 29.32 | 1.49. 1.52 |
| 8 | 37.32 | | 36.10 | 2.01s |
| 9 | 99.19 | | 99.42 | |
| 10 | 42.75 | | 42.12 | |
| 11 | 32.59 | α 3.36 d (17)<br>β 2.94 d (17) | 31.86 | α 2.91 d (17)<br>β 2.85 d (17) |
| 12 | 118.12 | | 117.23 | |
| 13 | 150.24 | | 154.97 | |
| 14 | 97.43 | 7.16s | 98.67 | 7.30s |
| 15 | 135.75 | – | 128.18 | – |
| 16 | 115.32 | – | 117.23 | – |
| 17 | 156.72 | – | 156.89 | – |
| 18 | 15.87 | 0.78 d (6) | 17.11 | 0.94 d (6) |
| 19 | 16.19 | 0.99 s | 17.65 | 0.99 d |
| 20 | 29.15 | 1.22 s | 29.20 | 0.95 s |
| 21 | 22.74 | 0.91 s | 22.39 | 0.90 s |
| 22 | 89.31 | 2.35s | 89.26 | 4.21s |
| 23 | 168.85 | | 169.10 | |
| 24 | 60.54 | 3.98 m (2H) | 62.45 | 4.28. 4.32 m (2H) |
| 25 | 46.02 | 3.68; 3.98 m | 44.78 | 3.75; 3.81 m |
| $CH_3O$-13 | 55.56 | 3.79 s | 55.98 | 3.79 s |
| $CH_3O$-22 | 53.28 | 3.68c | 56.14 | 3.68 |
| 24Ac-$CH_3$ | | | 20.83 | 3.89 |
| 24Ac-C- | | | 170.83 | |
| 3Ac-$CH_3$ | | | 21.21 | 2.03s |
| 3Ac-C | | | 170.86 | |

*Note: The spectrum of 13,22-Dimethoxystaxybothrin was recorded in deuteropyridine and 3O,24O-Diacetate –13,22-dimethoxystaxybothrin was recorded in deuterochloroform solvents. The chemical shifts, multiplicity and spin-spin (SSTC) coupling constant were determined from the 2M*$^1H$-$^1H$,$^1H$-$^{13}C$ *ЯМR correlation spectra. Abbreviations: s-singlet, d-doublet, t-triplet, dd-doublet-doublet, td-triplet-doublet, dt-doublet-triplet, tt-triplet-triplet, kd-quartet-doublet, m-multiplet*

## References

Hodgson, M.J., Morey, P., Leung, W.Y., Morrow, L., Miller, D., Jarvis, B.B., Robbins, H., Halsey, J.F., Storey, E. Building-associatedpulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*. J.Occup. Environ. Med. 1998. – 40. – P. 241–249.

Kuhn, D.M., Ghannoum, M.A. Indoor mold, toxigenic fungi, and *Stachybotrys chartarum:* Infectious disease perspective. Clin.Microbio. Rev. 2003. – 16. – P. 144–172.

Castlebury, L.A., Rossman, A.Y., Sung, G., Hyten, A.S., Spatafora, J.W. Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. Mycol. Res. 2004. – 108. – P. 864–872.

Johanning, E., Biagini, R., Hull, D., Morey, P., Jarvis, B., Landsbergis, P. Health and immunology study following exposure totoxigenic fungi (*Stachybotrys chartarum*) in a water–damaged office environment. Int. Arch. Occup. Environ. Health 1996. – 68. – P. 207–218.

Dearborn, D.G., Smith, P.G., Dahms, B.B., Allan, T.M., Sorenson, W.G., Montana, E., Etzel, R.A. Clinical profile of 30 infants withacute pulmonary hemorrhage in Cleveland. Pediatrics 2002. – 110. – P. 627–637.

Chung, Y.J., Copeland, L.B., Doerfler, D.L., Ward, M.D.W. The relative allergenicity of *Stachybotrys chartarum* compared to housedust mite extracts in a mouse model. Inhal. Toxicol. 2010. – 22. – P. 460–468.

Kock, J., Gottschalk, C., Ulrich, S., Schwaiger, K., Gareis, M., Niessen, L. Rapid and selective detection of macrocyclic trichotheceneproducing *Stachybotrys chartarum* strains by loop–mediated isothermal amplification (LAMP). Anal. Bioanal. Chem. 2021. – 413. – P. 4801–4813.

Vesper, S.J., Magnuson, M.L., Dearborn, D.G., Yike, I., Haugland, R.A. Initial characterization of the hemolysin stachylysin from *Stachybotrys chartarum.* Infect. Immun. 2001. – 69. – P. 912–916.

Tojiyeva S.N., Kamolov L.S. *Stachybotrys chartarum* zaharli zamburug'idan staxibotridialni ajratib olish va tuzulishini o'rganish. QarDUxabarlari. 2024-yil(1)2.69–76 p.

Tojiyeva S.N., Kamolov L.S., Ro'ziyeva Z.Q., Ziyodov Sh.U. Low molecular weight metabolites of fungi. 22-methoxystachybotrin from *Stachybotrys sp*. QarDUxabarlari. Ilmiy –nazariy, uslubiy jurnal.Qarshi 2022 yil. 5/1 sonli. – P. 41–49.

Tojiyeva S.N., Kamolov L., Naxatov I. Isolation of 3O-acetatestachybotridial from the toxic mushroom *Stachybotrys chartarum* Uz-21 and study of its structure. Universum, Химия и биология выпуск: – 10 (124). 2024. – P. 30–34.

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