STEROLS OF THE FUNGUS STACHYBOTRYS ALTERNAUS

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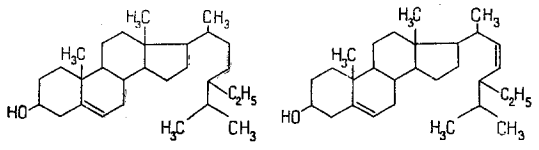
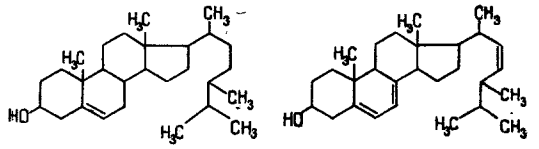
A toxic strain of the fungus Stachybotrys alternaus, family Dematiaceae, found in the Ukraine, attacks cellulose-containing substrates, especially crude fodder, causing disease and loss of animals [1].

In a study of the products of the activity of this fungus we became interested in the composition of the sterol fraction of the unsaponifiables. There is information in the literature according to which in numerous species of mycelial fungi (Aspergillus, Penicillium, Fusarium, Dendrodochium toxim, etc.) the sterol fraction is mainly ergosterol [1–3].

The sterol fraction of the fungus St. alternaus was obtained in the form of a crystalline substance with mp 133–134° C, $[\alpha]_D$ −42°. The UV spectrum of the sterol fraction showed the three maxima characteristic for ergosterol (IV): 271, 282, and 293 mμ. However, a quantitative evaluation with reference to a standard concentration of ergosterol showed that it amounted to ≈0.2% of the total mass of the sterol fraction. The IR spectrum (Fig. 1) contained two bands: ν 1020 and 1056 cm^{-1} due to the Δ^5-3β-hydroxy grouping of steroids [4]. At the same time, the band of the C—OH stretching vibrations (ν 1056 cm^{-1}) was somewhat broadened. This shows the possible presence in the fraction under study of a number of compounds possessing the Δ^5-3β-hydroxy structure. The increased intensity of the bands of the deformation vibrations of CH_2 groups (ν_{max} 1462 cm^{-1}) and CH_3 groups (ν_{max} 1387 cm^{-1}) as compared with cholesterol (the band of the O—H stretching vibration, ν 3260 cm^{-1}, was taken as a standard) shows an increase in the number of hydrocarbon groups in the molecule of the steroids in the fraction studied in comparison with the cholesterol molecule. We have previously shown the possibility of a quantitative functional-group analysis from the IR spectra of the steroids [5]. In the molecular region of the mass spectrum (Fig. 2) there are four peaks with m/e 414, 412, 400, and 396. The peak with m/e 396 corresponds to the molecular weight of ergosterol, and its percentage was determined from the UV spectra. In the NMR spectrum, the chemical shifts of the angular methyl groups at C_{18} (0.67 ppm) and at C_{19} (0.98 ppm) are the same as in cholesterol, and in the mass spectrum there is a strong peak with m/e 255 corresponding to the cholesterol molecule without the side chain and without a molecule of water. Thus, the compounds with mol wt 414, 412 and 400 are Δ^5-3β-hydroxy steroids with side chains at C_{17} which are larger than those of steroids of the cholestane series. The presence in the IR spectrum of a band with ν 974 cm^{-1} shows that the fraction studied contains a compound with the trans configuration of the protons at the double bond of the side chain [6], stigmasterol (II), with mol wt 412. The presence in the NMR spectrum of signals of protons of an ethyl group and the fragmentation of the degradation mass spectrum (see Fig. 2) characterize the sterol with a mass number of 414 as β-sitosterol (I), and the sterol with mol wt 400 as campesterol (III).



I II



III IV

Since, for the compounds mentioned, the routes of degradation under the action of electron impact are almost identical, and the melting points are similar, on the basis of the mass-spectrometric results it is possible to evaluate the quantitative ratio of these compounds in the sterol fraction of the fungus St. alternaus.

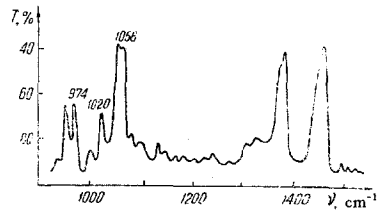


Fig. 1. IR spectrum of the sterol fraction of fungus St. alternaus.

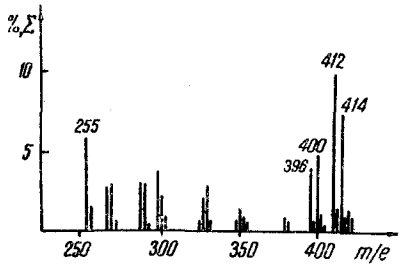


Fig. 2. Mass spectrum of the sterol fraction of the fungus St. alternaus in the region of the molecular ions.

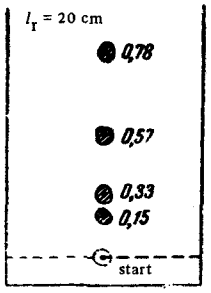


Fig. 3. Results of the thin-layer chromatography of the sterol fraction of the fungus St. alternaus.

In the sterol fraction of the unsaponifiable group of substances of the fungus under consideration 57% is β-sitosterol, 28% stigmasterol, and 15% campesterol. Usually only 0.2% ergosterol is found in the steroid fraction of mycelial fungi.

The presence in the mass spectrum of a fragment with m/e 396, i. e., a mass equal to ergosterol, may throw doubt on the relative amount of ergosterol in the fraction. But we consider the evaluation derived from the UV spectra to be more reliable. Furthermore, the fragment with m/e 396 may relate to β-sitosterol without a molecule of water. The specific rotation of the fraction ($[\alpha]_D$ −39.5°) calculated by an additive scheme agrees well with that found ($[\alpha]_D$ −42°), which shows the correctness of our quantitative evaluation of the composition of the fraction. A final confirmation of the composition of the sterol fraction was obtained by thin-layer chromatography (Fig. 3). The relative chromatographic mobilities of the four spots observed correspond to the sterols listed above and agree with literature data [7].

EXPERIMENTAL

The fungus St. alternaus was grown on previously sterilized barley straw at +23–25° C for 20 days. The mycelium with the spores was washed with water, the suspension was centrifuged, and the biomass was dried at 40–45° C (yield 8–9%). The lipids were extracted with methylene chloride. The sterol fraction was obtained by saponifying the lipids with ethanolic alkali [95% EtOH–50% KOH (5 : 1)]. The unsaponifiable fraction was washed out with petroleum ether. The solution was washed with 50% EtOH and then with water until it was neutral to phenolphthalein and was twice chromatographed on a column of alumina. The sterols were eluted with a chloroform–petroleum ether mixture (1 : 1). The fraction obtained was recrystallized three times from petroleum ether. The chromatographic separation of the sterols was carried out on plates coated with 85% silica gel (100 mesh) and 15% gypsum previously impregnated with undecane in an acetic acid–water (90 : 10) system. The UV spectra were taken on the SF-4A spectrophotometer, the IR spectra on a UR-10 instrument (solution in CCl_4 and tablets with KBr), the NMR spectra on a JNM-4H-100 (100 MHz) instrument, and the mass spectra on a MKh-1303 mass spectrometer. The measurement and interpretation of the mass spectra was carried out by V. G. Zaikin (Institute of the Chemistry of Natural Compounds, AS USSR).

CONCLUSIONS

1. The composition of the sterol fraction of the unsaponifiable group of substances of the fungus Stachybotrys alternaus has been studied. It has been shown that, unlike the sterol fraction of other mycelial fungi, the sterol fraction studied contains almost no ergosterol.

2. On the basis of UV, IR, mass, and NMR spectroscopy, optical activity, and chromatographic behavior, the substances present in the sterol fraction have been identified structurally, and the ratio of their quantities has been found.

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