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LOW-MOLECULAR-WEIGHT MUSHROOM METABOLITES. IV. SLIGHTLY POLAR COMPONENTS OF *Stachybotrys alternans*

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Slightly polar fractions of low-molecular-weight metabolites of Stachybotrys alternans *contain 10 components: 2(E)-decenal, 2(E)-undecenal, ionol, ethylpalmitate, ethylstearate, ethyloleinate, palmitic, stearic, and oleinic acids, and ergosterol.*

Key words: *Stachybotrys alternans*, fatty acids, ergosterol.

We have previously isolated four new products from the low-molecular-weight metabolites of *Stachybotrys alternans*. These are two N-containing compounds, stachybotrin and stachybotrin A, and two compounds without N, stachybotrolide and stachybotral [1-7]. Several slightly polar fractions in quantities of several milligrams accumulated during their isolation. In the present work, we identify the contents of two of these, which are denoted in the order of increasing polarity as fractions 1 and 2.

The ^{1}H and ^{13}C NMR and mass spectra showed that fraction 2 is a pure compound. Its PMR spectrum at high field contains signals of two tertiary and four secondary methyl groups. The ^{13}C NMR spectrum indicates that the compound contains two trisubstituted double bonds (141.33 and 119.65 ppm and 117.14 and 140.89 ppm) and one disubstituted double bond (132.17 and 136.15 ppm) in addition to a single functional oxygen as a secondary hydroxyl (69.91 ppm). These data enable us to identify this compound as a 24-methylsterol.

The mass spectrum of the sterol has a peak for the molecular ion at *m/z* 396. This molecular ion and fragment ions with *m/z* 363, 253, 157, 143, 81, 69, and 67 are characteristic of ergosterol [8]. The good agreement of the chemical shifts of C atoms in the ^{13}C NMR spectrum with those of ergosterol (Table 1) demonstrate that the compared compounds are identical [9].

Thus, we conclude that the sterol isolated by us is ergosterol (22E,24R-ergosta-5,7,22-trien-3β-ol).

Fraction 1 contained a mixture of slightly polar compounds. The components were identified using GLC/MS analysis. The results are given below:

Compound	Content, %
2(E)-Decenal	1.02
2(E)-Undecenal	0.29
Ionol	0.43
Ethylpalmitate	0.77
Ethylstearate	0.42
Ethyloleinate	0.81
Palmitic acid	41.72
Stearinic acid	34.91
Oleinic acid	18.75

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TABLE 1. ^{13}C and ^{1}H NMR Spectra of Ergosterol

C atom	δ_C	δ_H (J, Hz)	δ_C(lit. [9])
1	38.98		38.9
2	33.03		32.5
3	69.91	3.97 m	70.0
4	42.02	α2.84 ddd (14.5; 4.9; 2.4)	41.4
		β 2.69m	
5	141.33	-	141.0
6	119.65	5.51 m	119.7
7	117.14	5.73 (5.6; 2.4)	117.0
8	140.89	-	140.9
9	46.63		46.8
10	37.50	-	37.5
11	21.38		21.5
12	28.75		28.6
13	43.10	-	43.3
14	54.83		54.9
15	23.40		23.4
16	39.34		39.7
17	55.86		56.3
18	12.23	0.69 s	12.1
19	16.59	1.06 s	16.3
20	40.81		40.8
21	19.87	1.10 d (6.6)	19.7
22	132.17	5.27 t(7.4)	132.5
23	136.15	*	136.3
24	43.10		43.3
25	33.50		33.5
26	20.17	0.888 d (6.8)	20.0
27	21.30	0.896 d(6.8)	21.3
28	17.87	0.99 d (6.9)	17.7

*Signal overlapped by a broad water signal at 5.0 ppm. Assignment of H-22 and H-23 signals is arbitrary.

It can be seen that the mixture of slightly polar components includes nine compounds. Palmitic, stearinic, and oleinic acids and their ethyl esters represent >97% of the mixture. The fraction of the other three components is <2%.

Thus, another nine components of the low-molecular-weight metabolites of the toxic mushroom *Stachybotrys alternans* are identified.

EXPERIMENTAL

General observations have been published [3]. The following solvent systems were used: 1) hexane—diethylether (1:1), 2) C_6H_6—$CHCl_3$—ethylacetate (5:1:1).

^{1}H and ^{13}C NMR spectra were recorded on a UNITYplus 400 spectrometer in C_5D_5N (0 = TMS).

GLC/MS analysis was carried out on a Hewlett—Packard GCD (USA) chromato-mass spectrometer with a capillary column (60 m × 0.25 mm) packed with Innowax. The conditions and parameters have been published [10]. Analyses were performed in the Center for Research of Medicinal and Aromatic Plants and Medicines (TBAM) at Anadolu University (Eskisehir, Turkey) by Dr. T. Ozek, to whom the authors are grateful.

Isolation of Slightly Polar Components of *Stachybotrys alternans*. Mycelium of *Stachybotrys alternans* was separated

from the culture medium, dried, and extracted with ethanol. The extract was evaporated to dryness. The solid was chromatographed on a column and eluted successively with hexane and $CHCl_3$.

The fraction that was eluted by hexane was rechromatographed using system 1. A white crystalline substance was obtained (fraction 1). GLC/MS analysis showed that this fraction contained at least nine components.

The compound that was eluted by $CHCl_3$ was rechromatographed on a column using system 2. A homogeneous white crystalline substance was isolated (fraction 2). The contents of fraction 2 were identified as ergosterol on the basis of spectral data.

Ergosterol. $C_{28}H_{44}O$. Mass spectrum, *m/z* (%): M^+ 396 (53.1), 363 (18.8), 337 (9.4), 285 (12.5), 253 (18.8), 251 (31.3), 157 (21.9), 143 (18.8), 93 (53.1), 91 (37.5), 81 (93.8), 69 (100), 67 (40.6), 55 (93.8), 43 (43.8).

For the ^1H and ^{13}C NMR spectra, see Table 1.

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