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LOW-MOLECULAR-MASS METABOLITES OF FUNGI. III. STACHYBOTROLIDE FROM *Stachybotrys alternans*

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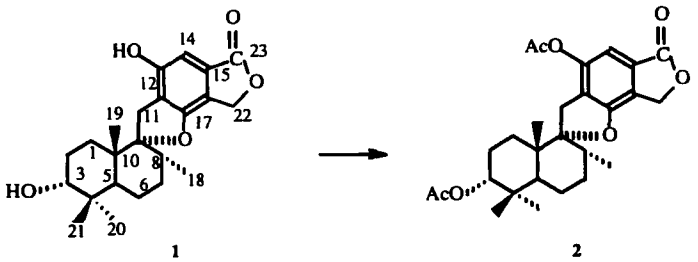
The new nitrogen-free compound stachybotrolide has been detected among the products of the vital activity of Stachybotrys alternans. *The structure of stachybotrolide has been determined on the basis of IR, mass, and ^1H and ^{13}C NMR spectra. To interpret the latter we have made use of DEPT, 2M NMR ^1H—^1H chemical shift correlation (^1H—^1H COSY or HMQC) and 2M NMR correlations of ^1H - ^{13}C long-range interactions (HMBC).*

Continuing a study of the low-molecular-mass metabolites of the lower fungus *Stachybotrys alternans* [1—4] grown under laboratory conditions, we have isolated a new compound, which has been called stachybotrolide (**1**). In the present paper we consider a determination of its structure.



1 **2**

The presence in the PMR spectrum of compound (**1**) (Table 1) of one-proton doublets of an *AB* system at 3.13 and 3.54 ppm (^2J = 17 Hz) and also of a one-proton singlet of an aromatic proton at 7.27 ppm, showing the presence of a spirobenzotetrahydrofuran system, permitted us to assign the new compound to the stachybotrin series [1, 4]. In view of this, in the high-field region of the ^1H NMR spectrum we observed the signals of four methyl groups characterizing the terpene part of the molecule. The mass-spectrometric fragmentation of stachybotrolide under electron impact, leading to the appearance of daughter ions with *m/z* 207, 189, and 135, also confirmed the conclusion that the carbon skeletons of stachybotrin and stachybotrolide are identical.

The elementary composition of stachybotrolide is $C_{23}H_{30}O_5$. In the IR spectrum of compound (**1**) we traced absorption bands at 3530 and 3220 cm^{-1}, which are characteristic for hydroxyl groups. The exhaustive acetylation of stachybotrolide with acetic anhydride in pyridine led to the diacetate (**2**). The ^1H NMR spectrum of the latter showed the resonance lines of two methyl groups of acetate functions at 2.13 and 2.41 ppm. Consequently, stachybotrolide contains one alcoholic and one phenolic hydroxyl group. The appearance of the peak of an ion with *m/z* 207 in the mass spectrum of stachybotrolide is evidence of the fact that the alcoholic hydroxyl group is present in the alicyclic part of the molecule.

In the PMR spectrum of stachybotrolide, H-5 resonates in the form of a doublet of doublets with the SSCCs $^3J_1$ = 13 and $^3J_2$ = 2.4 Hz at 2.56 ppm, which shows the α-axial orientation of the hydrogen atom concerned. Consequently, rings *A* and *B* are *trans*-linked. The good agreement of the chemical shifts of the carbon atoms and of the protons and their SSCCs in the

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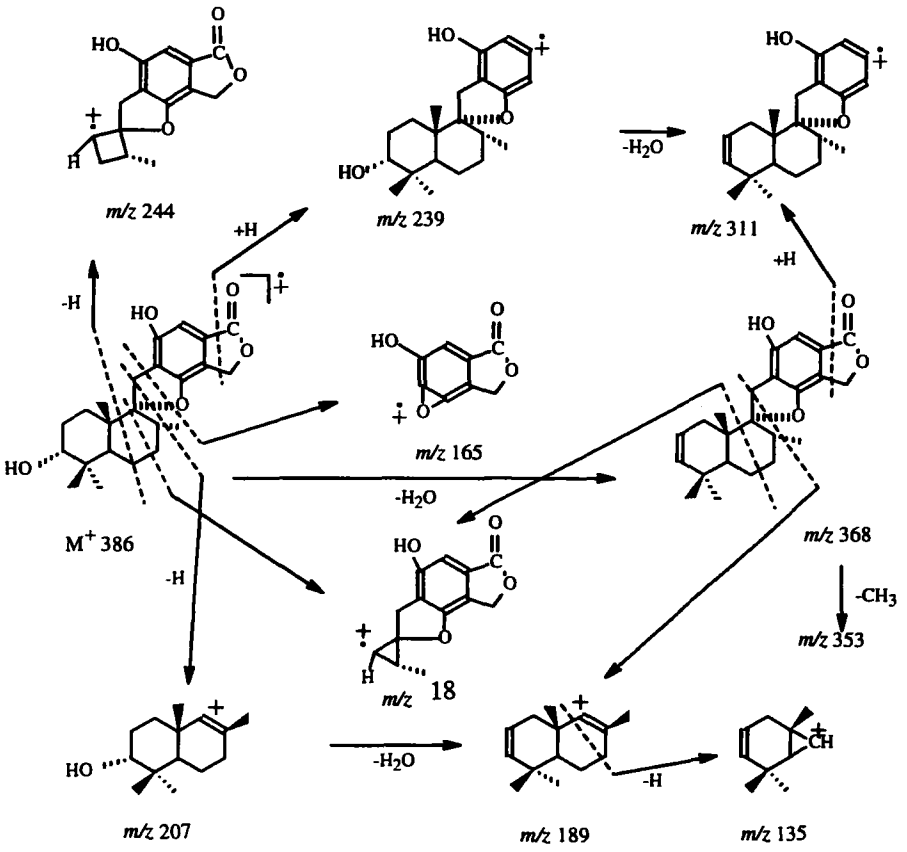
terpenoid part of the molecule of compound (1) with those of stachybotrin shows the identity of the stereochemistry and the other chiral centers and also of the conformations of rings *A* and *B* in the molecules of the compounds being compared. This means that stachybotrolide also contains a 3α-hydroxyl group. In fact, in the ^{1}H NMR spectrum of compound (1) there is at 3.61 ppm a signal of a proton geminal to a hydroxyl group at C-3 in the form of a triplet ($^3J = 2.7$ Hz) which is found in the spectrum of the diacetate (2) at 4.79 ppm in the form of a doublet of doublets ($^3J_1 = 3$; $^3J_2 = 2$Hz).



The ^{13}C NMR spectrum of the new metabolite (1) contains signals at 156.27 and 156.36 ppm characteristic of aromatic carbons bearing oxygen functions. One of them relates to a carbon atom with a phenolic hydroxyl group, and the other to a carbon atom linked to an epoxidic oxygen atom. In the spectrum of diacetate (2), these signals have undergone an upfield displacement and are observed at 148.44 and 155.97 ppm, respectively: In the HMBC spectrum of the same acetate (2), a correlation is observed between the signals of the 2H-11 atoms and the signals at 148.44 and 155.97 ppm, which shows that the latter belong to the C-13 and C-17 atoms, respectively. Consequently, the phenolic hydroxyl group is located at C-13. This conclusion is also confirmed by the upfield shift of the 2H-11 signals by 0.24 and 0.18 ppm on passing from compound (1) to its diacetate (2). This takes place in consequence of the elimination of the descreening influence of deuteropyridine because of the acetylation of the spatially close hydroxyl group through which the influence of deuteropyridine as solvent is manifested.

Under the influence of the acetyl group at C-13 in compound (2), the signal of the aromatic proton undergoes a downfield shift by 0.13 ppm as compared with that of compound (1). This shows that the functions under consideration are located vicinally, i.e., the aromatic proton is present at C-14. This conclusion is in complete agreement with the ^{13}C NMR spectra of stachybotrolide and stachybotrin, in which the C-14 chemical shifts have similar values.

In the IR spectrum of stachybotrolide we also observed an absorption band at 1774 cm^{-1} due to a γ-lactone function conjugated with an aromatic ring [5]. In agreement with this, in the ^{13}C NMR spectrum of compound (1) the signals of carbon atoms included in a lactone function are observed at 171.47 and 67.72 ppm. In the HMQC spectrum of compound (1), the latter signal correlates with two doublets of an *AB* system at 4.91 and 5.13 ppm. In the PMR spectrum of the diacetate (2) the signals of the protons under consideration are observed at 5.29 and 5.41 ppm. In the HMBC spectrum of the diacetate (2), the signals

mentioned correlate with a signal at 124.21 ppm assigned to C-16. This means that the protons under discussion of an *AB* system and the carbon atom linked with them and resonating at 67.60 ppm in the ^{13}C NMR spectrum of the diacetate (**2**) are atoms of the C-22 methylene group. Thus, we are justified in considering that the lactone function is that of a 22,23-olide. A correlation of the signals of the aromatic proton (H-14) with the signals of the carbon atom of the lactone carbonyl (C-23) in the same HMBC spectrum serves as additional confirmation of the position of the lactone function. This conclusion is also logical from biogenetic considerations in view of the fact that the carbonyl group in the stachybotrin molecule is present at C-15, and theC-22 methylene group at C-16.

TABLE 1. Details of the ^1H, ^{13}C, ^1H—^1H COSY, HMQC, and HMBC NMR Spectra of Stachybotrolide (**1**) and its Diacetate (**2**) (δ, ppm, C_5D_5N, 0 — TMS)

C atom	Compound				
	1		**2**		
	δ_C	δ_H, J(Hz)	δ_C	δ_H, J(Hz)	HMBC (C atom)
1	24.73	α 2.34 td (13.4; 3.5)	25.08	α 1.74	
		β 1.16 dt (13.4; 3.5)		β 1.07 dt (11.7;2.8)	19
2	26.09	α 1.76	22.63	α 1.64 dq (13.3)	
		β 1.99 tdd (13.4; 3.5; 2.7)		β 1.81 tdd (13; 3; 2)	1;19
3	74.76	3.61 td (2.7)	77.65	4.79 dd(3; 2)	1;5;21
4	38.25	-	36.99	-	
5	40.35	2.56 dd (13; 2.4)	41.39	2.18 dd (12.7; 2.8)	4;10;19;21
6	21.28	α 1.58	20.89	1.34	
		β 1.45 qd (13;4)		1.49	8;10
7	31.54	1.58; 1.70	31.15	1.54; 1.54	
8	37.74	1.76	37.17	1.73	
9	99.73	-	100.39	-	
10	42.79	-	42.49	-	
11	32.80	α 3.54 d (17)	32.59	α 3.30 d (17.5)	8; 10; 12; 13
		β 3.13 d (17)		β 2.95 d (17.5)	8;10;12; 13; 17
12	118.44	-	126.95	-	
13	156.27	-	148.44	-	
14	102.99	7.27 s	110.60	7.40 s	12; 13; 16; 23
15	127.99	-	128.42	-	
16	120.93	-	124.21	-	
17	156.36	-	155.97	-	
18	15.86	0.86 d (6)	15.68	0.81 d (6.6)	
19	16.20	1.00 s	15.89	0.87 s	5; 9
20	29.14	1.20 s	27.97	0.90 s	3; 4; 21
21	22.72	0.91 s	21.88	0.82 s	3; 4; 20
22	67.72	4.91; 5.13 d (14.6)	67.60	5.29; 5.41 (15)	16
23	171.47	-	170.21*	-	
CH_3			21.16	2.13 s	COO-3
COO-3			170.21*		
CH_3			20.71	2.41 s	COO-13
COO-13			168.61		

*The signals marked with an asterisk are superposed on one another. The proton chemical shifts given without SSCCs and multiplicities were determined from ^1H—^1H COSY and HMQC spectra.

Thus, the experimental results presented permit the conclusion that stachybotrolide has the structure illustrated by formula (**1**).

EXPERIMENTAL

For **General Observations**, see [1]. The solvent systems used were : 1) chloroform—methanol (70:1), and 2) benzene—methanol (60:1).

Isolation of Stachybotrolide. Mycelium of *Stachybotrys alternans*, separated from the culture liquid and dried, was extracted with diethyl ether. The residue after evaporation of the solvent was chromatographed on a column, with elution by system 1. A fraction was isolated the rechromatography of which gave stachybotrolide.

Stachybotrolide (1). $C_{23}H_{30}O_5$. IR spectrum (KBr, ν, cm^{-1}): 3530, 3220, 1774, 1749, 1664, 1624.

Mass spectrum, *m/z* (%): M^+ 386 (36.6), 368 (8), 329 (5), 315 (4), 311 (3), 244 (10), 230 (100), 207 (45), 203 (30), 189 (46.6), 165 (50), 135 (30).

For the NMR spectra, see Table 1.

Stachybotrolide 3,13-Diacetate (2) from (1). Stachybotrolide (14 mg) was acetylated with 0.25 ml of acetic anhydride in 0.5 ml of absolute pyridine at room temperature for 5 days. After evaporation of the solvent, the reaction products were chromatographed on a column in system 2. This gave 9.5 mg of the amorphous diacetate (**2**), $C_{27}H_{34}O_7$. Mass spectrum, *m/z*(%): M^+470 (1.8), 410 (6.4), 3.68 (2.7), 3.25 (2.7), 284 (6.4), 256 (18.5), 230 (12), 189 (22.2), 109 (25.9), 103 (72.2), 73 (100).

For the NMR spectra, see Table 1.

REFERENCES

1. L. S. Kamalov, S. F. Aripova, and M. I. Isaev, Khim. Prir. Soedin., 599 (1997).
2. L. S. Kamalov, S. F. Aripova, and M. I. Isaev, Khim. Prir. Soedin., Spets. Vypusk [Special Issue], 4 (1997).
3. L. S. Kamalov, S. F. Aripova, and M. I. Isaev, Khim. Prir. Soedin., Spets. Vypusk [Special Issue], 7 (1998).
4. L. S. Kamalov, S. F. Aripova, B. Tashkhodzhaev, and M. I. Isaev, Khim. Prir. Soedin., 666 (1998) [this issue].
5. K. Nakanishi, Infrared Absorption Spectroscopy. Practical, Holden-Day, San Francisco (1962) [Russian tranlation, Mir, Moscow (1965), p. 56].