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LOW-MOLECULAR-MASS METABOLITES OF FUNGI
iV. THE STRUCTURES OF STACHYBOTRIN A AND STACHYBOTRAL

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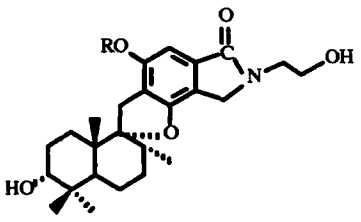
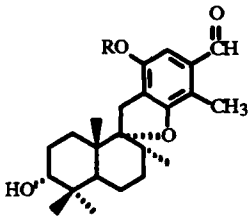
The structures of another two metabolites of the microscopic fungus Stachybotris alternans *— stachybotrin A and stachybotral — have been established on the basis of their spectral characteristics.*

Continuing an investigation of low-molecular-mass metabolites of the fungus *Stachybotris alternans* [1—6], we have determined the structures of two new compounds, which we have called stachybotrin A (1) and stachybotral (3).

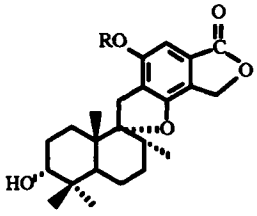
By rechromatographing the fraction preceding the isolation of stachybotrin (2) [2] on a column of silica gel we isolated a new metabolite of the fungus under study — stachybotrin A. A consideration of its ^1H and ^{13}C NMR spectra (Table 1) enabled us to assign the new compound (1) to the stachybotrin series.



1. R=CH_3
2. R=H



3. R=H
4. R=Ac



5. R=H
6. R=Ac

In the ^1H NMR spectrum of stachybotrin A, we observed a one-proton singlet of an aromatic proton at 7.16 ppm. In the same spectrum we traced the resonance lines of two protons of an *AB* system at 2.94 and 3.36 ppm with the SSCC ^2J = 17 Hz, assigned to the protons of the isolated C-11 methylene group. These facts indicated that the stachybotrin A molecule included a spirobenzofuran fragment trisubstituted in the aromatic ring.

The signals of three tertiary methyl groups and one secondary one in the high-field region of the PMR spectrum characterized the terpenoid part of the molecule of the substance under investigation (1). Also characteristic for this part of the molecule was a doublet of doublets with $^3J_1$ = 13 Hz, $^3J_2$ = 2.5 Hz, observed at 2.57 ppm, showing the *trans*-linkage of rings *A* and *B* [3].

A one-proton multiplet at 3.60 ppm was assigned to a proton geminal to a secondary hydroxy group, and it determined the position at C-3 and the β-equatorial orientation of the proton concerned [3, 5, 6]. The ^{13}C NMR spectrum of the new metabolite (1), containing a signal at 74.82 ppm, was in full agreement with this conclusion. Consequently, stachybotrin A also included a 3α-hydroxy group.

The facts given above and the chemical shifts of the carbon atoms of the terpenoid part of the molecule in the ^{13}C NMR spectrum, which were in very good agreement and practical coincidence with those of stachybotrin [2, 5] and of stachybotrolide (5) [6], showed the identity of the acyclic fragments of the compounds compared.

One-proton doublets of an *AB* system at 4.11 and 4.37 ppm with the SSCC ^2J = 17 Hz (2H-22), and also multiplets at

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3.68 and 3.98 ppm of one and three proton units, respectively, (2H-24 and 2H-25) permitted the conclusion that the side-chains, which including the C-22—C-25 atoms, were also identical. An analogous conclusion also followed from a comparison of the C-22—C-25 chemical shifts in the ^{13}C NMR spectra of stachybotrin and stachybotrin A.

A comparative analysis of the PMR spectra of compounds (1) and (2) showed that the spectrum of the former contained an additional three-proton singlet at 3.79 ppm. This signal belonged to a methoxy group. In agreement with this, in the ^{13}C NMR spectrum of compound (1) we traced a signal at 55.56 ppm. The magnitude of the chemical shift of the signal of the methoxy group in the PMR spectrum showed that the group under consideration was present in the aromatic ring.

It is interesting to note that the resonance lines of the 2H-11 protons in the ^{1}H NMR spectrum of stachybotrin A have undergone an upfield shift in comparison with those of stachybotrin and stachybotrolide similar to that which is observed in spectra of acetates of the latter obtained in deuteropyridine [5, 6]. This fact determines the position of the methoxy group in the molecule of compound (1) at C-13.

Thus, stachybotrin A is the 13-methyl ether of stachybotrin and has the composition $C_{26}H_{37}NO_5$ and the structure illustrated by formula (1).

During the isolation of stachybotrolide (5) [6], fractions accumulated that contained a chromatographically homogenous crystalline mixture of two substances. A consideration of the PMR spectrum of the mixture showed that it contained two sets of signals. One of them was assigned without difficulty to stachybotrolide. The other set belonged to a new component, which we have called stachybotral (3). Judging from the integral intensity of the signals, the mixture contained stachybotrin and stachybotral in a ratio of 1.4:1. Recrystallization from methanol led to an increase in the proportion of the second component of this mixture.

The ^{13}C and ^{1}H NMR spectra of stachybotral obtained from spectra of the mixture after the elimination of the indices of stachybotrin are given in Table 1. Attempts to separate the mixture through acetylation were also unsuccessful. Details of the PMR spectrum of the monoacetates (4) and (6), taken in deuterochloroform, are also included in Table 1.

As was to be expected, the mass spectrum of the mixture under study contained two groups of peaks: 1) M^+ 386, 368, 353, 329, 315, 311, 244, 230, 207, 203, 189, 165, 135; and 2) M^+ 372, 354, 339, 325, 216, 207, 189, 135, The first set belonged to stachybotrolide [6], and the second to stachybotral. The molecular ion M^+ corresponded to the elementary formula $C_{23}H_{32}O_4$.

In the PMR spectrum of compound (3) the signals of four methyl groups were observed in the interval of 0.85—1.24 ppm. In the same spectrum we noted one-proton doublets of an *AB* system at 3.00 and 3.40 ppm with $^{2}J = 16$ Hz (2H-11), and a singlet signal of an aromatic proton at 6.52 ppm. These observations showed that the new compound (3) also belonged to the group of compounds containing a combination of a spirobenzofuran system with a sesquiterpenoid drimane nucleus, analogous to stachybotrin A, stachybotrin, and stachybotrolide.

A one-proton doublet of doublets at 2.60 ppm with the SSCC $^{3}J_1 = 13$ and $^{3}J_2 = 2.7$ Hz, assigned to H-5, showed the *trans*-linkage of rings *A* and *B*.

Consequently the multiplet at 3.60 ppm was the signal of a proton geminal to a secondary hydroxy group, and it showed the β-orientation of this proton, located at C-3. This means that the corresponding hydroxy group had the α-orientation. The chemical shift of the secondary alcoholic carbon atom of 74.72 ppm in the ^{13}C NMR spectrum of compound (3), and also a triplet at 3.38 ppm with $^{3}J = 2.7$ Hz in the ^{1}H NMR spectrum of the monoacetate (4) ($CDCl_3$), confirmed the conclusion of the location and configuration of the hydroxy group under consideration.

A comparison of the chemical shifts of the carbon atoms of the terpene part of the stachybotral molecule with that of stachybotrin A, stachybotrin, and stachybotrolide revealed complete coincidence of the alicyclic parts of the compounds compared.

The alicyclic fragments of compounds (1—3), (5) were responsible for the daughter ions with *m/z* 207, 189, and 135 in the mass spectra [3, 5, 6]. It must therefore be assumed that in the mass spectrum of the mixture of substances (3) and (5) the peaks of these ions had a dual origin.

Acetylation of the mixture of substances (3) and (5) with acetic anhydride in pyridine led to a mixture of the monoacetates (4) and (6). The three-proton singlets at 2.30 and 3.33 ppm observed in the PMR spectrum of the monoacetates unambiguously determined the position of the acetate group in the aromatic ring at C-13 in each case. Consequently, stachybotral also contained a phenolic hydroxy group in the same position. This conclusion was confirmed by the ^{13}C NMR spectrum of stachybotral, which included the signal of an aromatic carbon atom at 159.92 ppm.

TABLE 1. Details of the ^{13}C and ^{1}H NMR Spectra of Compounds (**1—4, and 6**) (δ, ppm, 0 — TMS)

C atom	Compound						
	1		**2**	**3**		**4**	**6**
	δ_C	δ_H(J, Hz)	δ_C	δ_C	δ_H(J, Hz)	δ_H(J, Hz)	δ_H (J, Hz)
1	24.71	α 2.27 td (13; 3.5) β 1.10 dt (13; 3.4)	24.72	24.80			
2	26.07	β 1.98 tt (13; 3.4)	26.05	26.09*			
3	74.82	3.60 m	74.83	74.72	3.60 m	3.38 t (2.7)	3.40 t (2.7)
4	38.24	-	38.21	38.25*	-	-	-
5	40.39	2.57 dd (13; 2.5)	40.85	40.50	2.60 dd (13; 2.7)		
6	21.32	β 1.43 kd (13; 3.5)	21.33	21.38			
7	31.60		31.60	31.60			
8	37.32		37.34	37.34*			
9	99.19	-	98.72	99.47	-	-	-
10	42.75	-	42.75	42.79*	-	-	-
11	32.59	α 3.36 d (17) β 2.94 d (17)	32.86	32.80*	α 3.40 d (16) β 3.00 d (16)	α 3.07 d (17) β 2.69 d (17)	α 3.19 d (17) β 2.79 d (17)
12	118.12	-	117.60	111.35	-	-	-
13	150.24	-	155.39	159.92	-	-	-
14	97.43	7.16 s	101.80	111.86	6.52 s	6.43 s	7.11 s
15	135.75	-	135.66	129.20	-	-	-
16	115.32	-	113.22	111.71	-	-	-
17	156.72	-	156.88	141.86	-	-	-
18	15.87	0.78 d (6)	15.91	15.91	0.85 d (6.4)	0.76 d (6.8)	0.73 d (6.4)
19	16.19	0.99 s	16.21	16.27	0.97 s	0.989 s	0.993 s
20	29.15	1.22 s	29.11	29.20	1.24 s	0.995 s	1.01 s
21	22.74	0.91 s	22.72	22.72*	0.90 s	0.87 s	0.88 s
22	48.48	4.11; 4.37 d (17)	48.54	21.85	2.68 s	2.55 s	5.25; 5.32 d (14.5)
23	168.85	-	169.09	168.92	10.87 s	**	-
24	60.54	3.98 m (2H)	60.50	-	-	-	-
25	46.02	3.68 m; 3.98 m	45.95	-	-	-	-
CH_3O	55.56	3.79 s	-	-	-	2.30 s (CH_3COO)	2.33 s (CH_3COO)

The spectra of compound (**1—3**) were taken in deuteropyridine, and those of (**4**) and (**6**) in deuterochloroform. Signals marked with asterisks are superposed on the corresponding signals of stachybotrolide.

** The low-field part of the spectrum was not recorded.

The fourth oxygen atom is a component of an aldehydic carbonyl group the carbon atom of which resonated in the ^{13}C NMR spectrum of compound (**3**) at 168.92 ppm. In agreement with this, in the PMR spectrum of the compound concerned, (**3**), the signals of an aldehydic proton was observed at 10.87 ppm. From considerations of biogenesis, it may be assumed that the aldehydic function included the C-23 atom, since the carbonyl functions of stachybotrin A, stachybotrin, and stachybotrolide are functions of just this carbon atom.

Thus, the remaining unidentified atom, C-22, was represented by a methyl group. This was shown by a three-proton singlet in the ^{1}H NMR spectrum of stachybotral at 2.68 ppm and a signal in the ^{13}C NMR spectrum of the same compound at 21.85 ppm from the carbon atom of the methyl group under examination.

Hence, we are justified in concluding that stachybotral has the structure corresponding to formula (**3**). Some deviation of the chemical shifts of carbon atoms of the benzene nucleus in the ^{13}C NMR spectrum of stachybotral from those of stachybotrin A, stachybotrin, and stachybotrolide is due to the absence of a heterocyclic ring *E* in the molecule of stachybotral and, as a consequence of this, a different chemical nature of the C-22,C-23 function.

EXPERIMENTAL

For general observations, see [3]. We used the following solvent systems: 1) benzene—methanol (10:1); and 2) chloroform—methanol (70:1).

NMR spectra were obtained on a UNITY Plus 400 instrument in deuteropyridine and deuterochloroform (δ, ppm; 0 — TMS).

Stachybotrin A (1). The fractions collected immediately before the elution of stachybotrin (**2**) [3] were rechromatographed on a column, with washing by system (1). This led to the isolation of 4 mg of stachybotrin A (**1**) $C_{26}H_{37}NO_5$. For its ^{13}C and ^1H NMR spectra, see Table 1.

Stachybotral (3) and Stachybotrolide (5). During the isolation of stachybotrolide, chromatographically homogeneous crystalline fractions accumulated that consisted, according to their PMR spectra, of stachybotrolide and stachybotral in a ratio of 1.4:1.

For the mass spectrum of stachybotrolide, see [6].

Mass spectrum of stachybotral, *m/z*: M^+ 372 ($C_{23}H_{32}O_4$); 354, 339, 325, 216, 207, 189, 135.

For the ^{13}C NMR and ^1H NMR spectra of stachybotral (3), see Table 1.

The 13-Monoacetate of Stachybotral (4) and the 13-Monoacetate of Stachybotrolide (6). To 10 mg of the mixture of substances (**3**) and (**5**) in 1 ml of abs. pyridine was added 0.5 ml of acetic anhydride, and the reaction mixture was left at room temperature for 1 h. The residue after evaporation of the solvent was chromatographed on a column, with elution by system 2, which led to the isolation of 9.5 mg of a mixture of the monoacetates (**4**) and (**6**). For the ^1H NMR spectrum, see Table 1.

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