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LOW-MOLECULAR-WEIGHT METABOLITES OF FUNGI. II. REFINEMENT OF THE STRUCTURE OF STACHYBOTRIN

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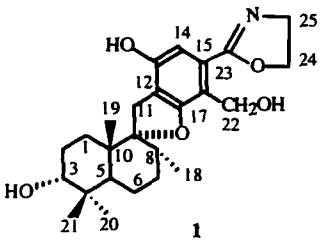
The structure of stachybotrin has been reconsidered in the light of ^1H, ^{13}C, ^1H—^1H COSY, HMQC, and HMBC NMR spectra, and the revised structure has been confirmed by x-ray structural analysis.

We have previously investigated the structure of stachybotrin, one of the metabolites of the fungus *Stachybotrys alternans*, ascribing to it the structure (**1**) [1]. Continuing our investigation, we have now made a refinement to the structure shown, the essence of which we expound in the present paper.

The ^1H and ^{13}C NMR spectra of stachybotrin and its diacetate (**3**), taken under identical conditions (C_5D_5N) showed that the chemical shifts of the 2H-24 protons and of the C-24 and C-25 carbon atoms underwent considerable changes on passing from stachybotrin to its diacetate (**3**) (Table 1). At the same time, no appreciable changes were observed in the chemical shifts of C-22 and 2H-22. Consequently, the C-22 atom is not the bearer of a primary hydroxy group. The downfield shifts of the C-24 and 2H-24 signals in the ^{13}C and ^1H NMR spectra of the diacetate (**3**) as compared with those of stachybotrin convincingly show that the primary hydroxy group in the stachybotrin molecule and the corresponding acetate group in the molecule of diacetate (**3**) are present at C-24. This means that the isolated C-24 and C-25 methylene groups, the protons of which compose an A_2BX system do not participate in the formation of the additional ring the necessity of which follows from the elementary composition of stachybotrin $C_{25}H_{35}NO_5$ [1].

As already shown [1], the function including the C-23 atom (169.09 ppm) may be a N,N-disubstituted carbamide group (>N—C=O) or azomethineoxy group (-N=C-O-) isomeric with it. The chemical shifts of C-22 (48.54 ppm) and C-25 (45.95 ppm) and also those of the hydrogen atoms attached to them, 2H-22 (4.09 and 4.35 ppm) and 2H-25 (3.65 and 3.90 ppm), in the ^{13}C and ^1H NMR spectra of stachybotrin show that the carbon atoms under consideration are linked to a hetero atoms — namely, a nitrogen atom. Consequently, the C-23 function is a N,N-disubstituted carbamide group, which, with the C-22 atom closes the heterocycle *E*. The correlation of the 2H-25 signals and the C-22 and C-23 signals in the HMBC spectrum of the diacetate (**3**) serves as additional confirmation of this conclusion.



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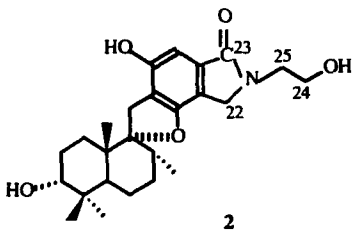

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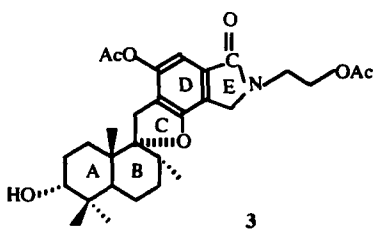

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TABLE 1. ^{1}H, ^{13}C, ^{1}H-^{1}H, COSY, HMQC, and HBMC NMR Spectra of Stahybotrin (2) and its Diacetate (3)* (δ, ppm, C_5D_5N, 0-TMS)

C atom	Compound				
	2		3		
	δ_C	δ_H(J, Hz)	δ_C	δ_H(J ,Hz)	HMBC (C atoms)
1	24.72	α 2.35 td (13; 3)	24.69	α 2.29 td (14; 4)	
		β 1.13 dt (13; 3)		β 1.15 dt (14; 4)	
2	26.05	α 1.70	26.06	α 1.75	
		β 1.95 tt (13; 3)		β 1.95	
3	74.83	3.54	74.74	3.60	1; 5
4	38.21	-	38.23	-	
5	40.85	2.56 dd (13; 2.4)	40.44	2.56 dd (12.8; 2.6)	4; 10; 19; 21
6	21.33	α 1.55	21.27	α 1.63	
		β 1.42 qd (13; 3.5)		β 1.43 qd (13; 4)	
7	31.60	α 1.70	31.56	α 1.70	
		β 1.55		β 1.60	
8	37.34	1.75	37.29	1.75	
9	98.72	-	99.86	-	
10	42.75	-	42.78	-	
11	32.86	α 3.50 d (16.7)	32.69	α 3.37 d (17.1)	8; 9; 10; 12; 13; 17; 8;
		β 3.09 d (16.7)		β 2.95 d (17.1)	9; 10; 12; 13; 17
12	117.60	-	124.25	-	
13	155.39	-	147.75	-	
14	101.80	7.31 s	108.55	7.39 s	12; 13; 16; 23
15	135.66	-	135.75	-	
16	113.22	-	119.05	-	
17	156.88	-	156.74	-	
18	15.91	0.80 d (5.8)	15.85	0.83 d (6.1)	7; 8; 9
19	16.21	0.97 s	16.17	0.96 s	1; 5; 9; 10
20	29.11	1.19 s	29.13	1.23 s	3; 4; 5; 21
21	22.72	0.88 s	22.71	0.91 s	3; 4; 5; 20
22	48.54	4.09; 4.35 d (16.7)	48.08	3.99; 4.29 d (17)	15; 16; 17; 23
23	169.09	-	167.85	-	
24	60.50	3.90 m (2H)	62.48	4.28 m (2H)	COO-24
25	45.95	3.65; 3.90 m	41.85	3.63; 3.88 m	22; 23; 24
CH_3	-	-	20.71	2.37 s	COO-13
COO-13			168.62		
CH_3	-	-	20.63	1.90 s	COO-24
COO-24			170.55		

*The chemical shifts given without multiplicities and SSCCs were determined from ^{1}H—^{1}H COSY and HMQC spectra.

Thus, stachybotrin has the structure illustrated by formula (2), and its mass spectrometric fragmentation can be represented in the form given by the scheme. The structure of stachybotrin has been confirmed by x-ray structural analysis. The spatial structure of the molecule is shown in Fig. 1. In the terpenoid part of the molecule, rings *A* and *B* are *trans*-linked and have chair conformation. The hydroxy group at C-3 and the methyl group at C-8 are α-oriented, while the methyl group at C-10 has the β-orientation. The aromatic ring with condensed heterocycles has a planar structure and is practically perpendicular to the plane of the terpenoid part. An inadequate set of experimental results prevents an analysis of the other geometric parameters of the molecule.

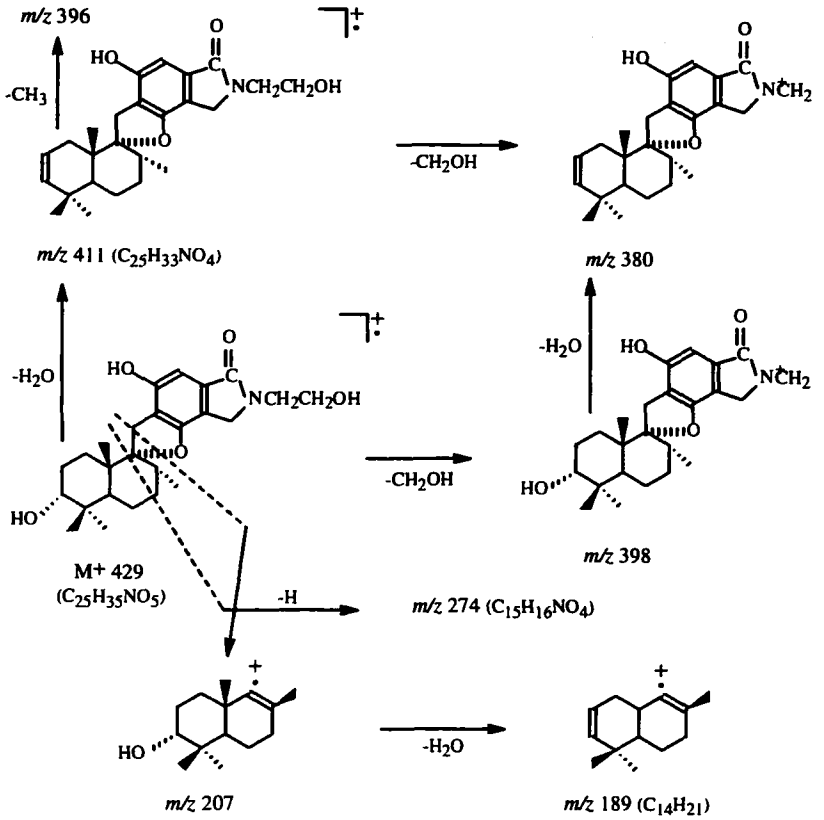

TABLE 2. Coordinates ($\times 10^3$) of the Atoms of the Stachybotrin Molecule

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
N	193(3)	491(2)	636(1)	C12	-122(3)	621(6)	367(2)
O1	257(2)	230(3)	201(1)	C13	-244(4)	655(7)	434(2)
O2	143(2)	540(2)	316(1)	C14	-189(4)	634(8)	533(2)
O3	-393(2)	705(3)	412(1)	C15	-44(4)	583(2)	533(1)
O4	-34(2)	563(1)	727(1)	C16	79(3)	548(7)	485(2)
O5	435(2)	637(5)	726(1)	C17	32(4)	572(2)	392(2)
C1	-29(3)	394(5)	192(2)	C18	181(4)	784(5)	281(2)
C2	-18(4)	298(9)	123(2)	C19	-30(3)	535(4)	62(2)
C3	178(6)	263(7)	112(2)	C20	501(3)	324(9)	83(2)
C4	295(4)	355(6)	64(2)	C21	258(4)	373(8)	-37(2)
C5	276(4)	463(9)	126(2)	C22	240(3)	498(9)	529(1)
C6	376(3)	567(0)	96(2)	C23	32(6)	556(2)	640(2)
C7	368(4)	657(3)	169(2)	C24	308(4)	453(0)	709(2)
C8	177(3)	697(6)	201(2)	C25	464(5)	518(5)	726(3)
C9	73(3)	592(2)	219(2)	O	602(3)	254(7)	441(2)
C10	76(7)	489(4)	149(2)	C	737(6)	342(8)	435(3)
C11	-127(5)	619(6)	259(2)				

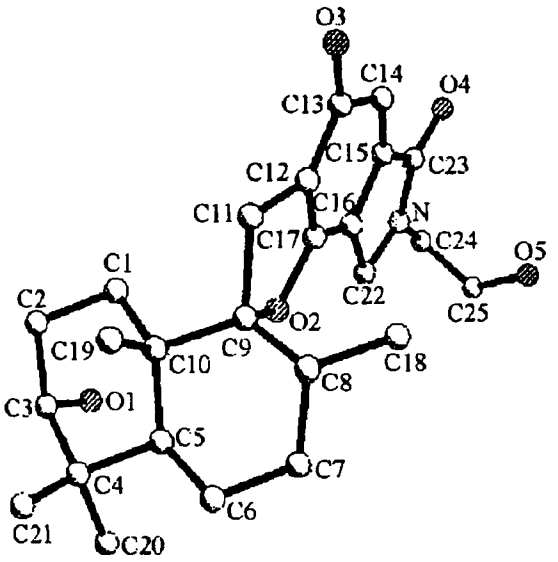

Fig. 1. Spatial structure of stachybotrin (**2**).

EXPERIMENTAL

NMR spectra were taken on Bruker AM-400 and UNITY Plus 400 instruments. The unit-cell parameters and the space group were determined and refined on a Syntex-P2 $_1$ diffractometer (CuK $_\alpha$-radiation): $a = 7.454$, $b = 12.011$, $c = 13.983$ Å, $\beta = 90.42°$, $d_{calc} = 1.140$ g/cm^3, space group P2$_1$, $Z = 4$. Because the microcrystals of stachybotrin had microscopic dimensions (of the order of 0.1 × 0.1 × 0.5 mm) it was impossinble to obtain a high-quality diffraction pattern. The number of independent and nonzero reflections with $I > 2\sigma(I)$ was 736. The structure was determined by the direct method using the SHELXS-86 program [2] (PC DOS version) with an increase in the number of variants to 500. Subsequent Fourier syntheses enabled all the nonhydrogen atoms to be localized. For the final ordering of the atoms we employed the spectral characteristics of stachybotrin. The structure was refined by the method of least squares (MLS) successively in the isotropic—anisotropic approximation by the SHELX-76 program [3]. The final value of the discrepancy factor, R, was 0.102 ($R_w = 0.098$). The coordinates of the nonhydrogen atoms from the last stage of MLS are gvien in Table 2. A molecule of methanol of crystallization is included in the independent part of the unit cell.

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3. G. M. Sheldrick, SHELX-76 Program for Crystal Structure Determination, Cambridge, England (1976).