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THE STRUCTURE OF TUBINGENSIN B: A CYTOTOXIC CARBAZOLE ALKALOID FROM THE SCLEROTIA OF ASPERGILLUS TUBINGENSIS

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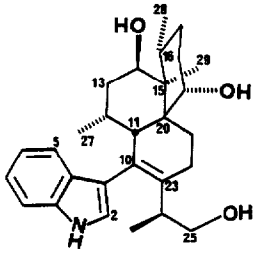
Abstract: Tubingensin B (4), a cytotoxic carbazole alkaloid with a novel ring system, was isolated from the sclerotia of the fungus Aspergillus tubingensis. The structure was assigned primarily on the basis of selective INEPT, homonuclear decoupling, and COSY experiments.

Sclerotia are specially-adapted morphological structures that are critical to the long-term survival and propagation of many fungal species.[1-5] We have reported the isolation of several antiinsectan indole diterpenes, including aflavinine derivatives (e.g., 1) and nominine (2), from the sclerotia of Aspergillus spp.[2-5] The natural concentrations of many of these compounds deter feeding by fungivorous insects which encounter sclerotia in nature. We have recently reported the isolation of an antiviral carbazole-containing natural product from Aspergillus tubingensis which we named tubingensin A (3).[6] Continued studies of the sclerotia of this organism have led us to the isolation of a new biologically active metabolite, tubingensin B. This compound contains a unique hexacyclic ring structure not previously reported as a component of a natural product. Details of the isolation, structure elucidation, and biological activity of tubingensin B are the subjects of this report.



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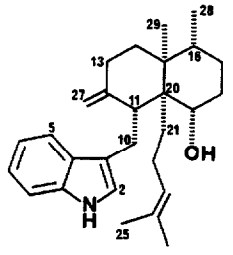

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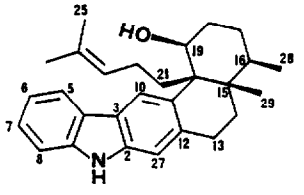

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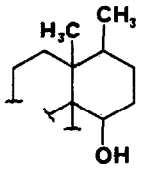
Sclerotia of A. tubingensis (NRRL 4700) were produced by solid substrate fermentation on corn kernels.[2] The hexane extract of the sclerotia exhibited antiinsectan activity, and was fractionated by HPLC (C_{18}) to afford several components, including three new aflavinine derivatives[5] and tubingensin A[6] (3). Further studies of this extract afforded an isomer of 3 which we named tubingensin B.[7] The molecular formula of tubingensin B was established as $C_{28}H_{35}NO$ on the basis of HREIMS and ^{13}C NMR data. Tubingensin B exhibited many spectral similarities with tubingensin A. Most notable were the relatively low intensity of the

quinolinium ion at m/z 130 in the mass spectrum, and the UV spectrum, which was characteristic of a carbazole moiety.[8] The ^{13}C NMR spectrum of tubingensin B indicated that this compound contained twelve vinylic/aromatic carbons. Because of the absence of evidence for alkyne or carbonyl functionalities, it was clear that tubingensin B must contain one more ring and one less double bond than tubingensin A.

Proton and carbon NMR data for tubingensin B are provided in Table I. Homonuclear decoupling and ^{1}H-^{1}H COSY experiments permitted establishment of the proton spin systems. Carbon-13 NMR assignments were determined by selective INEPT experiments, which afforded 2- and 3-bond C/H correlations[9], and by a heteronuclear shift correlation experiment (Table I). These data provided evidence for a 2,3-disubstituted carbazole substructure and for the partial structure a, a subunit common to 2 and 3. Comparison with the relevant data for tubingensin A and several aflavinine derivatives supported these assignments. In addition, the selective INEPT and COSY data clearly indicated the presence of a second isolated ethylene moiety and an isopropyl group attached to a third quaternary carbon.



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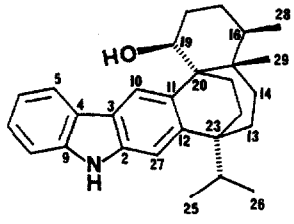

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The connectivity of these four units was assigned as shown in structure 4 on the basis of selective INEPT experiments (Table I). The isopropyl methine (H-24) and a methylene proton at C-13 both correlated with C-12 and 23. The C-H proton of the hydroxylated methine (H-19) correlated with C-11, and the aromatic proton singlets (H-10 and H-27) correlated with C-20 and C-23 respectively. These data and other correlations presented in Table I led us to propose the novel structure 4 for tubingensin B. The numbering system shown for 4 was chosen to facilitate spectral and biogenetic comparisons with 1-3. The relative stereochemistry shown for 4 was assigned initially by analogy to 1. This assignment was supported by ^{13}C NMR similarities with compounds 1-3 and by the results of a NOESY experiment (Table I). A NOESY interaction between H-14ax and H-16 indicates a cis relationship between C-14 and H-16, while a correlation between H_3-28 and H_3-29 supports their cis orientation. The NOESY correlations shown by *both* H-19 and H-18ax with H-10 are only possible if the molecule possesses the relative stereochemistry shown for positions 15 and 20. Finally, the relative configuration at C-23 must place the isopropyl group cis to C-19 due to geometrical requirements of the bridged ring system. Support for this conclusion was provided by a NOESY correlation between H-24 and H-27. The unusual upfield shift of the axial proton at C-14 (0.17 ppm) is also accounted for by this stereostructure. Dreiding molecular models show that this proton is oriented over the edge of the aromatic ring system when the conformation of the seven-membered ring places C-14 in an endo position.

Table I. Proton and Carbon-13 NMR Data for Tubingensin B (4)[a]

H/C#	^1H	^{13}C	Selective INEPT Correlations[b]	NOESY Correlations
1	7.86 (br s)	--		
2	--	137.95		
3	--	119.80		
4	--	123.65		
5	7.98 (br d; 7.8)	119.70	3, 7, 8[c], 9	
6	7.15 (ddd; 1.5, 7.3, 7.8)	119.19	4, 8	
7	7.32 (ddd; 1.1, 6.6, 7.3)	125.08		
8	7.30 (dd; 1.5, 6.6)	110.48		
9	--	139.62		
10	8.05 (br s)	116.83	2, 3, 4, 12, 20	14ax, 18ax, 19
11	--	134.80		
12	--	142.03		
13eq	1.40 (m)	38.36		
13ax	1.82 (ddd; 4.4, 12.7, 14.1)		12, 23	
14eq	1.24 (m)	38.08		
14ax	0.17 (ddd; 5.0, 13.4, 14.1)		13, 15, 16, 29	10, 16
15	--	40.67		
16	1.22 (m)	38.79		14
17	1.31 (m), 1.72 (m)	25.65		
18eq	2.11 (ddd; 3.2, 3.4, 10.7)	33.67	16, 20	
18ax	2.68 (m)			10
19	4.36 (br t)	76.65	11, 15, 17, 20	10
20	--	47.63		
21	1.76 (m)	27.70		
	2.59 (br dd; 3.6, 10.3)		11, 15, 20, 22	
22	1.44 (m), 1.66 (m)	25.63		
23	--	42.47		
24	2.41 (qq; 6.6, 6.8)	35.05	12, 23, 25, 26	27
25	1.00 (d; 6.6)	19.45	23, 24, 26	
26	1.09 (d; 6.8)	17.06		
27	7.32 (br s)	107.39	2, 3, 10[c], 11, 23	24
28	0.70 (d; 6.8)	16.89	15, 16, 17	29
29	1.15 (s)	14.08	14, 15, 16, 20	28

[a]Data recorded in $CDCl_3$ at 360 and 90.7 MHz, respectively. Carbon multiplicities were determined through a DEPT experiment, and are consistent with the assignments. [b]Each proton signal analyzed with the selective INEPT technique was individually subjected to three separate experiments, optimizing for 4, 7, or 10 Hz. Signals separated by less than 30 Hz were irradiated off-center in the appropriate direction to avoid ambiguous results. [c]Denotes a four-bond coupling. All other selective INEPT data represent 2- or 3-bond correlations.

Tubingensin B seems likely to arise from a biosynthetic intermediate common to 1-3, although its formation would have to involve a somewhat more complex and unusual rearrangement of the diterpenoid fragment. Tubingensin B exhibits mild activity against the crop pest Heliothis zea, causing 10% mortality when incorporated into a standard diet at 125 ppm. This compound also exhibits activity nearly identical to that of 3 in an assay against herpes simplex virus type 1 with an IC_{50} of 9 μg/mL, but was more cytotoxic to HeLa cells (IC_{50} 4 μg/mL vs. 23 μg/mL for compound 3). Aspergillus sclerotia are proving to be a rich source of new biologically active natural products. In contrast to the sclerotial metabolites of A. flavus and A. nomius, however, the indole diterpenoids produced by A. tubingensis are formed to some extent in liquid cultures. Interestingly, all of the new compounds reported from Aspergillus sclerotia so far have been indole diterpenoids.

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References and Notes

1. Willets, H. J. Biol. Rev. Cambridge Philos. Soc. 1971, 46, 387.
2. Wicklow, D. T.; Dowd, P. F.; TePaske, M. R.; Gloer, J. B. Trans. Br. Mycol. Soc. 1988, 91, 433.
3. Gloer, J.B.; TePaske, M.R., Sima, J.; Wicklow, D.T.; Dowd, P.F. J. Org. Chem., 1988, 53, 5457.
4. Gloer, J.B.; Rinderknecht, B.L.; Wicklow, D.T.; Dowd, P.F. J. Org. Chem. 1989, 54, 2530.
5. TePaske, M.R., Gloer, J.B.; Wicklow, D.T.; Dowd, P.F. Tetrahedron, in press.
6. TePaske, M.R., Gloer, J.B.; Wicklow, D.T.; Dowd, P.F. J. Org. Chem., in press.
7. A. tubingensis sclerotia (98 g) were exhaustively extracted with hexane to afford 474 mg of a light yellow oil. Tubingensin B (4; 54 mg), a light yellow crystalline solid, was isolated from this oil by HPLC (5μ C_{18} column; 250 X 10 mm; 90:10 MeOH-H_2O at 2.0 mL/min; retention time 23.6 min). Compound 4 has: mp 152-154°C; $[\alpha]_D$ -6.7° (c 0.80; $CHCl_3$); UV (MeOH) 338 (6700), 325 (2200), 299 (10100), 260 (10100), 237 (25500), 218 (17200); ^1H NMR and ^{13}C NMR data, Table I; EIMS (70 eV) 401 (M^+; rel. int. 91%), 383 (1.0), 358 (15), 340 (4), 330 (1.7), 314 (1.4), 300 (1.6), 288 (36), 260 (31), 246 (49), 230 (34), 218 (100), 204 (16), 191 (5), 180 (11), 167 (5), 144 (7), 130 (28); HREIMS, obs. 401.2733; calcd. for $C_{28}H_{35}NO$, 401.2720.
8. "Standard Ultraviolet Spectra", Sadtler Research Laboratories, Inc., V. 55, 1968, #13550.
9. Bax, A. J. Mag. Res. 1984, 57, 314.

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