in much better overall yield than that employing 6 (63% vs 13%), and the product is much more easily purified. This approach should be adaptable to the synthesis of optically active cannabinoids if racemization during the cyclization step can be prevented.

The initial synthetic design envisioned that the methyl ether would serve as a protecting group during elaboration of functionality at C-9. However, while it was possible to prepare the methyl ether of 1, conditions could not be found to regenerate the phenolic hydroxyl without affecting other portions of the molecule.[11] To circumvent this problem the more labile MOM ether of 8 (12) was employed. Although 8 could be prepared by demethylation of 9 (overall sequence: 10 → 11 → 9 → 8), a more efficient approach used the lithio derivative of the bis-MOM ether of olivetol (5, R = MOM) as the aromatic synthon. This procedure afforded pure enone 8 in 56% overall yield.

Dissolving metal reduction of MOM ether 12[6] and trapping of the enolate with *N*-phenyltriflimide[12] gave vinyl triflate 13 with moderate stereoselectivity (trans/cis ~ 3/1).[5] Palladium-mediated carboxylation to 14 followed by hydrolysis of the MOM ether[14] gave a mixture of acid 1 and its cis isomer (15). Careful chromatography failed to separate 1 and 15; however, pure acid 1 (22 mg) could be obtained from 52 mg of the mixture by crystallization. The spectral properties of synthetic acid 1 were identical with those of the natural material, and the overall yield of pure 1 from the bis-MOM ether of olivetol and apoverbenone (10) is 14%.

Acknowledgment. We thank the National Institute on Drug Abuse for support of this work under Grant DA-03590. We also thank Drs. Alan Schwartz of Hoffmann-LaRoche and Herbert H. Seltzman of the Research Triangle Institute for spectra of acid 1 and Melissa D. Lee for technical assistance.

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Articles

Tubingensin A: An Antiviral Carbazole Alkaloid from the Sclerotia of *Aspergillus tubingensis*

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Tubingensin A (3), a new carbazole alkaloid biogenetically related to the aflavinines, has been isolated from the hexane extract of the sclerotia of the fungus *Aspergillus tubingensis*. The structure of tubingensin A, which contains an unprecedented 9*H*-octahydronaphtho[3,4-*b*]carbazole ring system, was assigned through NMR decoupling, selective INEPT, and heteronuclear shift correlation experiments. Tubingensin A exhibits activity against the widespread crop pest *Heliothis zea* and displays in vitro antiviral activity against herpes simplex virus type 1.

Many fungi produce specially adapted morphological structures called sclerotia that are critical to the long-term survival and propagation of the species.[1-5] Sclerotia can remain dormant in soil for long periods of time, during which they are exposed to predation by fungivorous insects and arthropods.[2,3] Many vascular plants are known to selectively allocate secondary metabolites to important physiological structures as defenses against herbivore predation.[6] However, only the sclerotia of *Claviceps* spp. (which produce the ergot alkaloids) have been commonly explored for the production of unique, biologically active secondary metabolites.[7]

We have previously reported the isolation of four antiinsectan aflavinine derivatives (e.g., 1) that are selectively allocated to the sclerotia of *Aspergillus flavus*.[3,4] The typical sclerotial concentrations of these compounds are sufficient to deter feeding by fungivorous insects that encounter *A. flavus* sclerotia under natural conditions. A

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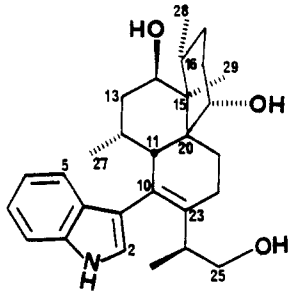

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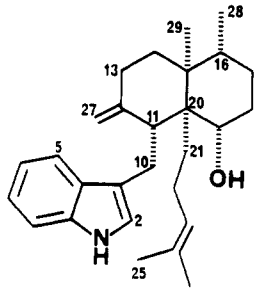

2

related compound, nominine (2), which we isolated from the sclerotia of *Aspergillus nomius*,[5] exhibits potent activity against the agriculturally important crop pest *Heliothis zea*.

Chemical studies of the sclerotia produced by *Aspergillus tubingensis* (Schober) Mosseray (NRRL 4700), a member of the *Aspergillus niger* taxonomic group,[8] have led us to the isolation of a new, biogenetically related carbazole alkaloid that we have named tubingensin A (3). Tubingensin A contains a unique 9*H*-octahydronaphtho-[3,4-*b*]carbazole pentacyclic ring structure not previously reported as a component of a natural product. Details of the isolation, structure elucidation, and biological activity of this compound are presented here.

Results and Discussion

Sclerotia of *A. tubingensis* were produced by solid substrate fermentation on autoclaved corn kernels. The hexane extract of these sclerotia exhibited potent antiinsectan activity, whereas the hexane extract of *A. flavus* was relatively inactive. Comparison of the mixtures by HPLC indicated the presence of a major chromatographic peak that was absent in the HPLC trace of the *A. flavus* hexane extract. Bioassays showed that this fraction was associated with significant anti-*Heliothis* activity, but spectroscopic examination revealed the presence of several components. Repeated reversed-phase HPLC of this fraction afforded the major component of the mixture, which we named tubingensin A.

The molecular formula of tubingensin A was established as $C_{28}H_{35}NO$ on the basis of HREIMS and ^{13}C NMR data. The ^{13}C NMR spectrum contained signals for 14 aromatic or olefinic carbons. This observation and the absence of any carbonyl or triple-bonded functionalities indicated that the remaining five unsaturations must be rings. Despite spectral similarities between tubingensin A and the aflavinines[4] and the detection of several aflavinine derivatives in *A. tubingensis*, the relatively low intensity of the quinolinium ion at m/z 130 in the mass spectrum suggested that this compound was not a monosubstituted indole. Furthermore, the UV spectrum revealed that tubingensin A possesses extended conjugation beyond a simple indole and specifically suggested that the compound contains a carbazole nucleus.[9]

Table I. Proton and Carbon-13 NMR Data for Tubingensin A (3)[a]

C	^1H	^{13}C	selective INEPT H/C correlations	selected ^1H–^1H NOE interactions[d]
1	7.81 (br s)		2, 3, 4, 9	27
2		137.84 (s)		
3		121.32 (s)		
4		123.78 (s)		
5	7.98 (br d; 7.8 Hz)	119.77 (d)	3, 4, 7, 9	
6	7.18 (dd; 3.9, 7.6, 7.8)	119.13 (d)	4, 5	
7	7.34 (m)[b]	125.27 (d)		
8	7.34 (m)[b]	110.41 (d)		
9		139.99 (s)		
10	7.92 (s)	118.45 (d)	2, 4, 12, 20	19
11		132.43 (s)		
12		135.08 (s)		
13	2.99 (ddd; 7.3, 12.9, 17.6; H_{ax})	27.06 (t)	11, 12, 15, 27	16
	2.88 (br dd; 6.6, 17.6; H_{eq})			27
14	1.52 (m)	29.42 (t)	12, 15, 20	
	2.01 (m)			
15		38.76 (s)		
16	1.74 (m)	32.58 (d)		13ax
17	1.17 (m)	25.36 (t)		28
	1.70 (m)			
18	1.66 (m)	29.61 (t)		
	2.05 (m)			
19	4.99 (br s)	71.35 (d)	15, 17, 20	10
20		47.23 (s)		
21	1.71 (m)	34.91 (t)		
	2.08 (m)			
22	1.76 (m)	23.10 (t)		
	2.06 (m)			
23	5.03 (dd; 6.6, 5.9)	125.00 (d)	22, 25, 26	26
24		131.48 (s)		
25	1.43 (br s)	17.62 (q)	23, 24	26
26	1.58 (br s)	25.62 (q)	23, 24	23, 25
27	7.11 (s)	110.65 (d)	3, 10,[c] 11, 13	1, 13eq
28	0.85 (d; 5.6)	16.24 (q)	15, 16, 17	29, 17
29	1.21 (s)	18.35 (q)	14, 15, 16, 20	28

[a] Data were recorded in $CDCl_3$ at 360 and 90.7 MHz, respectively. [b] The overlapping signals for the protons at C-7 and C-8 were well-resolved in benzene-d_6 and showed the expected multiplicities and J values. [c] A four-bond correlation. All other signals represent two- or three-bond correlations. [d] Does not include a number of cross peaks observed for overlapping ^1H signals or for protons that are scalar coupled.

Proton and carbon NMR data for tubingensin A are provided in Table I. Proton spin systems were established by homonuclear decoupling experiments conducted in two different solvents ($CDCl_3$ and C_6D_6). Carbon assignments were made on the basis of a heteronuclear shift correlation experiment, and a series of selective INEPT experiments,[5,10-12] (Table I) which afforded information about two- and three-bond C–H couplings. These assignments were supported by comparison with relevant data for the aflavinines and the carbazole-containing antibiotic carbazomycin A.[13] Firm evidence for a 2,3-disubstituted

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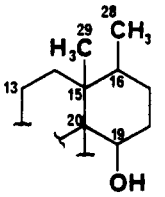
carbazole substructure was supplied by analysis of the ^1H NMR spectrum (Table I) and through selective INEPT correlation of H-5 with C-3, -4, -7, and -9, correlation of H-10 with C-2, -4, -12, and -20, and correlation of H-27 with C-3, -10, -11, and -13. One of these (C-10) represents a four-bond correlation. Four-bond couplings are well-precedented in aromatic and other π-systems, although they are usually small.[14] The absence of measurable coupling between H-10 and H-27 is consistent with their placement in a para orientation.

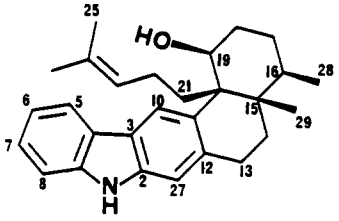
Partial structure a was established by the results of ^1H NMR decoupling experiments and by comparison of the NMR data to those obtained for the aflavinines.[4,5] Con-



a

firmation of this structural unit was obtained by long-range correlation of H_3-29 with C-14, -15, -16, and -20, and correlation of H_3-28 with C-15, -16, and -17. The protons at C-13 in this subunit were significantly downfield-shifted (2.99 ddd, 2.88 br dd), suggesting attachment of C-13 to an sp^2 center. The presence of a 4-methyl-3-pentenyl group was also clearly demonstrated by ^1H NMR decoupling experiments and carbon NMR data (C-21–C-26 in Table I).

Three additional observations resulting from selective INEPT experiments were instrumental in establishing the connectivity of these units. The downfield protons of the C-13–C-14 ethylene unit (H_2-13) were long-range coupled to two aromatic quaternary carbons (C-11 and -12), one aromatic methine (C-27), and an aliphatic quaternary carbon (C-15). The upfield protons of the ethylene unit (H_2-14) correlated with both aliphatic quaternary carbons (C-15 and -20) and one aromatic quaternary carbon (C-12). Finally, the downfield aromatic proton (H-10) correlated with the aliphatic quaternary carbon C-20. The only possible position of attachment for the 4-methyl-3-pentenyl group must then be C-20, although ^1H NMR signal overlap made confirming selective INEPT experiments ambiguous. These data led to proposal of the novel structure **3** for tubingensin A.



3

This assignment is supported by spectral comparison with the aflavinines and the *A. nomius* sclerotial metabolite nominine.[5,12] Biogenetic considerations also favor this structure. Tubingensin A apparently arises from the same condensation product of tryptophan and geranylgeraniol as do nominine and the aflavinines and would require only a different mode of ring closure than that leading to the aflavinines (e.g., connection of carbons 2 and 27 of structure **2**).[5,15] The numbering system shown for **3** was chosen to simplify structural, spectral, and biogenetic comparison with compounds **1** and **2**. In support of this suggested relationship, nominine was detected as a component of *A. tubingensis* sclerotia by HPLC analysis. However, neither nominine nor tubingensin A are present in the sclerotia of *A. flavus* or *A. parasiticus*. Interestingly, we have also encountered aflavinine derivatives in the sclerotia of *A. tubingensis*, but these compounds differ from the set of aflavinines produced by *A. flavus* and *A. parasiticus* with regard to the locations and orientations of substituents.[12]

The relative stereochemistry proposed for **3** is based on biogenetic considerations and ^{13}C NMR similarities with compounds **1** and **2** (for C-15–C-20, C-28, and C-29). Support for this assignment was provided by a NOESY experiment. A strong NOESY correlation between H-19 and H-10 indicated that these two protons are spatially close. Dreiding models indicate that H-19 must assume an equatorial position to account for such proximity, most likely in an orientation cis to C-11. The equatorial disposition of H-19 is in accord with the lack of trans-diaxial coupling between H-19 and neighboring protons. A correlation between the protons on C-28 and C-29 showed that these two methyls are gauche to each other. A cross peak correlating the axial proton at C-13 with a signal centered at 1.74 ppm (H-16) is consistent with a cis orientation for H-16 and C-14 and supports the proposed cis orientation for the methyl groups. The relative dispositions of the substituents at C-15 and C-16 with respect to those at C-19 and C-20 were partly verified by additional correlations, but overlap of key proton signals prohibited a complete stereochemical assignment for **3** on the basis of NOESY data alone. The three correlations described above are best accounted for by the relative stereochemistry proposed for **3** (with the adoption of a half chair-chair conformation), and this structure is fully consistent with all observed NOESY correlations.

Tubingensin A exhibits moderate activity against the widespread crop pest *Heliothis zea*, causing 11% mortality when incorporated into a standard diet at 125 ppm. Because of the structural novelty and physiological activity of **3**, an evaluation of this compound for other biological activities was undertaken. Tubingensin A exhibited activity in an in vitro assay against herpes simplex virus type 1 with an IC_{50} of 8 μg/mL. More extensive evaluation of compound **3** is under way, and further details of its biological activity will be reported elsewhere.

Comparative assays of various fungal parts (sclerotia, mycelia, and conidia) have previously shown that aflavinine derivatives and related compounds found in the sclerotia of *A. flavus*, *A. parasiticus*, or *A. nomius* are not found in other fungal parts and that neither sclerotia nor aflavinines are formed in liquid cultures of these fungi.[4,5] This situation closely parallels the selective allocation of the ergot alkaloids to the ergot (sclerotia) of *Claviceps* spp.[7] *A. tubingensis* is the only *Aspergillus* sp. we have encountered thus far that forms some sclerotia in liquid culture. Interestingly, it is also the only *Aspergillus* species we have encountered that forms significant quantities of indole diterpenoid metabolites, including **3**, in liquid culture.

Experimental Section

General Procedures. Sclerotia from a strain of *A. tubingensis* (NRRL 4700) were prepared by solid substrate fermentation on

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autoclaved dent field corn kernels at 28 °C and separated from substrate and other fungal material by using general procedures that have been previously described.[3] Sclerotial samples were stored at 4 °C until extraction. Proton and carbon NMR data were obtained in $CDCl_3$ on a Bruker WM-360 spectrometer, and chemical shifts were recorded by using the signal for the residual protiated solvent (7.24 ppm) as a reference. Carbon multiplicities were established by a DEPT experiment. One-bond C–H correlations were obtained by using an XHCORR pulse sequence optimized for 135 Hz. Proton signals studied with the selective INEPT technique were individually subjected to four separate experiments, optimizing for 4, 7, 10, or 15 Hz. HREIMS data were obtained on a VG ZAB-HF mass spectrometer, and the low-resolution spectrum was obtained on a VG TRIO 1 quadrupole instrument. Details of other experimental procedures and insect bioassays have been described elsewhere.[3-5,16]

Isolation and Properties of Tubingensin A. Sclerotia of *A. tubingensis* (500–750-μm diameter, 98.7 g) were produced by using 360 g of autoclaved corn kernels as substrate. The harvested sclerotia were ground with a mortar and pestle and triturated repeatedly with hexane (10 × 100 mL). The combined hexane extracts were filtered and evaporated to afford 474 mg of a yellow oil. This residue was subjected to crude preliminary separation by reversed-phase semipreparative HPLC (5-μm C_{18} column; 250 × 10 mm; 90:10 MeOH–H_2O at 2.0 mL/min). Fractions containing tubingensin A were rechromatographed on the same column at 85:15 MeOH:H_2O to afford 20 mg of tubingensin A (**3**) as a light yellow solid: mp 95–98 °C; HPLC retention time 17.7 min (90:10 MeOH–H_2O); $[\alpha]_D$ 13.6° (*c* 1.0, $CHCl_3$); UV (MeOH) 340 (ε 480), 326 (480), 302 (6780), 262 (6930), 239 (18 200), 218 nm (14 900); ^1H NMR and ^{13}C NMR, see Table I; EIMS (30 eV) 401 (M^+, rel intensity 38%), 318 (100), 300 (52), 260 (11), 246 (40), 234 (21), 232 (25), 220 (28), 206 (51), 180 (32), 146 (10), 130 (5); HREIMS, obsd 401.2698, calcd for $C_{28}H_{35}NO$ 401.2720.

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Thioanhydrides. 3. Synthesis, Properties, and Diels–Alder Reactions of Sulfur Analogues of 1,8-Naphthalic Anhydride†,[1]

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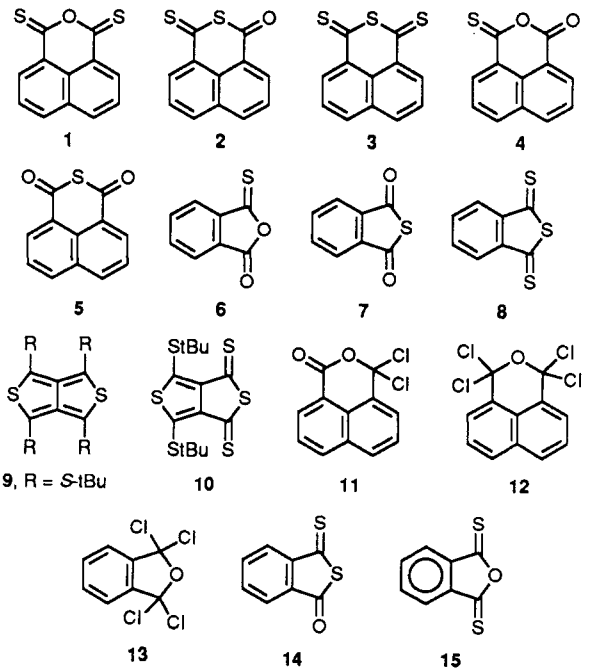
All five possible sulfur analogues of 1,8-naphthalic anhydride have been synthesized by practical procedures, starting from 1,8-naphthalic anhydride. The thionoanhydrides containing an oxygen bridge rearrange readily to thiolo isomers under tertiary amine catalysis, and all of the thionoanhydrides undergo (4 + 2) cycloadditions with norbornylene. In addition, other α-thiono-substituted naphthalenes have been probed in the norbornylene addition reaction, and some observations have been made concerning the mechanism of the Pedersen thionation reaction.

In contrast to the extensive chemistry of cyclic carboxylic anhydrides, very little is known concerning thiocarbonyl analogues containing either five-membered or six-membered rings.

In the five-membered series, thionophthalic anhydride **6** was obtained unexpectedly in 1967 from the reaction of phthaloyl chloride and hydrogen disulfide; it was quite unstable thermally and isomerized readily to the thiolo-anhydride **7**.[2] The rather more stable thionothiolophthalic anhydride **14** was reported by us in 1988 by an interesting reaction discussed in more detail below.[3] Trithiophthalic anhydride (**8**) has not yet been described, although the thiophene derivative **10**, which represents the first five-membered trithioanhydride, was reported in 1986 as a remarkable oxidation product of the stable thieno[3,4-*c*]-thiophene derivative **9**.[4]

The first six-membered thionoanhydrides (**1–3**), belonging to the 1,8-naphthalic anhydride series, were reported by us briefly in 1984.[5] We now present synthetic details of this work, as well as the synthesis of the remaining two sulfur analogues (**4** and **5**) of 1,8-naphthalic anhydrides, some mechanistic studies on the Pedersen thionation, and some expanded observations on the Diels–Alder addition of norbornylene to α-thiono-substituted naphthalenes.



Results and Discussion

Synthesis of the Five Possible 1,8-Naphthalic Thioanhydrides. Our initial thionation study involved the acid chloride of 1,8-naphthalic acid. This compound has been known for some time, but there was ambiguity

† For Part 2, see ref 3.

(1) A preliminary account of this work was presented in a plenary lecture by M. P. Cava at the 13th International Conference on Organic Sulfur Chemistry, held at Odense, Denmark, in August 1988.

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