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Atranones: Novel Diterpenoids from the Toxigenic Mold *Stachybotrys atra*

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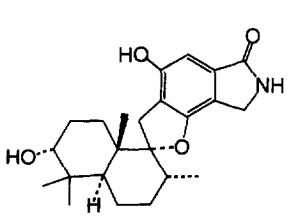
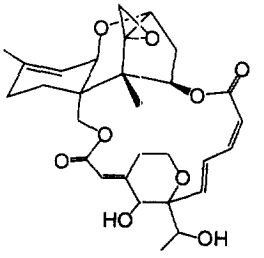
Abstract: The toxigenic mold *Stachybotrys atra* produces a new class of elaborated diterpenoid dolabellanes, the C_{24} atranones, which have several unusual structural features, including an enol-lactone ring system.

The filamentous fungus *Stachybotrys atra* (also known as *S. chartarum*) has a history of causing mycotoxicosis (stachybotryotoxicosis) in animals; horses are particularly sensitive to this mold which is a common contaminant of damp hay and straw [1]. Stachybotryotoxicosis in humans is rare and has been reported most commonly in workers who handle moldy straw and hay [2]. However, in 1986, Croft *et al.* reported an apparent episode of stachybotryotoxicosis in a family living in a water-damaged Chicago home that was infested heavily with *S. atra* [3]. Of some interest is the report of the strong association of *S. atra* with pulmonary hemosiderosis in infants in Cleveland, Ohio, that led to several fatalities [4]. However, the cause and effect relationship between *S. atra* and this syndrome is controversial [5,6].

Chemical investigations of *S. atra* have shown that this mold produces several types of biologically active classes of compounds, including immunosuppressants (e. g. stachybotryamide, Fig. 1) and the highly cytotoxic macrocyclic trichothecenes (e.g. satratoxin H, Fig. 1) [7]. A recent survey of *S. atra* isolates obtained from the Cleveland homes [8], coupled with unpublished data from our laboratory, have shown that all isolates of *S. atra* produce the immunosuppressant phenylspirodrimanes [7], but only about one-third of the isolates produce the trichothecene mycotoxins. In work directed at isolating new biologically active compounds from those non-trichothecene-producing *S. atra* cultures, we have isolated a set of novel compounds related in structure to the dolabellane diterpene ring system [9], but which is further elaborated by a four-carbon fragment with an unusual fused enol lactone system.



stachybotryamide



satratoxin H

Figure 1. *Stachybotrys atra* toxins.

Rice (850 g) inoculated with *S. atra* (Debrecen 5142, S-11) [10] was stored at room temperature (4 weeks) and extracted MeOH/$CHCl_3$ (1:1). The extract was triturated with hexane

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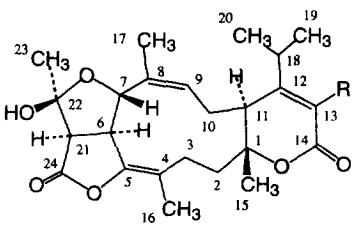
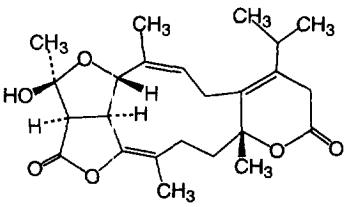
and the remaining insoluble material subjected to chromatography over polyethyleneimine silica gel [11]. The atranone-containing fractions gave distinctive orange spots upon developing the TLC plate with vanillin spray. Atranones A-C were purified by a combination of radial and HPLC chromatographies.

Atranone A was isolated as an amorphous white solid (90 mg, 179-184 °C) with composition $C_{24}H_{32}O_6$ determined by HREIMS (416.2212 requires 416.2199). The structure was elucidated by detailed analysis of the spectral data. Proton NMR data collected in $CDCl_3$ displayed unfortunate overlap, especially in the proton NMR methyl region(s). Although a structural solution was determined from these data, it is more expedient to discuss the data collected in deuterobenzene as the signal dispersion is remarkably improved (Table 1). The IR absorption at 1789 cm^{-1} and carbon resonance at δ 170.3 indicated an ester function, most likely a γ-lactone unit. An alcohol was evidenced by sharp absorptions at 3684 and 3599 cm^{-1}. A further IR absorption at 1706 cm^{-1} coupled with the carbon resonances δ 163.5, 166.8 and 114.7 as well as the lowest-field proton resonating at δ 5.82 suggested a β-substituted, α,β-unsaturated lactone group. This was also evidenced by the UV absorption at 224 nm (ε 10,500). Further unsaturation in the form of a trisubstituted double bond (^{13}C: δ 134.3, 128.9; ^{1}H: δ 5.23) and an oxygen bearing tetrasubstituted olefin (^{13}C: δ 145.7, 111.4) account for 5 of the 9 degrees of unsaturation; hence, the molecule is tetracyclic. Inspection of the DEPT and HMQC data indicated a partial molecular formula of $C_{24}H_{31}$, which was consistent with one exchangable OH proton. A combination of ^{1}H-^{1}H and ^{1}H-^{13}C COSY and HMBC ^{1}H-^{13}C long range correlation experiments established the main portion of the molecule to be a cycloundecadiene ring fused to an α,β-unsaturated δ-lactone ring. The remaining 4-carbon fragment is fused to the C5-C7 portion of the cycloundecadiene ring. However, we were unable to detect a 3-bond H7 to C22 coupling (HMBC) and thus were unable to say with certainty whether the γ-lactone-hemiketal bicyclic system was fused as shown in Figure 2, or whether this ring system was reversed, with the hemiketal portion being part of an enol ether. Fortunately, atranone C gave crystals suitable for X-ray diffraction analysis, which showed the C-4 unit to be the γ-enol-lactone/hemiketal bicyclic system (Fig. 2), a structural unit unique to the atranones. With the structure of atranone C secure, we were able to assign with confidence the structures of atranones A and B [12] based on the ^{1}H and ^{13}C NMR data, including NOESY correlations (Table 1). The double bond isomeric atranones A and C are observed (by HPLC analysis) in the crude extracts, and thus atranone A is not the result of an isomerization of atranone C during isolation.



atranone A: R = H
atranone B: R = OMe



atranone C

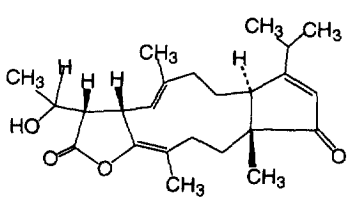
Figure 2. Atranones A-C produced by *Stachybotrys atra*.

Table 1. ^{1}H (500 MHz) and ^{13}C (100 MHz) NMR data for atranones A-C.[a,b]

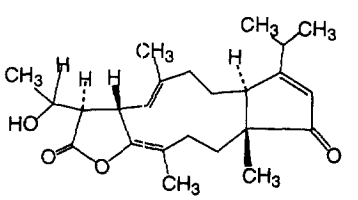
	Atranone A		Atranone B		Atranone C	
C#	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	84.7		84.0		86.6	
2a	39.2	1.27 ddd 14.0,12.5,2.7	39.2	1.22 m	39.1	1.39 m
2b		1.98 ddd 14.0,14.0, 5.2		1.94 ddd 14.5,14.5, 5.0		1.88 ddd 14.3,14.3,4.8
3a	26.8	1.54 m	26.5	1.50 brddd 14,14,2.3	27.2	1.61 ddd 13.3,13.3,2.7
3b		1.81 m		1.75 ddd 14.5,14.5,5.0		1.95 m
4	111.4		111.3		111.3	
5	145.7		145.7		145.4	
6	47.8	2.91 ddq 11.1,4.1,1.5	47.8	2.90 ddq 11.2,4.0,2.0	47.8	2.92 br ddq 11.4,4.6,2
7	80.7	5.16 d 4.1	80.9	5.14 d 4.0	80.9	5.18 d 4.6
8	134.3		134.3		133.3	
9	128.9	5.23 brdd 12.0, 4.1	128.9	5.27 brdd 12.5, 4.6	128.9	5.40 dd 12.0, 6.3
10a	23.4	1.46 m	24.4	1.45 m	25.6[c]	2.38 m
10b		1.82 m		1.82 ddd 13.0,12 5,1.9		2.56 dd 14.8, 12.0
11	44.0	2.47 brdd 7.9, 2.5	43.4	2.58 brd 8.1	129.5	
12	166.8		143.6		135.5	
13	114.7	5.82 d 2.5	142.5		29.3	2.51 brd 20.6
						2.87 d 20.8
14	163.5		160.1		168.9	
15	20.8	0.80 s, 3H	20.5	0.86 s, 3H	28.2	0.92 s, 3H
16	14.9	1.67 d 1.5, 3H	14.8	1.64 brs, 3H	14.7	1.63 brs, 3H
17	17.6	1.48 brs, 3H	17.6	1.46 s, 3H	17.8	1.53 brs, 3H
18	29.5	2.08 qq 6.7,6.8	29.1	2.19 qq 6.9,7.0	29.7	2.53 m
19	21.8	0.71 d 6.7, 3H	20.1	1.22 d 6.9, 3H	19.6	0.52 d 6.6, 3H
20	22.8	0.77 d 6.8, 3H	20.4	1.10 d 7.0, 3H	20.0	0.68 d 6.8, 3H
21	57.5	2.67 d 11.1	57.4	2.64 d 11.2	57.4	2.61 d 11.4
22	105.1		105.0		105.1	
23	25.6	1.40 s, 3H	25.6	1.33 s, 3H	25.6[c]	1.38 s, 3H
24	170.3		170.1		170.4	
OMe			58.9	3.63 s, 3H		

[a] ^{1}H-^{13}C correlations were determined by HMQC experiments. ^{13}C multiplicity was verified by DEPT experiment.
[b] NMR data were recorded in deuterobenzene at 25 °C. Proton resonances integrate for one proton unless otherwise indicated. Numbers following proton chemical shifts are coupling constants in Hz.
[c] DEPT and HMQC experiments verified the overlap of these two resonances.

During the course of this work, we have isolated several other compounds of related structure (to be reported in a full paper), including two new dolabellanes and atranones D and E (Fig. 3), in which the δ-lactone ring in the eastern portion of the atranones has been replaced by an α,β-unsaturated cyclopentenone ring. The bicyclo[9.3.0]tetradecane dolabellane ring system having the cyclopentenone substructure is uncommon [13], and the transformation of this ring system to the corresponding δ-lactone ring is unprecedented. Thus biosynthetically, atranones A-C and atranones D and E are related by what appears to be a Baeyer-Villiger oxidation of the latter, a transformation more commonly encountered in bacteria [14,15] than in fungi [16].



atranone D



atranone E

Figure 3. Atranones D and E produced by *Stachybotrys atra.*

The dolabellane diterpenes have previously been isolated from marine alga [17] and several lower plants such as liverworts [18], but only one report is listed of this class of compound being found produced by a mold [19]. Furthermore, the atranones are the first examples of dolabellanes that have been elaborated further by C-alkylation. Of particular note is our observation that *S. atra* isolates fall into two distinct classes: those that produce trichothecenes (*ca.* 1/3) and those that produce atranones (*ca.* 2/3). In no case in over 50 *S. atra* isolates (obtained from both North America and Europe) have we found an isolate that produces both these classes of natural products.

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References and Notes

1. J. Forgacs, "Stachybotryotoxicsis," in *Microbial Toxins,* Vol. VIII, S. Kadis, A. Ceigler, and S. J. Ajl (eds), Academic Press, New York, pp 95-128 (1972).
2. E.-L. Hintikka, "Human stachybotryotoxicosis," in *Mycotoxic Fungi Mycotoxins Mycotoxicoses*, Vol. 3, T. D. Wyllie and L. G. Morehouse (eds.), Marcel Dekker, New York, pp. 87-89 (1978).
3. W. A. Croft, B. B. Jarvis, and C. S. Yatawara, *Atmospheric Environment,* **20**, 549-552 (1986).
4. R. A. Etzel, E. Montana, W. G. Sorenson, G. J. Kullman, T. M. Allen, D. R. Olson, B. B. Jarvis, J. D. Miller, and D. G. Dearborn, *Arch. Pediatr. Adolesc. Med.*, **152**, 757-762 (1998).
5. F. Fung, R. Clark, and S. Williams, *J. Toxicol. Clin. Toxicol.*, **36**, 79-86 (1998).
6. J. Johanning, *J. Toxicol. Clin. Toxicol.*, **36**, 629-631 (1998).
7. B. B. Jarvis, J. Salemme, and A. Morais, *Natural Toxins*, **3**, 10-16 (1995).
8. B. B. Jarvis, W. G. Sorenson, E.-L. Hintikka, M. Nikulin, Y. Zhou, J. Jiang , S. Wang, S. Hinkley, R. A. Etzel, and D. G. Dearborn, *Appl. Environ. Microbiol.*, **64**, 3620-3625 (1998).
9. Y. Asakawa, *Prog. Chem. Org. Nat. Prod.*, **65**, 219-226 (1995).
10. B. B. Jarvis, Y.-W. Lee, C. S. Yatawara, and S. N. Cömezoglu, *Appl. Environ. Microbiol.*, **51**, 915-918 (1986).
11. B. B. Jarvis, *Phytochem. Anal.*, **3**, 241-249 (1992).
12. **Atranone A:** white solid; $[\alpha]_{20}$ (λ) +29 (589) (*c* 0.86, $CHCl_3$); mp 179-184 °C; UV λ_{max} nm (ε) 224 (10,500); IR ($CHCl_3$) 3684, 3598, 3036 (br), 1789, 1706, 1522, 1424, 1212 (br) cm^{-1}; HREIMS (%) 416.2212 (16) ($C_{24}H_{32}O_6$ requires 416.2199) 398 (5, M^+-H_2O), 374 (28, M^+-42), 373 (100, M^+-43), 355 (26, M^+-43-H_2O). **Atranone B:** white solid; $[\alpha]_{20}$ (λ) +25 (589) (*c* 1.5, $CHCl_3$); mp 213-218 °C, coloration at 200 °C; UV λ_{max} nm (ε) 231 (10,800); IR ($CHCl_3$) 3684, 3599, 3018 (br), 1790, 1704, 1522, 1424, 1213 (br), 1136 cm^{-1}; HREIMS (%) 446.2298 (44) ($C_{25}H_{34}O_7$ requires 446.2305), 404 (23, M^+-42), 403 (45, M^+-43), 363 (22), 319 (30), 221 (50), 149 (90), 112 (100). **Atranone C:** clear needles, recrystallized from MeOH/hexane; $[\alpha]_{20}$ (λ) +74 (589) (*c* 0.44, $CHCl_3$); mp 204-208 °C; UV end adsorption only; IR ($CHCl_3$) 3607, 2970, 2935, 1791, 1716, 1521, 1475, 1386, 1297, 1136 cm^{-1}; HREIMS (%) 416.2191 (34) ($C_{24}H_{32}O_6$ requires 416.2199), 398 (10, M-H_2O), 374 (33, M-42), 373 (91, M-43), 356 (22, M^+-43-H_2O), 268 (21), 227 (53), 183 (80), 131 (90), 81 (100).
13. M. Govindan, G. N. Govindan, and D. G. I. Kingston, *J. Nat. Prod.*, **58**, 1174-1184 (1995).
14. C. T. Walsh and Y.-C. J. Chen, *Angew. Chem. Int. Ed. Engl.*, **27**, 333-343 (1988).
15. J. L. C. Wright, J. Hu, J. L. McLachlan, J. Needham, and J. A. Walter, *J. Am. Chem. Soc.*, **118**, 8757-8758 (1996).
16. R. E. Minto and C. A. Townsend, *Chem. Rev.*, **97**, 2537-2555 (1997).
17. R. Duran, E. Zubia, M. J. Ortega, and J. Salva, *Tetrahedron*, **53**, 8675-8688 (1997).
18. T. Hashimoto, M. Toyota, H. Koyama, A. Kikkawa, M. Yoshido, M. Tanaka, S. Takaoka, and Y. Asakawa, *Tetrahedron Lett.*, **39**, 579-582 (1998) and references therein.
19. I. Jenny and H.-J. Borschberg, *Helv. Chim. Acta*, **78**, 715-731 (1995).