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MICROBIAL METABOLITES OF OPHIOBOLIN A AND ANTIMICROBIAL EVALUATION OF OPHIOBOLINS

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ABSTRACT.—Ophiobolin A [**1**], 3-anhydroophiobolin A [**2**], ophiobolin B [**3**], and ophiobolin L [**4**] were isolated from fermentation broths of *Cochliobolus heterostrophus*. Preliminary screening showed that a number of organisms were capable of metabolizing the sesterterpene ophiobolin A [**1**]. Large-scale transformations of ophiobolin A [**1**] with *Polyangium cellulosum* produced **6** and **7** while *Pseudomonas aeruginosa* produced **8**. Resting-cell preparations of *Penicillium patulum* afforded **9** and **10**. The structures of these metabolites were established by spectroscopic methods and by comparison of the spectral data with those of the starting material. The antimicrobial activity of the ophiobolins was also evaluated.

Ophiobolin A [**1**] and its analogues are a group of sesterterpene phytotoxins with unique chemical structures produced by the plant pathogen *Helminthosporium maydis* (1), as well as by other members of the same genus (2,3) and by *Aspergillus* spp. (4). Ophiobolins have a broad spectrum of biological activity and are active against nematodes (4), fungi, and bacteria (5,6). Levy *et al.* reported that in a phosphodiesterase inhibition assay, **1** inhibited calmodulin-activated cyclic nucleotide phosphodiesterase and the inhibitory activity was Ca^{2+} dependent, which implies that **1** might be a potent antitumor candidate since it was found that multidrug-resistant tumor cells have a greater intracellular concentration of calcium than non-resistant cells (8). Because there are no reports on the mammalian metabolism of ophiobolin A [**1**], we chose to use microbes as a tool to predict the mammalian metabolic pathway and to evaluate the biological activity of these metabolites (9). Ophiobolin A [**1**], 3-anhydroophiobolin A [**2**], ophiobolin B [**3**], and ophiobolin L [**4**], which is structurally related to ophiobolin H [**5**], were all isolated from *Cochliobolus heterostrophus* (ATCC 22246).

Five microbial metabolites of **1** were produced by three microorganisms. The metabolites have been identified as ophiobolin I [**6**], ophiobolin A lactone [**7**], ophiobolin B lactone [**8**], 6-*epi*-ophiobolin A [**9**], and 6-*epi*-ophiobolin L [**10**]. All of these metabolites possess weak to moderate anti-*Cryptococcus* activity, and strong activity against *Trichophyton mentagrophytes*, while metabolite **10** showed strong activity against *Staphylococcus aureus*. The isolation, structure elucidation, and antimicrobial evaluation of **1** and other naturally occurring ophiobolins [**2–4**], and the microbial metabolites of **1** are reported here.

RESULTS AND DISCUSSION

The starting substance ophiobolin A [**1**] was prepared by fermentation using a previously reported method (7) with some modification. *Cochliobolus heterostrophus* (ATCC 22246) was grown in modified Fries medium on a rotary shaker for 12 days, then the mycelia and the broth were extracted with Et_2O. After removal of the solvent, the extract was dissolved in a minimal amount of Me_2CO for crystallization of ophiobolin A [**1**]. Crude **1** was further purified by recrystallization from Me_2CO. Ophiobolin A [**1**] was identified by spectroscopic methods and by comparison with an authentic sample.

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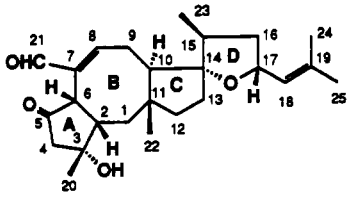

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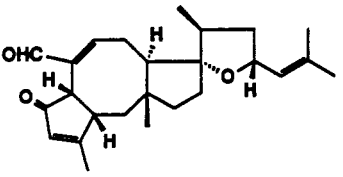

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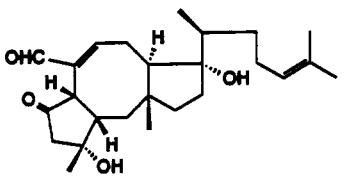

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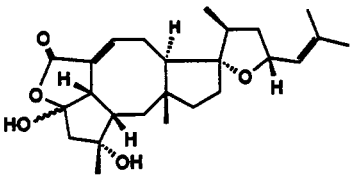

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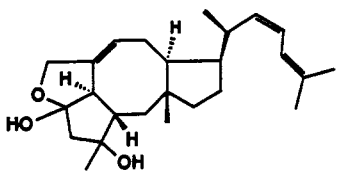

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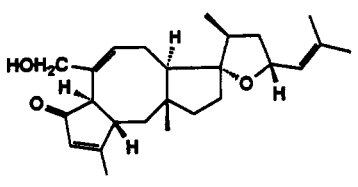
The residue resulting from the crystallization of ophiobolin A [**1**] was subjected to cc. Along with more of **1**, three other compounds were isolated. 3-Anhydroophiobolin A [**2**] and ophiobolin B [**3**] were identified by spectroscopic methods and by comparison



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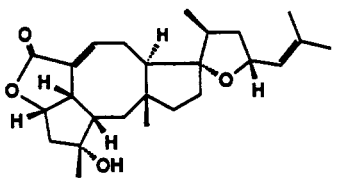

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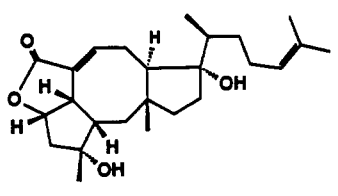

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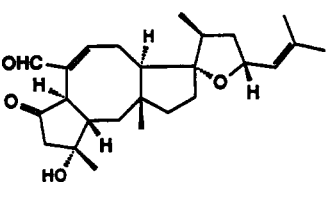

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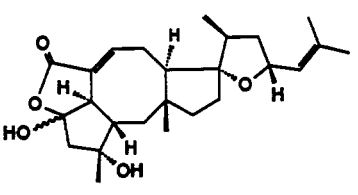

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with reported data, while **4** was identified as a previously undescribed ophiobolin analogue and was named ophiobolin L.

The ir spectrum of compound **4** revealed the presence of hydroxyl groups (ν max 3400 cm^{-1}). Fabms showed the molecular ion peak at *m/z* 416 and high-resolution fabms gave *m/z* 423.2716, corresponding to an elemental formula of $C_{25}H_{36}O_4Li$ (calcd 423.2723). The major differences in the ^{13}C-nmr spectra of **4** and ophiobolin A [**1**] (Table 1) were the appearance of a carboxylic signal at δ_C 171.0 and a new signal at δ_C 113.3 in the ^{13}C-nmr spectrum of **4**, which suggest that **4** is a lactone hemiketal derivative of **1**. The downfield signal at δ_C 113.3 is typical for a hemiketal. The signals at δ_C 131.2, 141.1, and 171.0 in the ^{13}C-nmr spectrum represent a conjugated system. Ophiobolin H [**5**], a similar hemiketal isolated from *Aspergillus ustus*, showed a downfield signal at δ_C 116.4 (5). The cis-A/B ring junction of **4** was assigned on the basis of nmr data. It has been observed from this study that C-1 and CH_3-22 always resonate at higher field in the ^{13}C-nmr spectrum when the A/B rings are cis rather than trans. This assignment was further confirmed by examining the 2D NOESY spectrum of **4**, which showed a strong nOe between H-2 and H-6. The stereochemistry at C-5 remains undetermined. The assigned ^{13}C-nmr data of **4** are summarized in Table 1. To our knowledge, **4**, named ophiobolin L, is a previously undescribed natural product.

The ^{13}C-nmr spectral data of **1** were assigned in 1975 by Radics *et al.* (10). Because unambiguous assignments for **1** are necessary for microbial transformation studies, it was deemed appropriate to reinvestigate these assignments using 2D nmr methods. A

TABLE 1. ^{13}C-Nmr Chemical Shifts of Ophiobolin A [**1**], 3-Anhydroophiobolin A [**2**], Ophiobolin B [**3**], Ophiobolin L [**4**], and the Microbial Metabolites **6–10**.[a]

Carbon	Compound								
	1	**2**	**3**	**4**	**6**[b]	**7**	**8**	**9**	**10**
1	35.2 (2)	36.4 (2)	37.2 (2)	35.1 (2)	47.2 (2)	35.1 (2)	36.9 (2)	42.1 (2)	41.8 (2)
2	50.1 (1)	48.2 (1)	50.8 (1)	50.9 (1)	52.7 (1)[1]	51.8 (1)	52.1 (1)	49.5 (1)	51.6 (1)
3	76.6 (0)	176.1 (0)	76.8 (0)	79.9 (0)	180.8 (0)	80.2 (0)	79.9 (0)	76.6 (0)	80.6 (0)
4	54.7 (2)	131.4 (1)	54.8 (2)	51.3 (2)	131.0 (1)[3]	46.6 (2)	46.2 (2)	54.7 (2)	53.8 (2)
5	217.0 (0)	207.2 (0)	217.2 (0)	113.3 (0)	210.2 (0)	81.4 (1)	81.5 (1)	216.6 (0)	112.0 (0)
6	48.2 (1)	47.9 (1)	48.4 (1)	50.5 (1)	51.1 (1)[1]	44.4 (1)	44.2 (1)	48.0 (1)	49.3 (1)
7	141.6 (0)	139.4 (0)	141.6 (0)	131.2 (0)	134.5 (0)[2]	130.8 (0)	130.6 (0)	142.5 (0)	135.7 (0)
8	162.8 (1)	159.7 (1)	163.3 (1)	141.1 (1)	130.2 (1)[3]	139.8 (1)	140.5 (1)	157.7 (1)	141.8 (1)
9	23.5 (2)	30.7 (2)	24.1 (2)	23.4 (2)	28.0 (2)	23.4 (2)	23.8 (2)	29.4 (2)	28.4 (2)
10	60.5 (1)	53.8 (1)	65.5 (1)	60.9 (1)	53.4 (1)	61.2 (1)	66.6 (1)	53.3 (1)	52.8 (1)
11	42.6 (0)	44.3 (0)	44.7 (0)	42.4 (0)	42.1 (0)	42.5 (0)	44.3 (0)	41.5 (0)	41.9 (0)
12	40.8 (2)	39.4 (2)	39.9 (2)	41.3 (2)	41.6 (2)	41.5 (2)	40.3 (2)	42.0 (2)	40.6 (2)
13	43.0 (2)*	42.8 (2)	32.6 (2)	42.8 (2)	42.2 (2)	42.9 (2)	32.1 (2)	42.6 (2)	42.6 (2)
14	94.6 (0)	94.7 (0)	87.0 (0)	95.2 (0)	96.3 (0)	95.0 (0)	86.9 (0)	96.0 (0)	96.3 (0)
15	36.7 (1)	36.7 (1)	40.2 (1)	36.6 (1)	35.2 (1)	36.6 (1)	39.9 (1)	35.2 (1)	35.2 (1)
16	30.0 (2)*	30.7 (2)	32.2 (2)	30.2 (2)	30.7 (2)	30.4 (2)	32.0 (2)	31.0 (2)	30.8 (2)
17	70.8 (1)	71.4 (1)	26.2 (2)	70.9 (1)	71.9 (1)	70.9 (1)	26.2 (2)	72.0 (1)	71.8 (1)
18	125.3 (1)	126.7 (1)	124.2 (1)	126.0 (1)	127.0 (1)	125.9 (1)	124.5 (1)	126.8 (1)	126.4 (1)
19	136.0 (0)	134.5 (0)	131.8 (0)	135.3 (0)	134.7 (0)[2]	135.4 (0)	131.1 (0)	135.2 (0)	127.9 (0)
20	25.4 (3)*	25.7 (3)	25.5 (3)	25.7 (3)	17.2 (3)	25.7 (3)	25.3 (3)	25.7 (3)	24.7 (3)
21	195.8 (1)	193.8 (1)	196.2 (1)	171.0 (0)	67.4 (2)	171.3 (0)	171.9 (0)	194.0 (1)	170.8 (0)
22	17.8 (3)	18.2 (3)	18.6 (3)	17.4 (3)	22.3 (3)	18.2 (3)	17.8 (3)	22.8 (3)	23.1 (3)
23	18.1 (3)	17.4 (3)	16.4 (3)	17.8 (3)	16.4 (3)	17.4 (3)	16.2 (3)	16.1 (3)	15.7 (3)
24	18.1 (3)	18.2 (3)	17.7 (3)	18.2 (3)	18.1 (3)	18.0 (3)	17.5 (3)	18.1 (3)	18.2 (3)
25	25.7 (3)*	25.7 (3)	25.7 (3)	25.8 (3)	25.8 (3)	25.8 (3)	25.5 (3)	25.8 (3)	25.9 (3)

[a]The number in parentheses indicates the number of attached hydrogens as determined by the ATP and DEPTGL experiments. Assignments bearing the same numerical superscript may be reversed. The resonances designated by * in **1** reflect differences with previous assignments (10).

[b]The assignments were made by comparison with analogues.

series of 2D nmr experiments including COSY, HETCOR, LR-HETCOR, and NOESY was performed and confirmed all previous assignments except for C-20, C-25, and C-13, C-16, which we have reversed from those previously reported (10). Even though the chemical shifts for C-20 and C-25 are quite close, a clear nOe between H-18 and CH_3-25 allowed them to be distinguished. The methylene carbons C-12, C-13, and C-16, could be distinguished in the ^{13}C-nmr spectrum by noting the strong correlations between H-17 and one of the H-16 protons by COSY. HETCOR then allowed assignment of C-16. Also, there was a long-range ^1H-^{13}C correlation between the C-22 methyl group and the protons at C-12, which allowed assignment of C-12 and thus C-13 as well. Assignments of the signals for the methylene carbons C-4, C-9, and C-1 were straightforward, based on analyses of the 2D data. Thus, the unambiguously assigned ^{13}C-nmr data for **1** are summarized in Table 1.

Because it is well known that ophiobolin A [**1**] will undergo epimerization at C-6 and/or anhydro formation in ring A, it was anticipated that the normal microbial transformation procedures (9) might have to be modified. Substrate controls (**1** + media) clearly showed the presence of additional spots that were subsequently identified as 3-anhydroophiobolin A [**2**] (major) and 6-*epi*-3-anhydroophiobolin A (minor, tlc only). Allowing the Stage I culture to achieve a large cell mass before addition of ophiobolin A [**1**] tended to minimize anhydro formation. Thus, the transformations were carried out after Stage I cultures were grown for 72 h. The transformations were carried out for only four to six days, after which time the cultures were extracted.

A total of 85 microorganisms was evaluated for their ability to metabolize ophiobolin A [**1**] using this modified procedure. Tlc analyses of these culture extracts identified seven microorganisms that were capable of producing at least five metabolites. *Polyangium cellulosum* (ATCC 29610), *Pseudomonas aeruginosa* (ATCC 15442), and *Penicillium patulum* (ATCC 24550) were selected for preparative-scale transformations.

After six days of incubation of **1** with *P. cellulosum*, the Et_2O extract of the culture broth afforded two compounds ([**6**] and [**7**]) on purification by cc. The ^1H-nmr spectrum of **6** showed a pair of AB doublets at δ_H 4.15 and 3.89 (J=11.9 Hz), the disappearance of the aldehyde proton from **1**, the shift of the methyl group at C-20 from δ_H 1.24 to 2.06, and the appearance of a new olefinic signal at δ_H 5.94. These observations were consistent with anhydro formation in ring A and reduction of the aldehyde to a primary alcohol. The ^{13}C-nmr data also confirmed these observations, showing new signals for a methylene group at δ_C 67.4, and olefinic carbon signals at δ_C 131.0 and 180.8, and an upfield shift of the carbonyl carbon to δ_C 210.2. Analysis of these data strongly suggested structure **6**, which has been previously reported as ophiobolin I (3). The relative stereochemistry at C-6 was confirmed by a NOESY experiment that showed a strong nOe correlation between H-6 and H-9α and no correlations between H-6 and H-2 and H-6 and CH_3-20. These data agree well with those reported for ophiobolin I [**6**] (3).

The ir spectrum of **7** showed a strong absorption band at ν max 1752 cm^{-1}, suggesting an α,β-unsaturated γ-lactone that could result from oxidation of C-21 and reduction of C-5. The ^{13}C-nmr data confirmed the absence of the aldehyde and ketone carbonyls. A lactone carbonyl signal at δ_C 171.3 and a methine at δ_C 81.4 were present. The remaining ^1H- and ^{13}C-nmr data were very similar to those of **1**, suggesting that no other changes had occurred. Strong nOe's between H-6 and H-2, H-6 and H-5, and H-6 and CH_3-20 suggested the relative stereochemistry as shown in **7**. Compound **7** was previously prepared by Canonica and coworkers by chemical conversion (11). Rossi and Tuttobello also isolated this compound as a metabolite of *C. miyabeanus* growing under modified conditons with a supplement of methionine in the fermentation broth (12). The spectral data are consistent with those reported by Rossi and Tuttobello. This is the first time that the relative stereochemistry of **7** has been confirmed.

TABLE 2. Antibacterial and Antifungal Evaluation of Ophiobolin A [1] and Its Derivatives.[a]

Organism	Test compound						
	1	3	4	6	7	8	10
Candida albicans	+++	−	+	−	−	−	−
Cryptococcus neoformans	++	±	++	+	+	+	±
Trichophyton mentagrophytes	++++	+++	NT	+++	+++	+++	+++
Escherichia coli	−	−	−	−	−	−	−
Staphylococcus aureus	+++	++	−	−	−	−	++++
Mycobacterium intracellulare	++	−	++	++	++	−	−

[a]Inhibitory zones were recorded in mm, measured from the edge of the well to the edge of the zone, the codes were assigned as follows: −, no inhibitory zone; ±, ≤1 mm; +, 1–2; ++, 3–6 mm; +++, 7–12 mm; ++++, >12 mm; NT, not tested. Amphotericin B and streptomycin sulfate were used as positive antifungal and antibacterial controls, respectively.

A preparative-scale transformation of **1** with *P. aeruginosa* led to the isolation of **8** as the major metabolite. A comparison of the spectral data of **8** with those of **7** immediately suggested the same functionalities in rings A and B, but differences in rings C and D were evident from the nmr data. The nmr signals for **8** were nearly identical to those observed for ophiobolin B [**3**]. The key difference between **1** and **8** was the C-17 signal, which appeared at δ_C 26.2 in **8** as a methylene rather than a methine at δ_C 70.8 as in **1**.

Finally, transformation of **1** using resting cells (13) of *P. patulum* in buffered solution produced metabolites **9** and **10** after three days of incubation. Metabolite **9** was identified as 6-*epi*-ophiobolin A (2,14) from spectral data and by comparison with an authentic sample.

A fabms of **10** (molecular ion at *m/z* 416) suggested that a single oxygen atom had been added to the starting material. The ^{1}H- and ^{13}C-nmr spectra of **10** and ophiobolin L [**4**] were very similar (Table 1). The difference of the ^{13}C-nmr chemical shift between C-1 and CH_3-22 indicated there was a trans-A/B ring junction in **10**. A 2D NOESY spectrum did not show nOe effects between H-6 and H-2, and H-6 and CH_3-20, while a nOe was observed between H-6 and H-9α. Thus, **10** was assigned as an epimer of ophiobolin L at C-6 and thus named as 6-*epi*-ophiobolin L.

Previous studies of the biological activities of the ophiobolins showed a variety of activities (4,6,7), inclusive of an antimicrobial spectrum for ophiobolin A [**1**] (6). The antifungal and antimicrobial activities of the naturally occurring ophiobolins and the microbial metabolites were evaluated. Most of the metabolites showed weak to moderate anti-*Cryptococcus* activity and strong activity against *Trichophyton mentagrophytes*. Metabolite **10** had improved in vitro activity against *Staphylococcus aureus* in the agar-well diffusion assay. The antimicrobial results are summarized in Tables 2 and 3.

TABLE 3. Minimum Inhibitory Concentration (MIC) of Ophiobolin A [1] Against Fungi.[a]

Organism	MIC (μg/ml)	
	1	Amphotericin B
Candida albicans	12.5	0.78
Aspergillus flavus	25	3.12
Trichophyton mentagrophytes	12.5	0.78
Torulopsis cremoris	0.20	3.12
Torulopsis petrophilum	1.56	0.20

[a]MIC values are in μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Fisher digital melting point analyzer model 355 or a Thomas Hoover capillary melting point apparatus and were uncorrected. The optical rotation data were collected using a Jasco DIP 370 digital polarimeter. Cc was performed using Si gel (70–270 mesh), and tlc with precoated Si gel 60 F_{254}. Tlc plates were developed in CH_2Cl_2-Me_2CO (9:1) and were visualized under uv light or using 10% H_2SO_4. Ir spectra were recorded in KBr (unless otherwise stated) using a Perkin-Elmer 281 B infrared spectrophotometer. Mass spectral data were collected in the Department of Chemistry, University of Kansas, Lawrence. ^{1}H- and ^{13}C-nmr spectra were obtained on a Varian VXR 300 spectrometer with standard pulse sequences operating at 300 MHz and 75 MHz, respectively. All nmr spectra were recorded in $CDCl_3$, the chemical shift values are reported in ppm units, and the coupling constants are in Hz. Carbon multiplicities were determined by DEPTGL experiments. All ^{13}C-nmr assignments were based on DEPTGL, HETCOR, long-range HETCOR (5 and 10 Hz), and/or comparisons within the compound series. An authentic sample of ophiobolin A [**1**] was purchased from Sigma Chemical Company.

OPHIOBOLIN A [**1**].—Ophiobolin A [**1**] was isolated from *Cochliobolus heterostrophus* (ATCC 22246) using slightly modified conditions as reported previously (7) and was identified using spectroscopic methods and by comparison with an authentic sample. Briefly, the fungus was grown in 4 liters of modified Fries medium (0.4% potato dextrose extract was added) (7) on a Gyrotory shaker (150 rpm, New Brunswick Scientific Co., New Jersey) at room temperature for 12 days. The fermentation broth was separated by filtration. The filtrate and mycelia were extracted with Et_2O. The Et_2O extract was dried on anhydrous Na_2SO_4 overnight. After removal of the Et_2O under reduced pressure, the residue was dissolved in Me_2CO for crystallization. The crude crystals were rinsed with Et_2O. After repeating the crystallization procedure two more times, colorless crystals of **1** were obtained. Typically 800–1200 mg of pure **1** were obtained from 4 liters of culture media, which are considerably better than yields previously reported (100–200 mg per 4 liters) (7). Mp 170–172°; $[\alpha]^{25}$D +265.5° (c=1.0, $CHCl_3$), [lit. (1) mp 182°, $[\alpha]$D +270°]; ir ν max 3500, 1730, 1690, 1660, 1625 cm^{-1}; ^{1}H nmr δ 0.78 (3H, s, Me-22), 1.04 (3H, d, J=7.0 Hz, Me-23), 1.31 (3H, s, Me-20), 1.65 (3H, d, J=1.2 Hz, Me-24), 1.69 (3H, d, J=1.2 Hz, Me-25), 1.28 (1H, dd, J=14.8 and 12.5 Hz, H-1), 1.36 (1H, m, H-12), 1.56 (1H, m, H-16), 2.00 (1H, ddd, J=13.8, 12.5, and 8.7 Hz, H-16), 1.6–1.8 (5H), 2.1 (1H, m, H-15), 2.2 (1H, m, H-9), 2.3 (1H, m, H-2), 2.4 (1H, m, H-9), 2.44 (1H, d, J=19.3 Hz, H-4), 2.74 (1H, d, J=19.3 Hz, H-4), 3.21 (1H, d, J=10.8 Hz, H-6), 3.20 (1H, s, OH), 4.37 (1H, ddd, J=8.7, 8.7, and 6.0 Hz, H-17), 5.10 (1H, d, J=8.7 Hz, H-18), 7.16 (1H, dd, J=8.5 and 8.5 Hz, H-8), 9.18 (1H, s, H-21); ^{13}C-nmr data, see Table 1; fabms m/z 407 [M+Li], 401 [M+1].

OTHER OPHIOBOLINS.—The mother liquor resulting from the crystallization of ophiobolin A [**1**] was subjected to Si gel cc, eluting with Me_2CO in CH_2Cl_2 (7 and 15%). Three other compounds were isolated along with ophiobolin A [**1**] and were identified as 3-anhydroophiobolin A [**2**] (250 mg/4 liters broth), ophiobolin B [**3**] (100 mg/4 liters broth), and ophiobolin L [**4**] (200 mg/4 liters broth).

3-Anhydroophiobolin A [**2**].—Yellow amorphous solid; $[\alpha]^{25}$D +82.3° (c=1.0, $CHCl_3$); ir ($CHCl_3$) ν max 1700, 1687, 1620 cm^{-1}; ^{1}H nmr δ 0.74 (3H, s, Me-22), 1.01 (3H, d, J=7.0 Hz, Me-23), 1.63 (3H, d, J=1.2 Hz, Me-24), 1.69 (3H, s, Me-25), 2.12 (3H, s, Me-20), 3.85 (1H, d, J=6.9 Hz, H-6), 4.43 (1H, ddd, J=6.7, 6.7, and 6.7 Hz, H-17), 5.13 (1H, m, H-18), 6.11 (1H, m, H-4), 7.06 (1H, dd, J=8.5 and 8.5 Hz, H-8); ^{13}C-nmr data, see Table 1. These data are consistent with previously reported data, and an authentic sample was prepared from ophiobolin A [**1**] as described earlier (15).

Ophiobolin B [**3**].—Colorless crystals (Me_2CO), mp 165–167°; $[\alpha]^{25}$D +236.4° (c=1.0, $CHCl_3$), [lit. (14) mp 174°, $[\alpha]$D +273°]; ir ν max 3460, 1720, 1659, 1620 cm^{-1}; ^{1}H nmr δ 0.83 (3H, d, J=7.0 Hz, Me-23), 0.90 (3H, s, Me-22), 1.36 (3H, s, Me-20), 1.62 (3H, s, Me-24), 1.70 (3H, s, Me-25), 2.3 (1H, m, H-9), 2.49 (1H, d, J=19.2 Hz, H-4), 2.53 (1H, m, H-9), 2.80 (1H, d, J=19.2 Hz, H-4), 3.25 (1H, d, J=10.8 Hz, H-6), 5.12 (1H, m, H-18), 7.22 (1H, dd, J=7.2 and 7.2 Hz, H-8), 9.23 (1H, s, H-21); ^{13}C-nmr data, see Table 1. These data are consistent with those previously reported (10,16). Fabms m/z [M+Li] 409, [M+1] 403; hrfabms m/z [M+Li] 409.2954 (calcd for $C_{25}H_{38}O_4Li$, 409.2930), [M+1] 403.2859 (calcd for $C_{25}H_{39}O_4$, 403.2848).

Ophiobolin L (amorphous) [**4**].—$[\alpha]^{25}$D +92.3° (c=0.1, $CHCl_3$); ir ν max 3400, 1732, 1675 cm^{-1}; ^{1}H nmr δ 0.81 (3H, s, Me-22), 1.03 (3H, d, J=6.9 Hz, Me-23), 1.21 (3H, s, Me-20), 1.65 (3H, s, Me-24), 1.70 (3H, s, Me-25), 3.24 (1H, br d, J=9.3 Hz, H-6), 4.40 (1H, ddd, J=9.0, 7.0, and 7.0 Hz, H-17), 5.12 (1H, br d, J=8.9 Hz, H-18), 6.93 (1H, m, H-8); ^{13}C-nmr data, see Table 1; fabms m/z 423 [M+Li]; hrfabms m/z 423.2716 (calcd for $C_{25}H_{36}O_5Li$, 423.2723).

MICROORGANISMS.—Cultures were lyophilized and kept in a deep freezer at the Department of Pharmacognosy, University of Mississippi. Subcultures were maintained on agar slants and stored at 4°.

Cultures were obtained from either American Type Culture Collection, (ATCC, Rockville, Maryland) or Northern Regional Research Laboratories (NRRL, Peoria, Illinois).

MEDIA.—The medium used for microbial transformations was composed of 2% dextrose, 0.5% peptone, 0.5% yeast extract, 0.5% NaCl, and 0.5% K_2HPO_4 in distilled H_2O, and was autoclaved at 121°, 15 psi for 15 min. For the resting cell experiment, 0.05 M pH 7.1 phosphate buffer was used.

TRANSFORMATION AND ISOLATION PROCEDURES WITH *P. CELLULOSUM* (ATCC 29610).—Ophiobolin A (**1**, 280 mg) was dissolved in 4 ml DMSO and distributed evenly among 4 2-liter Erlenmeyer culture flasks, each containing 500 ml of 72-h-old stage I culture. The entire broth was pooled and blended after 6 days, and then extracted with Et_2O (4×500 ml). The organic layer was dried over anhydrous Na_2SO_4 overnight. A dark residue was recovered after removing the solvent under reduced pressure. The residue was subjected to Si gel cc (20 g, 1×20 cm) with 6% Me_2CO/CH_2Cl_2 as eluent. Metabolites **6** (16 mg) and **7** (26 mg) were isolated.

Ophiobolin I (amorphous) [**6**].—$[\alpha]^{25}D$ +48.6° (c=1.0, $CHCl_3$); ir ν max 3450, 1680, 1657, 1613 cm^{-1}; ^1H nmr δ 0.97 (3H, s, Me-22), 1.00 (3H, d, J=7.0 Hz, Me-23), 1.64 (3H, d, J=1.2 Hz, Me-24), 1.68 (3H, d, J=1.2 Hz, Me-25), 2.06 (3H, s, Me-20), 2.7 (1H, m, H-2), 3.64 (1H, d, J=3.0 Hz, H-6), 3.88 (1H, d, J=11.9 Hz, H-21), 4.15 (1H, d, J=11.9 Hz, H-21), 4.56 (1H, ddd, J=6.7, 6.7, and 6.7 Hz, H-17), 5.12 (1H, dq, J=8.7 and 1.2 Hz, H-18), 5.76 (1H, m, H-8), 5.94 (1H, br s, H-4); ^{13}C-nmr data, see Table 1; fabms m/z 391 [M+Li].

Compound **7**.—Amorphous; $[\alpha]^{25}D$ +38° (c=0.1, $CHCl_3$); ir ν max 3460, 1753, 1708 cm^{-1}; ^1H nmr δ 0.83 (3H, s, Me-22), 1.05 (3H, d, J=7.1 Hz, Me-23), 1.24 (3H, s, Me-20), 1.67 (3H, d, J=1.2 Hz, Me-24), 1.71 (3H, d, J=1.2 Hz, Me-25), 1.83 (1H, dd, J=15.0 and 5.5 Hz, H-4), 2.16 (1H, m, H-15), 2.25 (1H, d, J=15.0 Hz, H-4), 2.34 (1H, dd, J=12.0 and 9.0 Hz, H-9), 2.7 (1H, m, H-2), 3.54 (1H, ddd, J=9.8, 7.1, and 2.4 Hz, H-6), 4.42 (1H, ddd, J=8.5, 8.5, and 6.0 Hz, H-17), 4.95 (1H, dd, J=7.0 and 5.6 Hz, H-5), 5.13 (1H, dq, J=8.5 and 1.2 Hz, H-18), 6.95 (1H, ddd, J=8.6, 7.5, and 2.2 Hz, H-8); ^{13}C-nmr data, see Table 1; fabms m/z [M+Li] 407, [M+1] 401; hrfabms m/z [M+Li] 407.2765 (calcd for $C_{25}H_{36}O_4Li$, 407.2774), [M+1] 401.2669 (calcd for $C_{25}H_{37}O_4$, 401.2692).

TRANSFORMATION AND ISOLATION PROCEDURES WITH *P. AERUGINOSA* (ATCC 15442).—Ophiobolin A (**1**, 560 mg) was dissolved in 8 ml DMSO and distributed evenly among 8 2-liter Erlenmeyer culture flasks, each containing 500 ml of 48-h-old stage I culture. The entire broth was pooled after 5 days incubation on a Gyrotory shaker (150 rpm, room temperature), and then extracted with Et_2O (4×800 ml). The organic layer was dried over anhydrous Na_2SO_4 overnight. A dark residue was recovered after removing the solvent under reduced pressure. The residue was subjected to Si gel cc (20 g, 1×20 cm) with 6% Me_2CO/CH_2Cl_2 as eluent, giving 70 mg of metabolite **8**.

Compound **8**.—Mp 178–179° (Me_2CO); $[\alpha]^{25}D$ +14° (c=0.1, $CHCl_3$); ir ν max 3440, 1735, 1725 cm^{-1}; ^1H nmr δ 0.80 (3H, d, J=7.0 Hz, Me-23), 0.90 (3H, s, Me-22), 1.0 (2H, m, H-16), 1.23 (3H, s, Me-20), 1.32 (1H, dd, J=10.4 and 5.0 Hz, H-12), 1.5 (1H, m, H-15), 1.59 (3H, s, Me-24), 1.67 (3H, s, Me-25), 1.79 (1H, dd, J=14.8 and 5.7 Hz, H-4), 1.9 (1H, m, H-10), 2.0 (1H, m, H-2), 2.1 (1H, m, H-9), 2.25 (1H, d, J=14.8 Hz, H-4), 2.47 (1H, dd, J=12.3 and 8.6 Hz, H-9), 3.52 (1H, ddd, J=9.2, 7.0, and 2.2 Hz, H-6), 4.94 (1H, dd, J=7.0 and 5.7 Hz, H-5), 5.12 (1H, ddq, J=7.1, 7.1, and 1.3 Hz, H-18), 6.95 (1H, ddd, J=8.6, 8.6, and 2.2 Hz, H-8); ^{13}C-nmr data, see Table 1; fabms m/z 409 [M+Li], 403 [M+1]; [M+1] hrfabms m/z [M+Li] 409.2925 (calcd for $C_{25}H_{38}O_4Li$, 409.2930), 403.2827 (calcd for $C_{25}H_{39}O_4$, 403.2848).

TRANSFORMATION AND ISOLATION PROCEDURES WITH *P. PATULUM* (ATCC 24550).—Cell mycelia, 72-h-old, grown in 4 liters of medium, were collected and washed with distilled H_2O and phosphate buffer. The cells were blended and resuspended in 2 liters pH 7.1 buffer (4 Erlenmeyer flasks) and ophiobolin A (**1**, 400 mg), dissolved in 6 ml DMSO, was distributed evenly among the culture flasks. The entire solution was pooled and blended after 3 days, and then extracted with Et_2O (4×600 ml). The organic layer was dried over anhydrous Na_2SO_4 overnight. A white solid residue was recovered after removing the solvent under reduced pressure. The residue was subjected to Si gel cc (20 g, 1×20 cm) with Me_2CO/CH_2Cl_2 (6%, 15%) as eluent. Metabolites **9** (13 mg) and **10** (16 mg) were isolated.

6-epi-*Ophiobolin A (amorphous)* [**9**].—^1H nmr δ 0.84 (3H, s, Me-22), 1.03 (3H, d, J=6.9 Hz, Me-23), 1.41 (3H, s, Me-20), 1.66 (3H, d, J=1.2 Hz, Me-24), 1.70 (3H, d, J=1.2 Hz, Me-25), 2.38 (1H, m, H-4), 2.60 (1H, dd, J=13.8 and 4.2 Hz, H-10), 2.77 (1H, m, H-9), 3.04 (1H, d, J=16.8 Hz, H-4), 3.35 (1H, br d, J=10.5 Hz, H-6), 4.60 (1H, ddd, J=9.0, 7.0, and 7.0 Hz, H-17), 5.13 (1H, dq, J=9.0 and 1.5 Hz, H-18), 6.87 (1H, m, H-8); ^{13}C-nmr data, see Table 2.

6-epi-*Ophiobolin L (amorphous)* [**10**].—$[\alpha]^{25}D$ +26° (c=0.4, $CHCl_3$); uv λ max 223 nm; ir ν max 3400,

1740, 1735, 1670, 1610 cm^{-1}; ^1H nmr δ 0.81 (3H, s, Me-22), 1.01 (3H, d, J=6.9 Hz, Me-23), 1.24 (3H, s, Me-20), 1.68 (3H, d, J=1.2 Hz, Me-24), 1.72 (3H, d, J=1.2 Hz, Me-25), 2.07 (1H, d, J=14.9 Hz, H-4), 2.25 (1H, d, J=15.0 Hz, H-4), 3.46 (1H, br d, J=10.5 Hz, H-6), 4.60 (1H, ddd, J=9.0, 7.0, and 7.0 Hz, H-17), 5.22 (1H, br d, J=8.9 Hz, H-18), 6.94 (1H, m, H-8); ^{13}C-nmr data, see Table 1; fabms *m/z* 423 [M+Li]; [M+Li] hrfabms *m/z* 423.2725 (calcd for $C_{25}H_{36}O_5Li$, 423.2723).

QUALITATIVE AND QUANTITATIVE ANTIMICROBIAL EVALUATION.—An agar-well diffusion assay as previously described (17) was used in evaluating the antimicrobial activities of the metabolites against pathogenic fungi and bacteria. Agar plates were streaked with a solution of each 24-h-old test organism cultured in Sabouraud dextrose broth or Eugon broth. Cylindrical plugs were removed from the plates by means of a sterile cork borer to produce wells with a diameter of approximately 10 mm. A solution (100 μl) of each pure compound at a concentration of 1 mg/ml was added to the well. The antimicrobial activities were recorded as the width (in mm) of the inhibition zone measured from the edge of the well to the edge of the inhibition zone following incubation of the plates at 37° for 24 or 48 h. The antimicrobial agents amphotericin B and streptomycin were included as standards in the assay. The MIC values (in μg/ml) were determined as previously described (17).

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