# Ophiobolins G and H: New Fungal Metabolites from a Novel Source, *Aspergillus ustus*

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Two new metabolites, ophiobolin G ($C_{25}H_{34}O_2$) and ophiobolin H ($C_{25}H_{38}O_3$), were isolated from *Aspergillus ustus*. The structure of ophiobolin G was unequivocally established by single-crystal X-ray diffraction studies, whereas that of ophiobolin H was deduced from $^1H$ and $^{13}C$ NMR studies. Both compounds are unusual in that the ring fusion between rings A and B is trans (as opposed to cis in other fungal ophiobolins) and there is a C16–C17 cis double bond. Both inhibited etiolated wheat coleoptile growth at $10^{-3}$, $10^{-4}$, and $10^{-5}$ M. Both inhibited growth of *Bacillus subtilis* cultures, but ophiobolin H was a more potent inhibitor at rates above 250 μg/disk than ophiobolin G. Neither inhibited growth of *Escherichia coli*. Ophiobolin H also induced hyperacusia in day-old chicks at rates up to 375 mg/kg, but there was no mortality.

In 1957, cochliobolin, a substance toxic to rice seedlings, was isolated from *Helminthosporium oryzae* (=*Cochliobolus miyabeanus*, perfect stage) (Orsenigo, 1957). Later, ophiobolin A and related compounds were extracted from *H. oryzae* and other *Helminthosporium* spp. (Ishibashi and Nakamura, 1958; Ishibashi, 1961, 1962a,b), and the complete structure and stereochemistry were reported (Nozoe et al., 1965; Canonica et al., 1966). Cochliobolin and ophiobolin A were found to be identical. Subsequent work led to the isolation of ophiobolin B (Canonica et al., 1966; Nozoe et al., 1966) and ophiobolin C from *Helminthosporium* species. Ophiobolin D (cephalonic acid) was isolated from *Cephalosporium caerulens* (Itai et al., 1967). Ophiobolin E appears not to have been isolated; but ophiobolin F was obtained from *Cochliobolus heterostrophus* (Nozoe et al., 1968).

The occurrence of the ophiobolane structure has not been limited to fungal sources. The wax secreted by the scale insect *Ceroplastes albolineatus* was shown to contain the sesterterpenes ceroplastol, ceroplasteric acid (Iitaka et al., 1968), and albolic acid (Ríos and Gomez G., 1969). Each structure contained the A, B, and C rings of the ophiobolins found in the fungi and are, therefore, very close, differing only minimally structurally in the nature of the substituents on each ring.

Earlier, during our exploration of fungi to find new plant growth inhibiting and phytotoxic substances, we reported the discovery of two new metabolites, pergillin (Cutler et al., 1980) and dihydropergillin (Cutler et al., 1981), from *Aspergillus ustus*. We now report the isolation, identification, and biological activity of two new metabolites, ophiobolin G (I; Figure 1) and ophiobolin H (II; Figure 1), from the identical organism.

## MATERIALS AND METHODS

**Production, Isolation, and Purification of Ophiobolin G and H.** *A. ustus* (Bainier) Thom and Church (ATCC Accession No. 38849) was isolated from greenhouse-produced edible pod pea seed (*Pisum Sativum* var. macrocarpon cv. Oregon sugarpod) grown in Georgia and cultured on potato–dextrose agar for 2 weeks at 26 °C. The organism was then transferred in sterile distilled water to 2.8-L flasks containing shredded wheat medium (Kirksey and Cole, 1974). Cultures were maintained at 26 °C for 12 days and then extracted with acetone. Approximately 300 mL of acetone was added to each flask, the contents were macerated with a Super Dispax homogenizer, and the pulp was placed on Whatman No. 1 filter paper in a Büchner funnel and filtered under vacuum. The filtrate was reduced to the aqueous phase under vacuum at 50 °C, and the latter was twice extracted, each time with an equal volume of ethyl acetate. Ethyl acetate fractions were combined, dried over anhydrous sodium sulfate, and reduced in volume to a viscous liquid under vacuum at 50 °C. This was added to the top of a silica gel (70–230 mesh) chromatography column (9.0 × 10 cm) that had been slurry packed in benzene. Stepwise elution followed with 1.2 L each of benzene, diethyl ether, ethyl acetate, acetone, and acetonitrile. Each solvent drained to the top of the silica before addition of the next solvent. Each fraction was reduced in volume under vacuum, and aliquots were tested for inhibitory properties in the etiolated wheat coleoptile bioassay. Since the benzene and diethyl ether fractions inhibited coleoptile growth, they were combined and added to a silica gel (70–230 mesh) chromatography column (3.5 × 47 cm) slurry packed in benzene. Thereupon, 600 mL of benzene percolated through the column, and this was followed by a linear gradient of benzene to ethyl acetate (1.0 L of benzene and 1.0 L of ethyl acetate). Fractions of 25 mL were collected and 25-μL aliquots were bioassayed. Tubes 32–42 and tubes 54–72 exhibited activity. Each set was composited, reduced in volume, and subjected to further chromatography.

**Ophiobolin G (I) Purification.** The first set (32–42) was subjected to $C_{18}$ reverse-phase open column chromatography (packing obtained from a Waters Associates Prep-Pak 500 $C_{18}$ cartridge) in a 3 × 20 cm column. The $C_{18}$ was packed in acetonitrile–water (1:1 v/v), and the chromatography was effected with the same solvent. Twenty-five-milliliter fractions were collected and 25-μL aliquots were bioassayed. Tubes 3–5, inclusive, contained biologically active material. They were composited, added to a silica gel (70–230 mesh) chromatography column (2.0 × 30 cm) packed in benzene, and eluted with benzene–ethyl acetate (9:1 v/v). Biological activity was found in tubes 10–16. When these were combined and reduced in volume, 680 mg of gellike material resulted. Again, this was added to a chromatographic column (3.0 × 25 cm)

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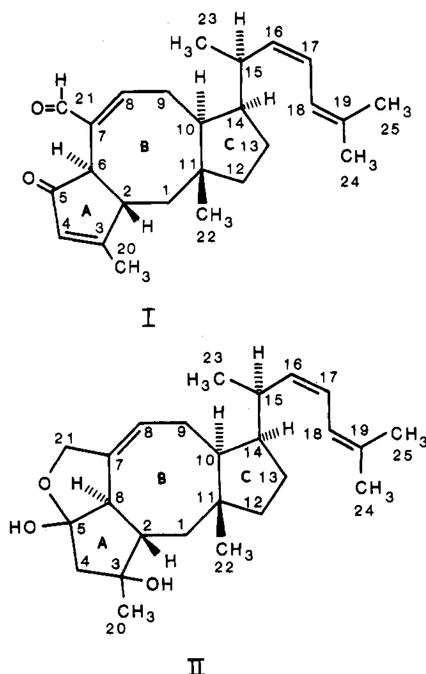

**Figure 1.** Structure of ophiobolin G (I) and ophiobolin H (II).

filled with Florisil (100–200 mesh) packed in benzene. A linear gradient elution from benzene to benzene–ethyl acetate (9:1 v/v) (500 mL of benzene to 500 mL of benzene–ethyl acetate) followed after an initial head of 300 mL of benzene had percolated through the column, and 25-mL fractions were collected. After completion of the elution, 250 mL of acetone was passed through the column, and this, upon reduction in volume, yielded about 0.2 mL of clear, viscous, biologically active material. This was added to a further Florisil column (2.0 × 31 cm) packed with benzene–ethyl acetate (9:1 v/v) and initially treated with 100 mL of this eluent, followed by a gradient of benzene–ethyl acetate (9:1 v/v) to acetone (250 mL of each). The biologically active material was contained in tubes 3–6, which were combined, reduced in volume, and stored at 5 °C, until crystals formed.

**Ophiobolin H (II) Purification.** The second set (54–72, vide supra) was reduced in vacuo to a small volume and placed on an RP-2 (silanized silica gel 60, 70–230 mesh) silica gel chromatography column (3.5 × 30 cm) packed in acetonitrile–water (1:1 v/v), and 800 mL of the solvent mixture was passed through the column during which 25-mL fractions were collected. Aliquots from tubes 3–27 inhibited wheat coleoptile growth. Therefore, these were pooled, reduced in volume, and chromatographed on a $C_{18}$ column (3.5 × 30 cm) developed with acetonitrile–water (1:1 v/v), and 25-mL fractions were collected. Activity was found in tubes 9–20; so these were combined and reduced to a small volume, acetone was added, and the mixture was stored at 5 °C. Within a month, fine crystals formed.

**Physical and Chemical Analyses.** Purification steps were monitored for each metabolite by thin-layer chromatography with silica gel 60, F-254 (E. M. Laboratories, Inc.) plates developed in benzene–ethyl acetate (9:1 v/v). Plates were viewed at 254 nm, then sprayed with anisaldehyde, and heated at 100 °C until the chromogenic responses were complete (Stahl, 1965). Melting points, obtained on a Kofler block, were uncorrected.

Ultraviolet (UV) spectra of I and II were obtained from $10^{-5}$ M solutions in 95% ethanol with a Beckman Model 35 spectrophotometer. Infrared (IR) spectra were obtained with a Beckman IR 4210 spectrophotometer fitted with a 4× beam condenser, and samples were prepared as thin films on KBr windows.

Proton and $^{13}C$ nuclear magnetic resonance spectra were obtained on a Varian Associates XL-300 spectrometer operating at 300 and 75 MHz, respectively. Samples were prepared in 5-mm sample tubes (75 mg/0.5 mL) with $CDCl_3$ as the solvent and $Me_4Si$ as the internal reference. The $^1H$ spectra were run with a 3-kHz sweep width, a 90° pulse, a 4-s delay between pulses, and 16K data points. The free induction decay (FID) was zero-filled to 32K data points before Fourier transformation. Homonuclear spin-decoupling experiments were performed to aid in the assignment of the $^1H$ spectra. Both homonuclear-correlated and homonuclear J two-dimensional NMR experiments (Aue et al., 1976) were run to further aid in the assignment of the chemical shifts.

Conditions for the $^{13}C$ NMR spectra were a 17-kHz sweep width, a 45° pulse angle, a 2-s delay between pulses, and 16K data points. A line broadening of 0.5 Hz was applied to the FID before Fourier transformation. Both APT (Patt and Shoolery, 1982) and DEPT (Pegg et al., 1982) $^{13}C$ spectra were run to determine the carbon multiplicities. A heteronuclear correlated two-dimensional experiment (Bodenhausen and Freeman, 1977; Bax et al., 1980) was run on II to correlate the assignments of the $^1H$ and $^{13}C$ spectra.

Low-resolution (LRP) mass spectra were obtained with a Hewlett-Packard 5985B mass spectrometer. Samples were introduced by direct insertion probe (DIP) for both electron impact (EI) and chemical ionization (CI). The latter used methane as the reagent gas. The temperature program was 20–200 °C at 20 °C/min, the scan rate was 266–267 amu/s, and the mass ranges were 50–500 amu for EI and CI, respectively.

Crystals of I and II were obtained from ethyl acetate and acetone, respectively, for melting point studies. However, crystals of ophiobolin G suitable for single-crystal X-ray were formed from acetone at −15 °C as clear thick rods. Preliminary diffraction experiments indicated that the space group symmetry was $P2_1$, with cell constants of $a$ = 10.829 (4) Å, $b$ = 10.497 (1) Å, $c$ = 11.654 (3) Å, and $\beta$ = 94.21 (3)° for $Z$ = 2. Upon standing for several days, the crystals became translucent, indicating that the crystals were solvated and were losing solvent slowly. Consequently, a fresh crystal used for data collection was covered with a thin coat of epoxy to prevent solvent loss. Of the 1907 reflections measured with an automatic four circle diffractometer with graphite monochromated Cu K$\alpha$ radiation ($\lambda$ = 1.5418 Å), 1617 were observed and corrected for Lorentz, polarization, and absorption effects. A multisolution tangent formula approach to phase solution gave an initial model that was subsequently refined by using Fourier methods and least-squares techniques (Frenz, 1981; Main, 1980; Johnson, 1970). The function minimized was $\sum\omega(|F_o| - |F_c|)^2$ with $\omega = (1/\sigma F_o)^2$.

**Bioassay.** The primary bioassay for detecting the presence of I and II was the etiolated wheat coleoptile (Hancock et al., 1964) with *Triticum aestivum* L., cv Wakeland grown on moist sand in the dark for 4 days at 22 ± 1 °C. Details have been reported (Cutler et al., 1982). Aliquots of 25 $\mu$L from column chromatography eluates were added to each test tube and evaporated to dryness under nitrogen, and 2 mL of phosphate–citrate buffer plus 2% sucrose at pH 5.6 (Nitsch and Nitsch, 1956) was added. Ten coleoptile segments were put into each tube, and tubes were placed in a roller-tube apparatus that rotated at 0.25 rpm for 24 h at 22 °C in the dark. All biological preparation was done under a safelight at 540 nm (Nitsch and

Nitsch, 1956). At completion of the tests, coleoptiles were placed on a glass plate in a photographic enlarger, their images were magnified 3× (Cutler and Vlitos, 1962), and data were collected and statistically analyzed (Kurtz et al., 1965). Purified preparations of I and II were bioassayed in concentrations ranging from $10^{-3}$ to $10^{-6}$ M.

Bean, corn, and tobacco plants were also challenged with both metabolites. Seven-day-old bean seedlings, 7-day-old corn seedlings, and 6-week-old tobacco seedlings, all greenhouse grown, were treated with I and II at $10^{-2}$, $10^{-3}$, and $10^{-4}$ M in aqueous phase. Bean and tobacco plants were sprayed with 1 mL of each metabolite in aerosol. Corn was treated by pipetting 100 μL of each solution into the innermost sheath of the plants. Details of the assay have been described (Cutler et al., 1982).

Bacteriostatic assays were run with *Bacillus subtilis* and *Escherichia coli*. Petri dishes (10 × 1.5 cm) containing Diagnostic Sensitivity Test Agar (Oxoid) were streaked with either *B. subtilis* or *E. coli*, and disks impregnated with I or II were added to the agar surface. Solutions of I and II were made in acetone, added to disks, dried, and then placed in the assay. Plates were incubated 18 h at 37 °C. Controls consisting of solvent-treated disks and standards of erythromycin were included. Treatments ranged from 25 to 1000 μg. Levels of inhibition were recorded (Bailey and Scott, 1962).

One-day-old chicks were used for the vertebrate bioassay. Dosing was by crop intubation. Samples were prepared in corn oil by dissolving I and II in acetone and then mixing with corn oil. Acetone was removed by vacuum distillation at 70 °C until no further acetone distilled; then the collector was emptied, dried, and replaced, and the sample was further distilled for 10 min. All acetone was thus removed and controls were prepared identically (Kirksey and Cole, 1974). Chicks were dosed with 1 mL of corn oil preparation/chick up to a maximum concentration of 375 mg/kg of body weight for both I and II.

## RESULTS AND DISCUSSION

**Physical and Chemical Characteristics.** The amount of each metabolite isolated from 56 flasks of *A. ustus* grown on shredded wheat medium after 12 days was 270 mg of I and 752 mg of II. While we have reported the isolation procedure using activated silica gel chromatography, later studies have shown that hydrated silica gel (5%) affords better separation of the metabolites.

The $R_f$ value for I was 0.24–0.30 when developed in benzene–ethyl acetate (9:1 v/v) on silica gel 60, F-254 plates, and a bright magenta color developed when plates were sprayed with anisaldehyde reagent and heated at 100 °C. Metabolite II had an $R_f$ 0.08–0.12 in the same system, but on treatment with anisaldehyde and heat, a brick-red spot appeared that slowly changed to magenta on further heating. Both I and II appeared as dark slate colored spots at 254 nm. Melting points for I were 131–133 °C: it was noted that solvent of crystallization boiled off at 94–104 °C and the appearance of the crystals changed from rods to clumps of fine needles. Also, the crystals changed from being transparent to translucent. Melting points for II were 125–128 °C.

UV spectra obtained from ethanol solutions were $\lambda_{max}^{EtOH}$ 235 nm (log ε = 4.56) for I and $\lambda_{max}^{EtOH}$ 243 nm (log ε = 4.29) for II. The IR spectrum for I indicated the values 3360 (w, br), 2960, 2930, 2870, 2730 ($CH_3$, $CH_2$), 1705, 1685 (carbonyl), 1648, 1615, 1440, 1412, 1380 ($CH_3$), 1315, 1295, 1175, 1158, 1140, 1102, 1025, 990, 942, 882, 758, 690; for II values were 3400 (br, OH), 2955, 2920, 2865 ($CH_3$, $CH_2$), 1728, 1672, 1442 ($CH_2$), 1370 ($CH_3$), 1290, 1225, 1088, 1052, 1012, 985, 932, 874, 855, 815, 755, 682.

**Table I. Carbon-13 and Proton Chemical Shifts of Ophiobolin G and Ophiobolin H[a]**

| carbon | ophiobolin G (I) | ophiobolin H (II) |
|---|---|---|
| 1 | 35.67 (~1.4, m) | 35.88 (1.35, m) |
| 2 | 46.28 (4.162, m) | 50.69 (2.256, d, t, J = 2.2, 11.8 Hz) |
| 3 | 177.41 | 79.97 |
| 4 | 130.59 (6.059, s) | 50.79 (2.079, 2.150, J = 13.4 Hz) |
| 5 | 207.05 | 116.39 |
| 6 | 48.23 (~4.162, d) | 52.82 (3.128, d, J = 11.1 Hz) |
| 7 | 137.58 | 138.89 |
| 8 | 160.34 (7.073, m) | 122.97 (5.598, m) |
| 9 | 27.77 (2.530, 1.67, m) | 24.86 (2.487, 1.694, m) |
| 10 | 48.78 (~1.4, m) | 54.97 (1.694, m) |
| 11 | 45.99 | 43.39 |
| 12 | 40.22 (~1.4, m) | 42.89 (~1.3, 1.5, m) |
| 13 | 29.59 (~1.7, m) | 26.56 (~1.7, ~1.5, m) |
| 14 | 48.23 (~2.07, m) | 47.05 (2.042, m) |
| 15 | 35.30 (2.530, m) | 35.39 (2.690, m) |
| 16 | 120.12 (5.987, m) | 120.27 (5.985, m) |
| 17 | 137.33 (5.122, m) | 137.75 (5.193, m) |
| 18 | 122.28 (5.975, m) | 121.62 (5.973, m) |
| 19 | 135.62 | 134.89 |
| 20 | 24.42 (2.217, s) | 25.49 (1.209, s) |
| 21 | 194.91 (9.388, s) | 76.57 (4.453, 4.567, J = 12.8 Hz) |
| 22 | 18.64 (0.791) | 18.65 (0.892, s) |
| 23 | 20.58 (0.871, d, J = 6.6 Hz) | 20.15 (0.874, d, J = 6.6 Hz) |
| 24 | 18.09 (1.744, s) | 18.01 (1.723, s) |
| 25 | 26.43 (1.828, s) | 26.36 (1.801, s) |

[a] In ppm downfield from $Me_4Si$. Proton shifts and coupling constants are in parentheses.

Proton and $^{13}C$ NMR were consistent for the structure of I obtained by X-ray crystallography. However, II did not give crystals suitable for X-ray analysis, and the structure was determined by NMR studies and comparison with I.

The $^{1}H$ and $^{13}C$ NMR chemical shifts of I are given in Table I. Assignment of the $^{1}H$ spectrum was accomplished by comparison with previously reported data for ophiobolins (Nozoe et al., 1965, 1966, 1968; Canonica et al., 1966) and from homonuclear decoupling experiments. The assignment of the $^{13}C$ NMR spectrum was aided by APT and DEPT experiments to determine carbon multiplicities, comparison with previously reported $^{13}C$ NMR data for ophiobolins A–D (Radics et al., 1975), and comparison with the assignment of ophiobolin H (vide infra).

The $^{1}H$ and $^{13}C$ NMR spectra of II showed many similarities with those of I, as well as some obvious differences. The $^{1}H$ spectrum indicated the presence of five methyl groups. However, the chemical shifts indicated the absence of a double bond between C3 and C4. This was further confirmed by the presence of an isolated AB pattern at 2.08 and 2.15 ppm (J = 13.4 Hz), indicating a similarity of this part of the molecule to that of ophiobolin C (Nozoe et al., 1966). The aldehyde group on C21 in I was absent in the spectrum of II. A broad AB pattern at 4.45 and 4.57 ppm (J = 12.8 Hz) was present in the spectrum of II. These chemical shifts were similar to those reported for a hemiketal derivative of an ophiobolin reported previously (Nozoe et al., 1967). Extensive homonuclear decoupling experiments and a homonuclear correlated two-dimensional spectrum were run on II to determine the coupling constant connectivities.

The $^{13}C$ spectrum of II indicated the presence of 25 carbons. A DEPT spectrum gave the multiplicities as five methyls, six methylenes, nine methines, and five quaternary carbons and accounted for thirty-six hydrogens. From the chemical shifts it was clear that the aldehyde at C21

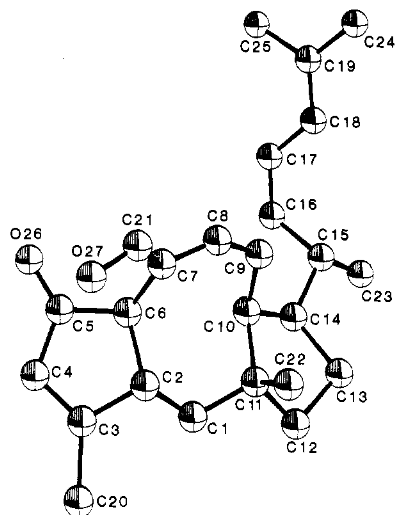


**Figure 2.** A perspective drawing of ophiobolin G (I) with hydrogens omitted for clarity. The absolute configuration was not determined but has been drawn to coincide with the other ophiobolins.

and ketone at C5 in I were not present in II. There were three double bonds indicated for II with an additional downfield peak whose chemical shift (116.39 ppm) was characteristic of a carbon with two oxygens and two carbons attached. Two additional peaks, a methylene and quaternary at 76.57 and 79.97 ppm, were in the correct chemical shift range for carbons with one oxygen attached.

From comparison of all the spectroscopic data of II with those of I and the previously reported ophiobolins, the structure given in Figure 1 is suggested for II. The NMR data are given in Table I. A heteronuclear correlated two-dimensional $^{13}C$ NMR spectrum was run to correlate the $^{13}C$ NMR shifts with the $^{1}H$ chemical shifts. The quaternary carbons were assigned to be consistent with the data for other ophiobolins.

Mass spectral analyses by EI for I gave a molecular ion peak ($M^+$) at $m/z$ 366 and a computer-generated formula $C_{25}H_{34}O_2$ with prominent ion fragment peaks at $m/z$ 348 $(M - H_2O)^+$ and 337 $(M - CHO)^+$ and a base peak at 109. The CI gave an M + 1 at $m/z$ 367. For II, the EI data showed a molecular ion peak ($M^+$) at $m/z$ 386 and formula $C_{25}H_{38}O_3$ with ion fragments at $m/z$ 368 $(M - H_2O)^+$, 353 $(M - H_2O - CH_3)^+$ 350 $(M - 2H_2O)^+$, 335 $(M - 2H_2O - CH_3)^+$, 259, and 241 and a base peak at 109. The CI gave an M − 1 at $m/z$ 385, with fragments at $m/z$ 369 and 351 and a base peak at 109.

X-ray diffraction studies of I indicated that a badly disordered molecule of acetone was present in the crystal lattice that was modeled by eight positions with occupancies of 0.5. Positions were found for all hydrogens except for those on C25, C24, and C20. The final unweighted R factor was 0.079. Tables containing the final fractional coordinates, bond distances, and bond angles may be found in the supplementary material (see paragraph at end of paper regarding supplementary material). Figure 2 is a computer-generated drawing of I calculated from the X-ray experiments. Thus, I is a sesterterpene with an identical carbon skeleton to that of all the other ophiobolins. However, the structure of I differs in several respects from the other members of the family. For instance, in all previously described fungal ophiobolins that have a saturated junction between rings A and B the fusion is cis. In contrast, I has a trans junction, and NMR studies imply that II has a similar configuration. Also, I and consequently II are the only members having a double bond between C16 and C17, and that double bond is cis.

**Table II. Inhibitory Effect of Ophiobolins G and H on *B. subtilis* in Vitro**[a]

| compd | inhibition zone (diameter), mm,[b] for concentration, μg/disk | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1000 | 750 | 500 | 350 | 250 | 100 | 75 | 50 | 25 |
| ophiobolin G | 12 | 12 | 12 | 13 | 11 | 10 | 11 | 10 | 0 |
| ophiobolin H | 20 | 30 | 30 | 14 | 16 | 0 | 0 | 0 | 0 |

[a] Minimum of duplicate assays. [b] Inhibition >15 mm. Moderate inhibition 10–15 mm. No inhibition <10 mm. Standard: erythromycin 15 μg; inhibition 33 mm.

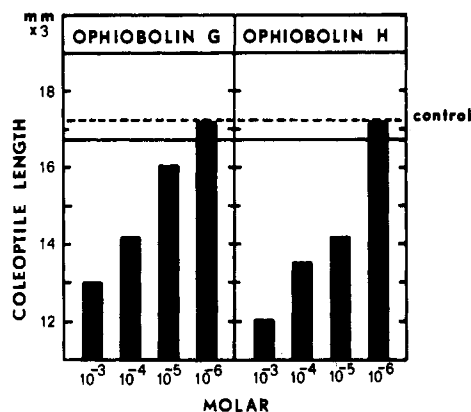


**Figure 3.** Effects of ophiobolin G (I) and ophiobolin H (II) on the growth of wheat (*T. aestivum* L., cv Wakeland) coleoptiles. Significant inhibition ($P < 0.01$): below solid line. Control: broken line.

**Bioassays.** Etiolated wheat coleoptile growth was significantly inhibited ($P < 0.01$) by solutions of I and II at $10^{-3}$, $10^{-4}$, and $10^{-5}$ M (Figure 3). Specifically, I inhibited coleoptiles 81, 53, and 23% and II 99, 70, and 58% relative to controls at $10^{-3}$, $10^{-4}$, and $10^{-5}$ M, respectively. Thus, of the two structures the hemiacetal II was more inhibitory than the ketoaldehyde I. Bean, corn, and tobacco plants were not visibly affected by treatment with I at $10^{-2}$, $10^{-3}$, and $10^{-4}$ M. However, II caused complete necrosis of the innermost corn leaf in each plant treated at $10^{-2}$ M within 5 days, but all other leaves appeared normal. Bean and tobacco plants were unaffected by II.

*B. subtilis* growth was inhibited by both I and II. However, I inhibited *B. subtilis* moderately at various concentrations from 1000 down to 50 μg/disk and II inhibited strongly at the higher concentrations but the end point of activity was reached at 250 μg/disk (Table II). The inhibition zones for II were irregular and indicated a diffusion or pH effect in the media. Zones for I were uniformly circular. Neither compound inhibited *E. coli*.

Metabolite I induced no visible effects in day-old chicks at concentrations up to 375 mg/kg. However, within 1 h of dosing with II at 375 mg/kg, chicks refused feed and were extremely nervous for 8 h. They exhibited symptoms of hyperacusia, and it would appear, therefore, that II increased the irritability of the sensory neural mechanism. The threshold dosage was ca. 250 mg/kg for induction of the response. There was no mortality and chicks had fully recovered in 24 h.

From analyses of Drieding models it is implied that the C5 OH is situated cis to the C6 proton and, therefore, trans to the C2 proton in II. However, the stereochemistry at C3 is uncertain at this time, but previous reports (Nozoe et al., 1966) would suggest that the C3 OH is cis to the C2 proton and consequently the methyl group is trans in II.

Further points need to be resolved. First, do other species of *Aspergillus* contain these unique ophiobolins, and do they also occur in other fungi? Second, what is the

biological role of these compounds? Third, what is the optimum incubation time, temperature, and medium for the production of these metabolites?

**Registry No.** I, 90108-63-7; II, 90108-64-8.

**Supplementary Material Available:** Fractional coordinates and temperature factors (Table I), bond distances (Table II), and bond angles (Table III) (4 pages). Ordering information is given on any current masthead page.

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# Thermal Degradation of Sodium Dodecyl Sulfate

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Pyrolysis at 700 °C of a technical grade of sodium dodecyl sulfate that contained an alkyl group distribution of 56.7% dodecyl, 37.5% tetradecyl, and 5.8% hexadecyl resulted in a 65% weight loss and produced 47% of condensable material and 18% of noncondensable gases (largely $SO_2$). The condensable products consisted of the corresponding isomeric alkenes, primary alcohols, and ethers. A substantial amount of the mixed ether, dodecyl tetradecyl ether, was formed along with didodecyl and ditetradecyl ethers. In a cytotoxicity screening procedure using cultured Hamster embryo cells, some of the condensable pyrolyzate components were found to be more toxic than aniline.

Sodium dodecyl sulfate, a widely used surfactant, may be found in the chemical residues on certain crops as a result of the application of agricultural chemical formulations. In the case of tobacco crops, these residues will be subjected to high temperatures during the use of the tobacco (smoking) and may be expected to undergo pyrodegradation. In related work we have found that the nature of the "inert" ingredient in commercial formulations of agricultural chemicals can make a significant difference in the cell toxicity of the pyrolyzate obtained on thermolysis of the formulation. The thermal decomposition of sodium dodecyl sulfate (SDS) and homologues has been investigated extensively under a variety of conditions. Aqueous solutions of SDS are converted completely to the corresponding alcohols at 270 °C in a GC injection port (Malin and Chapoteau, 1981) while pyrolysis alone at 650 °C is reported to produce 1-alkenes and the corresponding alcohols (Liddicoet and Smithson, 1965). When the SDS is mixed with phosphorus pentoxide and pyrolyzed at 400 °C, only 1-alkenes and internal alkenes of the same carbon number are obtained (Lew, 1967). The pyrolysis of sodium octyl sulfate in mixture with KOH at 400 °C leads to isomeric alkenes, alcohols, and ethers (Nakagawa, 1968), and it is stated without details that the same kinds of

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