tase.[7,8] Thus, $O_2^{\bullet-}$ may act as an activating agent for the S-oxygenation of ethionamide in vivo. Replacement of the carbonyl oxygen of amides by a sulfur atom enhances the reactivity of $O_2^{\bullet-}$ by at least a factor of 1000.

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Some New Phytotoxic Ophiobolins Produced by *Drechslera oryzae*

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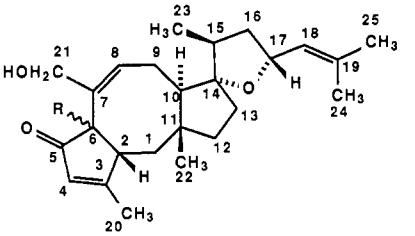
Several previously unreported phytotoxic compounds were isolated from the fungus *Drechslera oryzae*, a plant pathogen of rice. The compounds were characterized as 6-epiophiobolin I (**1**), ophiobolin J (**3**), 8-deoxyophiobolin J (**4**) by spectroscopic analyses and comparisons with recently determined ophiobolin I (**2**).

A plant pathogenic fungus, *Drechslera oryzae* (*Helminthosporium oryzae*) causes brown leaf spot disease on rice.[1] The first work on the phytotoxins of this organism was reported in the mid 1960's by Nozoe and co-workers,[2,3] who elucidated the structures of ophiobolin A and B. As a consequence of our recent findings of new ophiobolins from *D. maydis* and *D. sorghicola*,[4] we undertook a more detailed investigation of the phytotoxic metabolites of *D. oryzae* because the sexual stages of all of these fungi are genetically compatible and they are likely to produce the same or related compounds. Six ophiobolins (A, 6-epi A, B, I, 25-hydroxy I, and 3-anhydro-6-epi A)[5] were characterized from culture broth of *D. oryzae* and identified with 400-MHz ^{1}H NMR and high-resolution mass spectra (HRMS). However, three previously unreported ophiobolins were isolated from this fungus and their structures were characterized with spectroscopic methods, which is the subject of this report.

Our earlier work had defined the structure of ophiobolin I (**2**) by single-crystal X-ray diffraction methods,[4] and it serves as a convenient starting point for the characterization of the new ophiobolins. The chromatographic behavior of **1** was very similar to that of **2**. Its molecular formula was determined by HRMS as $C_{25}H_{36}O_4$ and its fragmentation pattern closely resembled that of **2**. The ^{1}H NMR showed a doublet at δ 3.55 (J = 6.5 Hz) assigned for H6 and a multiplet at δ 3.17 for H2 and these were consistent with cis ring fusion, whereas a coupling constant



1: R = Hα

2: R = Hβ

of J = 2.6 Hz exists in the case of **2** which is trans ring fused. A doublet shifted to low field at δ 6.04 (J = 8.1 Hz) for H8 and a doublet of H6 indicated the position of a double bond on C7. These combined with the other signals led to the assignment of **1** as the 6-epimer of **2**. An exposure of **1** in methanol solution with 1 N HCl[6] afforded a single product which was identified as **2** by chromatographic analyses (HPLC and TLC), ^{1}H NMR, and HRMS. These results implied the stereochemical structure of **1** to be the same as to that of **2** on C2, C10, C11, C14, C15, and C17.

Ophiobolin J **1** has a molecular formula of $C_{25}H_{36}O_4$ as determined by HRMS. The 400-MHz ^{1}H NMR of **3** was similar to that of **2**, but there were differences primarily in the A–B ring fusion region. The olefinic proton H8 at δ 5.78 and the bridgehead proton H6 at δ 3.67 of **2** were replaced by a signal at δ 4.68 in **3**. The bridgehead proton at C2 of **3** appeared at δ 4.04 (broad doublet, J = 11.2 Hz) rather than a multiplet at δ 2.53 as in **2**. These changes suggest that the double bond moved to C6 from C7 also

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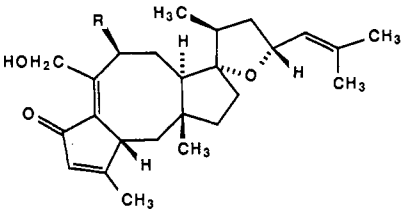
Table I. Assignments of Two- and Three-Bond Couplings in the Long-Range ^1H–^{13}C COSY (500 MHz) Experiments

obsd ^1H (chem shifts, δ)	two-bond coupling with C (Hz)	three-bond coupling with C (Hz)
H20 (2.10)	C3 (177.5)	C4 (131.2), C2 (44.3)
H21 (4.52 and 4.41)	C7 (149.1)	C6 (138.5), C8 (74.8)
H22 (1.16)	C11 (41.6)	C1 (49.8), C12 (41.3)
H23 (1.03)	C15 (36.0)	C14 (95.9), C16 (42.3)
H24 and H25 (1.64 and 1.70)	C19 (134.5)	C18 (126.8)

placing an oxygen substituent at C8. This was fully verified by additional NMR analysis.



3 : R = OH

4 : R = H

The 500-MHz 1H NMR of **3** showed signals for an olefinic primary alcohol at δ 4.52 and 4.41 (H21), olefinic protons at δ 6.02 (H4) and 5.14 (H18), and a tetrahydrofuran ring proton (H17) at δ 4.48. In the ^1H–^1H COSY spectrum,[7] an allylic coupling was observed between H4 and H20, H18 and H25, respectively. The signal at δ 4.68 due to H8 showed cross peaks with methylene groups (δ 2.13 and 1.87). A signal at δ 4.48 (H17) had correlations with both geminal protons attached to C16 (δ 1.70 and 1.76) and an olefinic proton of H18 (δ 5.14). The cross peaks of a geminal methylene protons at δ 1.92 and 1.08 with δ 4.04 (H2) were interpreted as the H1–H2 interactions. A DEPTGL[8] spectrum of the 125-MHz ^{13}C NMR of **3** revealed five methyl, six methylene, and seven methine carbon signals among the 25 carbons. The ^1H–^{13}C COSY[9] spectrum clarified the carbon signal assignments. Methyl and methylene carbons were assigned as follows: five methyl carbons at δ 25.8 (C24), 21.7 (C20), 18.1 (C25), 17.3 (C21), 16.2 (C23); six methylene carbons at δ 62.4 (C22), 49.8 (C1), 42.3 (C16), 41.3 (C12), 30.8 (C9), 30.6 (C13). The remaining carbons were assigned by a long-range ^1H–^{13}C COSY[10] experiment (Table I). The three bond couplings of H20 at δ 2.10 with the methine carbon at δ 44.3 and H22 at δ 1.16 with δ 52.4 were assigned as C2 and C10, respectively. Also the correlation of H21 at δ 4.52 and 4.41 with δ 74.8 (C8) could be distinguished from a similar signal at δ 71.6 (C17). Six olefinic carbons were identified in this fashion as C7 (δ 149.1), C6 (δ 138.5), C19 (δ 134.5), C4 (δ 131.2), C18 (δ 126.8), and C14 (δ 95.9) (Table I). The presence of two hydroxyl groups was confirmed by derivatization of **3** with *p*-bromobenzoyl chloride in dry pyridine to give a bis(*p*-bromobenzoate). The stereochemistry at C8 was also implied after comparing it to the coupling constant of C8 of ophiobolin D whose structure had been determined by single-crystal X-ray diffraction methods[11] and shown to have an allylic β-hydroxyl group at C8. Although the gross stereochemistry of **3** could not be clearly elucidated by NMR experiments, there is no reason for us to believe that it is different than ophiobolin D because it also is produced by *D. oryzae*. Furthermore, other ophiobolins from this source, including crystalline A and I (**2**) were determined by X-ray[4,11] analyses and their NMR spectra are related to **3**.

The molecular formula of the less polar deoxyophiobolin J **4** was determined by HRMS as $C_{25}H_{36}O_3$—losing one oxygen from J **4**. The ^1H NMR spectrum closely resembled that of **3**, however, a signal at δ 4.68 (H8) of **3** was not present in **4** and H2 resonated at δ 3.17 with a large coupling constant (J = 12.0 Hz) similar to that of **3**. These spectral data were fully consistent with the structure of **4** as 8-deoxyophiobolin J including its relative stereochemistry.

Generally, these new ophiobolins cause yellowish to reddish lesions on rice cultivars in a nonselective manner at 10^{-4} M. They also effectively reduce, by 50%, dark CO_2 fixation in rice leaf pieces in the range of 10^{-4}–10^{-5} M.[4] It is possible that these newly found compounds might play an important role in the development of disease symptoms by this fungus when it attacks rice.[5]

Experimental Section

^1H NMR 1D spectra were recorded with a JEOL GX-400 and Bruker WM-250 NMR spectrometers. ^{13}C NMR spectra and all 2D experiments were performed on a Varian VXR-500 NMR spectrometer. Chemical shifts were taken in δ units to the residual $CHCl_3$ in the $CDCl_3$ (δ relative to TMS).

The homonuclear proton chemical shift correlation 2D (COSY) experiment used 512 separate values of the evolution period with six transients per increment (three-transient phase-cycling), acquired for 0.218 s per transient using a 0.6-s relaxation period. The data were collected at a controlled temperature of 20.0 °C. The data were processed in the absolute value mode as a 2048 × 2048 matrix with squared sine-bell weighting (maximum at 0.068 s in t2 and 0.054 s in t1) with symmetrization about the diagonal following the 2D transform. The total experiment time was 65 min.

The long-range heteronuclear $^{13}C/^1H$ 2D chemical shift correlation experiment used 256 200-transient increments with a 0.272-s acquisition time per fid and a 0.6-s relaxation delay at 20.0 °C. The data were processed in the absolute value mode as a 8192 × 512 matrix with Gaussian line-broadening (time constant 0.145 s) in t2 and a combination of Gaussian (time constant 0.32 s) and sine-bell broadening (time constant 0.106 s, shifter negatively by 0.098 s in t). Total acquisition time was 13.2 h.

Data were processed using the VXR-500 data system employing a 3/160 Sun Microsystems computer and plotted with a Hewlett-Packard LaserJet printer.

Mass spectra were obtained from an Hitachi M-80 mass spectrometer and VG Analytical Model VG 7070E mass spectrometers. Optical rotations were obtained on a Perkin-Elmer Model 241MC polarimeter. R_f value were determined with Kieselgel 60 F_{254} HPTLC glass plates (E. Merck, Darmstadt, West Germany) in (A) $CHCl_3$/MeOH (14:1) and (B) CH_3Ph/AcOEt (1:1).

Toxin Production and Purification. The mycelium of *D. oryzae* was inoculated into modified M-1-D culture broth[4] and then shaken at 200 rpm for 3 weeks at 26 °C under luminescence. The culture filtrate obtained after filtration of culture broth was extracted with EtOAc, and the extracts were evaporated to dryness under reduced pressure and subjected to flash column chromatography[12] using $CHCl_3$/MeOH (25:1) and CH_3Ph/EtOAc (2:1).

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Purities of toxins were checked on HPLC by reverse-phase Lichrosorb RP-18 (4 × 250 mm) (E. Merck) in CH_3CN/H_2O (65:35) detected by UV (254 nm). Ophiobolin A and 6-epi A were identified by NMR and MS spectra.

Toxin Bioassays. A leaf puncture bioassay test similar to that previously reported for corn and sorghum was conducted on rice cultivars S-201, Samnam, Nakdong, Aichi-asahi, ES-18, and IR-8.[4]

6-Epiophiobolin I (1): colorless oil (0.5 mg/8 L); R_f 0.50 (A) and 0.21 (B); $[\alpha]_D$ −4° (*c* 0.4, $CHCl_3$); EILRMS, *m/z* (relative intensity) 384 (21), 366 (85), 266 (35), 257 (31), 199 (27), 165 (90), 109 (100); EIHRMS, $C_{25}H_{36}O_3$ (M^+; obsd *m/z* 384.2665, calcd *m/z* 384.2666) and $C_{25}H_{34}O_2$ ($M^+ - H_2O$; obsd *m/z* 366.2559, calcd *m/z* 366.2560); ^1H NMR (250 MHz) δ 6.04 (1 H, d, *J* = 8.1 Hz, H8), 5.99 (1 H, s, H4), 5.09 (1 H, d, d, *J* = 1.2, 9.9 Hz, H18), 4.40 (1 H, d, d, d, *J* = 5.5, 8.8, 8.8 Hz, H17), 3.87 (2 H, s, H21), 3.55 (1 H, d, *J* = 6.5 Hz, H6), 3.17 (1 H, m, H2), 2.05 (3 H, s, H20), 1.70 (3 H, s, H24), 1.67 (3 H, s, H25), 1.04 (3 H, d, *J* = 7.1Hz, H23), 0.79 (3 H, s, H22).

Identification of ophiobolin I (2): R_f 0.47 (A) and 0.34 (B); EIHRMS, $C_{25}H_{36}O_3$ (M^+; obsd *m/z* 384.2665, calcd 384.2666) and $C_{25}H_{34}O_2$ ($M^+ - H_2O$; obsd *m/z* 366.2559, calcd 366.2560); ^1H NMR (250 MHz) δ 5.93 (1 H, s, H4), 5.76 (1 H, d, *J* = 4.1 Hz, H8), 5.11 (1 H, d, *J* = 8.7 Hz, H18), 4.55 (1 H, d, d, *J* = 7.3, 15.6 Hz, H17), 4.13 (1 H, d, *J* = 11.8 Hz, H21), 3.88 (1 H, d, *J* = 11.8 Hz, H21), 3.64 (1 H, d, *J* = 2.6 Hz, H6), 2.50 (1 H, d, d, *J* = 2.6, 14.6 Hz, H2), 2.19 (1 H, q, d, *J* = 7.0, 13.4 Hz, H15), 2.05 (3 H, s, H20), 1.67 (3 H, s, H24), 1.63 (3 H, s, H25), 0.99 (3 H, d, *J* = 7.0 Hz, H23), 0.96 (3 H, s, H22).

Acid Treatment of 1. A solution of 1 (1 mg) in methanol (1 mL) was added to the solution of 1 N HCl (1 mL) in methanol and then placed at room temperature for 18 h. The reaction mixture was poured into aqueous $NaHCO_3$ and then extracted with ethyl acetate. After evaporation of the organic layer under nitrogen, the residue was purified with preparative TLC ($CHCl_3$/MeOH, 14:1) to afford a single product. It was identified as ophiobolin I (2) with ^1H NMR, HRMS, TLC, and HPLC.

Ophiobolin J (3): colorless oil (20 mg/8 L); R_f 0.29 (A) and 3.31 (B); $[\alpha]_D$ +48° (*c* 1.7 $CHCl_3$); EILRMS, *m/Z* (relative intensity) 400 (6), 382 (27), 364 (10), 356 (22), 300 (24), 283 (40), 282 (31), 255 (22), 191 (33), 165 (8c), 110 (63), 109 (100); EIHRMS, $C_{25}H_{36}O_4$ (M^+; obsd *m/z* 400.2611, calcd 400.2614) and $C_{25}H_{34}O_3$ ($M^+ - H_2O$; obsd *m/z* 382.2502, calcd *m/z* 382.2509); ^1H NMR (400 MHz) δ 6.02 (1 H, t, *J* = 1.2 Hz, H4), 5.14 (1 H, d, d, d, *J* = 1.2, 1.5, 8.8 Hz, H18), 4.68 (1 H, d, d, *J* = 3.8, 6.2 Hz, H8), 4.52 (1 H, d, *J* = 13.9 Hz, H21), 4.51 (1 H, d, d, *J* = 8.8, 15.6 Hz, H17), 4.41 (1 H, d, *J* = 13.9 Hz, H21), 4.04 (1 H, br d, *J* = 11.2 Hz, H2), 2.10 (3 H, d, *J* = 0.5 Hz, H20), 1.70 (3 H, d, *J* = 1.2 Hz, H24), 1.64 (3 H, d, *J* = 1.2 Hz, H25), 1.16 (3 H, s, H22), 1.03 (3 H, d, *J* = 6.8 Hz, H23); ^{13}C NMR (125 MHz) δ 202.3 (s, C5), 177.5 (s, C3), 149.1 (s, C7), 138.5 (s, C6), 134.5 (s, C19), 131.2 (d, C4), 126.8 (d, C18), 95.9 (s, C14), 74.8 (d, C8), 71.6 (d, C17), 62.4 (t, C21), 52.4 (d, C10), 49.8 (t, C1), 44.3 (d, C2), 42.3 (t, C16), 41.6 (s, C11), 41.3 (t, C12), 36.0 (d, C15), 30.8 (t, C9), 30.6 (t, C13), 25.8 (q, C24), 21.7 (q, C22), 18.1 (q, C25), 17.3 (q, C20), 16.2 (q, C23).

8-Deoxyophiobolin J (4): colorless needle crystal (1.2 mg/8 L); mp 138–140 °C; R_f 0.56 (A) and 0.49 (B); $[\alpha]_D$ +8° (*c* 0.15, $CHCl_3$); EILRMS, *m/z* (relative intensity) 384 (18), 366 (27), 302 (15), 284 (30), 266 (19), 201 (39), 199 (30), 165 (83), 109 (100); EIHRMS, $C_{25}H_{36}O_3$ (M^+; obsd *m/z* 384.2663, calcd *m/z* 384.2666) and $C_{25}H_{34}O_4$ ($M^+ - H_2O$; obsd *m/z* 366.2558, calcd *m/z* 366.2560); ^1H NMR (250 MHz) δ 6.00 (1 H, s, H4), 5.65 (1 H, d, *J* = 9.0 Hz, H18), 4.52 (1 H, d, d, *J* = 7.4, 15.7 Hz, H17), 4.36 (1 H, d, *J* = 13.0 Hz, H21), 4.31 (1 H, d, *J* = 13.0 Hz, H21), 3.17 (1 H, d, *J* = 12.0 Hz, H2), 2.07 (3 H, s, H20), 1.66 (3 H, s, H24), 1.60 (3 H, s, H25), 1.06 (3 H, s, H22), 0.99 (3 H, d, *J* = 6.9 Hz, H23).

Bis(*p*-bromobenzoate) of 3: R_f 0.75 ($CHCl_3$/MeOH, 50:1); ^1H NMR (250 MHz) δ 7.91 (1 H, d, *J* = 8.3 Hz, Ar H), 7.80 (1 H, d, *J* = 8.3 Hz, Ar H), 7.56 (1 H, d, *J* = 8.3 Hz, Ar H), 7.51 (1 H, *J* = 8.3 Hz, Ar H), 6.00 (1 H, s, H4), 5.95 (1 H, d, *J* = 5.8 Hz, H8), 5.73 (1 H, d, *J* = 13.4 Hz, H21), 5.65 (1 H, d, *J* = 13.4 Hz, H21), 5.04 (1 H, d, *J* = 8.5 Hz, H18), 4.51 (1 H, d, d, *J* = 7.2, 15.0 Hz, H17), 3.74 (1 H, d, *J* = 10.9 Hz, H2), 2.02 (3 H, s, H20), 1.57 (3 H, s, H24), 1.53 (3 H, s, H25), 1.19 (3 H, s, H22), 1.02 (3 H, d, *J* = 6.8 Hz, H23).

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Stereochemistry of the *N*-Methyl Group in Salts of Tropane Alkaloids

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^1H and ^{13}C NMR slow exchange limit spectra of atropine sulfate/mesylate, homatropine hydrobromide, and benztropine mesylate solutions concur in showing that, at equilibrium, the equatorial:axial *N*-CH_3 diastereomeric mixture was ca. 7:1 (D_2O) and ca. 18:1 (CD_2Cl_2). A similar preponderance of equatorial *N*-CH_3 stereochemistry was observed for cocaine salts in both solvents (only equatorial isomer noted in D_2O ^{13}C NMR spectrum, while ca. 18:1 equatorial:axial ratio found in CD_2Cl_2). The *N*-CH_3 orientation in scopolamine hydrobromide was strikingly solvent sensitive and underwent a reversal from an equatorial:axial *N*-CH_3 ratio of ca. 1:18 (D_2O) to ca. 18:1 (CD_2Cl_2). Solid-state CP-MAS ^{13}C NMR spectra of crystalline equatorial *N*-CH_3 (8*s*)-atropine sulfate and axial *N*-CH_3 (9*r*)-scopolamine hydrobromide confirmed the stereochemical assignments for major and minor diastereomers in solution.

Introduction

Atropine [*dl*-hyoscyamine[3] (1)], scopolamine [*l*-hyoscine[4] (2)], and homatropine[5] (*dl*-3) are antimuscarinic (or atropinic) drugs that inhibit the action of acetylcholine on structures innervated by postganglionic parasympathetic nerves.[6] *l*-Hyoscyamine (1) and *l*-hyoscine (2) are natural products isolated from belladonna plants [*atropa bella-*

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