Identification of an Aliphatic Epoxide and the Corresponding Dihydrodiol as Novel Congeners of Zearalenone in Cultures of *Fusarium graminearum*

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The mycotoxin zearalenone (ZEN) is produced by various *Fusarium* fungi and frequently found as a contaminant in food and feed. There are reports in the literature that several closely related analogues of ZEN are also formed in cultures of *Fusarium* species. We have therefore analyzed the organic extract from a 40 day culture of *Fusarium graminearum* by LC-DAD-MS and detected 15 compounds, which could be congeners of ZEN because of their ultraviolet, mass spectroscopy, and tandem mass spectroscopy spectra. In addition to confirming the previously reported α- and β-stereoisomers of 5-hydroxy-ZEN and 10-hydroxy-ZEN, we identified seven ZEN congeners for the first time. One of the major novel congeners was shown by nuclear magnetic resonance spectroscopy and chemical synthesis to have the structure of an aliphatic ZEN epoxide, whereas two minor products proved to be the corresponding dihydrodiols. In addition, three stereoisomers of a cyclization product of the dihydrodiols, carrying a spiro-acetal group, were identified as fungal products for the first time. The latter may be artifacts, because the ZEN epoxide and dihydrodiol are unstable under acidic conditions and rearrange easily to the spiro-acetal compounds.

INTRODUCTION

Zearalenone (ZEN) is a macrocylic β-resorcylic acid lactone (**Figure 1**) with pronounced estrogenic activity, produced by a variety of *Fusarium* fungi, including *Fusarium graminearum* (*Gibberella zeae*), *Fusarium culmorum*, *Fusarium cerealis*, *Fusarium equiseti*, and *Fusarium crookwellense* (*1*, *2*). These are common soil fungi in countries with temperate and warm climate that infest predominantly corn but also barley, oats, wheat, sorghum, millet, and rice. As cereals are colonized in the field, toxin production mainly takes place before harvesting but may also occur during storage if the crop is not dried and handled properly (*1*, *2*).

ZEN was first isolated 1962 by Stob et al. (*3*) from cultures of *F. graminearum* as the active compound accounting for the estrogenicity of such cultures in immature female pigs and mature ovariectomized mice. The chemical structure of ZEN was elucidated in 1966 by Urry et al. (*4*). In 1972, Bolliger and Tamm (*5*)

reported on the identification of four ZEN-related fungal metabolites from cultures of *F. graminearum*, i.e., 13-formyl-ZEN, 5,6-dehydro-ZEN, and the two epimers of 5-hydroxy-ZEN (**Figure 2**). The formation of 5-hydroxy-ZEN was subsequently confirmed (*6*−*8*), and further congeners of ZEN were disclosed (**Figure 2**), i.e., the α- and β-epimers of zearalenol (ZEL) (*7*−*9*), 5-hydroxy-ZEL (*10*), the epimers of 10-hydroxy-ZEN (*11*), the epimers of zearalanol (ZAL), *cis*-ZEN, and the epimers of *cis*-ZEL (*9*).

To use the reported fungal metabolites of ZEN as reference compounds for studies of the mammalian metabolism of ZEN, we have analyzed the extract from a *F. graminearum* culture for congeners of ZEN by HPLC and LC−MS/MS. As we will report in this paper, several hitherto unknown fungal metabolites were observed in the course of our study. Of particular interest is the identification of a novel aliphatic epoxide of ZEN and the corresponding dihydrodiol, as well as several cyclization products of the dihydrodiol.

MATERIALS AND METHODS

Standards and Chemicals. ZEN was purchased from Fermentek (Jerusalem, Israel) and had a purity of 98% according to HPLC analysis. 13-Hydroxy-ZEN and 15-hydroxy-ZEN were synthesized from ZEN as

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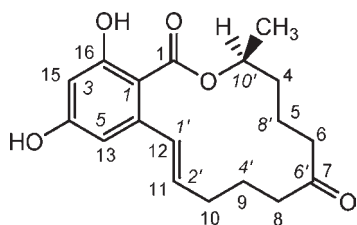
Figure 1. Chemical structure of ZEN. For numbering the different positions, an older system (1–6 and 1′–10′, numbers in italics) and the more recent IUPAC system (1–16) are indicated.
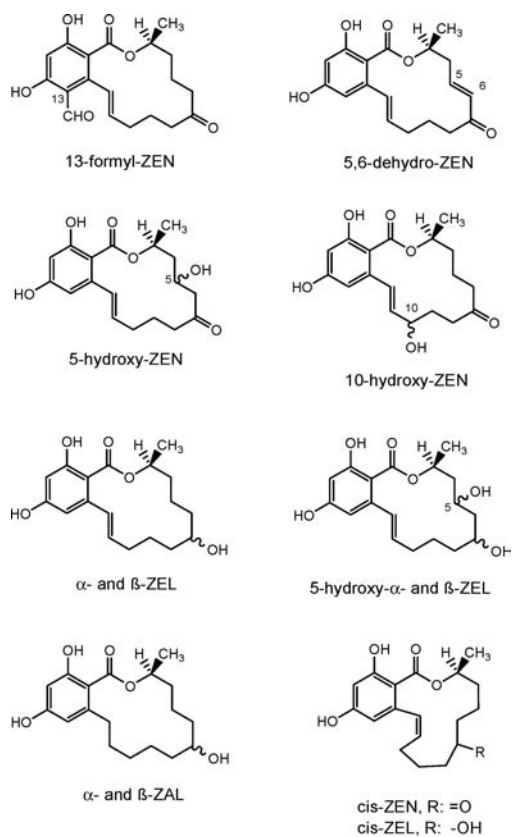

Figure 2. Chemical structures of congeners of ZEN previously reported in the literature.

reported previously (*12*). 5-Hydroxyzearalanone (5-hydroxy-ZAN) has recently been identified as a fungal product (*13*) and was provided by B. Wha Son (Pukyong National University, Busan, Korea). All chemicals and reagents not specifically mentioned were of the highest quality available and purchased from Sigma/Aldrich/Fluka (Taufkirchen, Germany). HPLC grade acetonitrile was from Acros Organics (Geel, Belgium).

Synthesis of ZEN-11,12-oxide. A solution of 100 μmol of pure *m*-chloroperbenzoic acid in 1 mL of CH_2Cl_2 was added to 50 μmol of ZEN dissolved in 1.5 mL of CH_2Cl_2. After 5 h at 20 °C, the yellow solution was extracted with 1 mL of a 10% aqueous solution of K_2CO_3, washed with 1 mL of water, and dried over anhydrous Na_2SO_4. The nearly colorless CH_2Cl_2 solution, according to LC-DAD-MS analysis, contained ~55% ZEN (according to TIC, eluting at 22.0 min), ~20% ZEN-11,12-oxide (eluting at 19.4 min), and ~25% of a mixture of 13- and 15-hydroxy-ZEN (eluting together at 17.8 min).

ZEN-11,12-oxide: $[M - H]^-$ *m/z* 333; MS^2 of *m/z* 333, 333 (3), 315 (61), 305 (23), 289 (100), 277 (53), 271 (20), 261 (8), 247 (34), 246 (16), 245 (12), 235 (9), 233 (11), 203 (18), 191 (23), 175 (30), 165 (42), 164 (25), 147 (32).

13- and 15-hydroxy-ZEN: $[M - H]^-$ *m/z* 333; MS^2 of *m/z* 333, 333 (100), 315 (25), 305 (6), 289 (43), 261 (8), 219 (9), 201 (14), 191 (57).

Synthesis of ZEN-11,12-dihydrodiol. The fresh solution of 120 μmol of OsO_4 in 1 mL of pyridine was added to 100 μmol of ZEN dissolved in 1 mL of pyridine. The reaction mixture turned to dark violet within minutes. After 18 h at 20 °C in the dark, 2 mL of an aqueous solution of 60 mg (270 μmol) of $K_2S_2O_5$ was added and the mixture stirred for 4 h, followed by extraction with 5 mL of $CHCl_3$. According to LC-DAD-MS analysis, the $CHCl_3$ extract (which still contained some pyridine) was devoid of ZEN and contained one large peak (85% according to TIC, eluting at 11.9 min) and one small peak (15%, eluting at 9.1 min), both with an $[M - H]^-$ ion at *m/z* 351 consistent with the structure of ZEN-11,12-dihydrodiols.

ZEN-11,12-dihydrodiols. Large peak: $[M - H]^-$ *m/z* 351; MS^2 of *m/z* 351, 333 (6), 307 (2), 165 (100), 147 (4), 137 (5). Small peak: $[M - H]^-$ *m/z* 351; MS^2 of *m/z* 351, 333 (8), 307 (2), 165 (100), 137 (5).

The $CHCl_3$ extract (0.5 mL) was magnetically stirred with an equal volume of 5 N aqueous HCl for 1 h at 20 °C to extract the pyridine and then washed with 0.5 mL of water. A slight precipitate dissolved after the addition of 0.2 mL of ethyl acetate. LC-DAD-MS analysis of the organic phase showed that the two dihydrodiols had disappeared and one product with an $[M - H]^-$ ion at *m/z* 333 and a retention time of 17.1 min had formed (see Results and Discussion).

Acid-Catalyzed Hydrolysis of ZEN Congener P16. A solution of 0.5 mg of P16 in 100 μL of methanol was mixed with 20 μL of 1 N aqueous HCl at 20 °C and analyzed by LC-DAD-MS after 30, 60, and 90 min.

Hydrogenation of P7 and P10. Approximately 50 μg each of P7 and P10 were isolated by HPLC, separately dissolved in 1 mL of methanol, and magnetically stirred under a hydrogen atmosphere at 20 °C for 12 h in the presence of a small amount of PtO_2. The catalyst was then removed by centrifugation and the supernatant used for LC-MS/MS analysis.

Oxidation with MnO_2. The solution of the ZEN congener (~0.5 mg) in 2 mL of acetone was magnetically stirred with ~50 mg of fresh MnO_2 at 20 °C for 6 h. The MnO_2 was then separated from the supernatant in a laboratory centrifuge and washed twice with 1.5 mL of acetone each, and the combined organic solutions were evaporated to dryness, redissolved in 1 mL of methanol, and analyzed by LC-MS/MS.

Large-Scale Cultivation of *F. graminearum*. Three 5 L flasks, each filled with 143 g of expanded vermiculit and 500 mL of Hidy medium, were inoculated with *F. graminearum* strain DSM 4528 and kept in a dark incubator at 25 °C. Flasks, vermiculit, and medium had been previously sterilized at 121 °C for 20 min. The starter cultures used for inoculation were obtained by growing spores of *F. graminearum* on potato-dextrose-agar plates for 5 days at 25 °C in an incubator. Each week, the content of the 5 L flasks was mixed by using a metal claw and vigorous shaking, and an aliqot of ~2 g was removed with a spatula for analysis of the amount of mycotoxin. The aliquots were homogenized with 2 mL of a 7:3 (v/v) mixture of methanol and 150 mM potassium acetate buffer (pH 5.0), followed by centrifugation and dilution of the supernatant with methanol for LC-DAD-MS analysis.

The total content of the three 5 L flasks (~1800 g) was worked up after 40 days when the production of the mycotoxins appeared to plateau (see Results and Discussion). Portions of 200 g each were mixed with 200 mL of acetate buffer, homogenized using a small kitchen mixer, diluted with 500 mL of methanol, and magnetically stirred for 1 h. Solid materials were then removed by filtration and the filtrates concentrated with a rotary evaporator to remove the methanol. The remaining aqueous phases were subsequently extracted twice with 200 mL of ethyl acetate each and the two combined extracts dried over anhydrous $MgSO_4$ for 12 h. Finally, all the ethyl acetate extracts were filtered, combined, and concentrated in vacuo by using a rotary evaporator to yield a brown oil.

Column Chromatography on Silica Gel. For partial purification, the crude fungal extract was dissolved in 25 mL of an 8:2 (v/v) benzene/ethyl acetate mixture and chromatographed on silica gel in six portions. Each chromatography run was conducted in a glass column with a 24 mm inside diameter filled with 15 g of silica gel 60 (Merck AG, Darmstadt, Germany) suspended in an 8:2 (v/v) benzene/ethyl acetate mixture, using a hood to avoid exposure to the solvents. The column was first eluted with 70 mL of the same solvent mixture and then with 150 mL of ethyl acetate.

HPLC Analysis. Analytical HPLC was conducted on a Hewlett-Packard 1100 system equipped with a binary pump and a photodiode array detector, and HP ChemStation Rev.A.07.01 for data collection and analysis was used. Separation was conducted on a 250 mm × 4.6 mm (inside diameter), 5 μm, reversed-phase Luna C18 column (Phenomenex,

Torrance, CA). Solvent A was deionized water adjusted to pH 3.0 with formic acid, and solvent B was acetonitrile. A linear solvent gradient was used, changing from 30 to 70% B over 30 min and then to 100% B over 3 min. After the column had been eluted with 100% B for 6 min, the initial level of 30% B was reached in 1 min and held for 5 min before the next injection. The flow rate was 1 mL/min. When analytical HPLC was used to purify some of the ZEN congeners (see Results and Discussion), the respective HPLC fractions were collected, the acetonitrile was removed on a rotary evaporator, and the compounds were extracted from the remaining aqueous phases with ethyl acetate.

For preparative HPLC, a Shimadzu CBM-20A preparative HPLC with a 250 mm × 10 mm (inside diameter), 5 μm, reversed-phase Luna C18(2) column (Phenomenex) was used. Solvent A was deionized water, and solvent B was methanol. ZEN (37.1 min) was separated from its congeners using the following gradient: 60% B from injection to 25 min, then to 70% B over 10 min, and then to 100% B over 3 min at a flow rate of 4 mL/min. P15 and P16 coeluted at 22.2 min under these conditions. The methanol was removed from the HPLC fractions using a rotary evaporator, and the compounds were extracted from the aqueous phases with ethyl acetate. Separation of P15 (21.3 min) and P16 (26.9 min) was achieved under isocratic conditions using 50% B and a flow rate of 2 mL/min.

LC-MS/MS Analysis. LC-MS experiments were performed using a LXQ Linear Ion Trap MSn system (Thermo Fisher Scientific Inc., Waltham, MA) operated in the negative electrospray ionization (ESI) mode. Nitrogen was used as sheath gas and auxiliary gas with flow rates of 30.0 and 15.0 L/min, respectively. The spray voltage was 4.5 kV, and the capillary temperature was 350 °C. Ion optics were automatically tuned with a 10 μM solution of ZEN in methanol. MS/MS of the $[M - H]^-$ ions was conducted at CID 35 (35% of 5 kV). The same column and solvents were used as in HPLC but with a different solvent gradient, changing from 30 to 100% B over 30 min. The initial level of 30% B was then reached within 1 min and held for 5 min before the next injection. The flow rate was 0.5 mL/min.

NMR Spectroscopy. ^1H and two-dimensional (2D) NMR data were acquired on a Bruker AVANCE 600, ^{13}C NMR or Bruker DRX 500 NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany). Signals are reported in parts per million referenced to the deuterated solvent as indicated. For structural elucidation and NMR signal assignment, 2D NMR experiments, such as 2D nuclear Overhauser effect spectroscopy (NOESY), correlated spectroscopy (COSY), heteronuclear multiple-quantum correlation (HMQC), and heteronuclear multiple-bond correlation (HMBC), were conducted. Pulse programs for the experiments were taken from the Bruker software library.

NMR Data

(i) ZEN (numbering according to Figure 1). ^1H NMR (600 MHz, CD₂Cl₂): δ 1.40 (d, ^3J = 6.0 Hz, 3 H, 2-CH₃), 1.55 (m, 1 H, H-9), 1.66 (m, 2 H, H-4), 1,79 (m, 2 H, H-5), 2.11−2.29 (m, 4 H, H-6, H-8, H-9, H-10), 2.39 (m, 1 H, H-10), 2.67 (m, 1 H, H-6), 2.90 (ddd, ^3J = 18.8 Hz, ^3J = 12.2 Hz, ^2J = 2.4 Hz, 1 H, H-8), 5.02 (m, 1 H, H-3), 5.75 (ddd, ^3J = 15.3 Hz, ^3J = 10.6 Hz, ^3J = 3.6 Hz, 1 H, H-11), 6.38 (d, ^4J = 2.6 Hz, 1 H, H-15), 6.49 (d, ^4J = 2.6 Hz, 1 H, H-13), 6.62 (br, 1 H, OH-14), 7.06 (d, ^3J = 15.3 Hz, 1 H, H-12), 12.10 (s, 1 H, OH-16). ^{13}C NMR (125 MHz, CD₂Cl₂): δ 20.5 (C-2), 21.0 (C-9), 22.3 (C-5), 31.0 (C-10), 34.7 (C-4), 36.6 (C-8), 43.0 (C-6), 73.6 (C-3), 102.2 (C-15), 103.8 (C-17), 108.4 (C-13), 132.6 (C-11), 133.0 (C-12), 144.1 (C-18), 160.9 (C-14), 165.4 (C-16), 171.4 (C-1), 212.2 (C-7).

(ii) P16 (ZEN-11,12-oxide). Because of the overlap of signals in the aliphatic region of the proton spectrum, the signals were assigned via HMQC and HMBC. Because the signals are obscured in the aliphatic region, the assignment contains a slight uncertainty. The numbering follows that of **Figure 4**. ^1H NMR (600 MHz, CD₃CN): δ 1.34 (d, ^3J = 6 Hz, 3 H, 2-CH₃), 1.39 (m, 1 H, H-10), 1.47 (m, 1 H, H-4), 1.56 (m, 1 H, H-9), 1.60 (m, 1 H, H-4), 1,68 (m, 1 H, H-5), 1.78 (m, 1 H, H-5), 1,99 (m, 1 H, H-10), 2.13 (m, 2 H, H-6 + H-9), 2.26 (ddd, ^3J = 18.1 Hz, ^3J = 7.0 Hz, ^2J = 2.1 Hz, 1 H, H-8), 2.64 (m, 2 H, H-6 + H-11), 2.74 (ddd, ^3J = 18.1 Hz, ^3J = 11.9 Hz, ^2J = 2.1 Hz, 1 H, H-8), 4.45 (d, ^3J = 1.8 Hz, 1 H, H-12), 5.23 (m, 1 H, H-3), 6.31 (s, 2 H, H-13 + H-15), 8.02 (br, 1 H, OH-14), 12.02 (br, 1 H, OH-16). ^{13}C NMR (125 MHz, CD₃CN): δ 19.2 (C-9), 20.3 (C-2), 21.8 (C-5), 29.6 (C-10), 33.9 (C-4), 37.5 (C-8), 42.2 (C-6), 57.0 (C-12), 64.3 (C-11), 73.6 (C-3), 102.3 (C-15), 104.1 (C-17), 104.4 (C-13), 143.7 (C-18), 162.7 (C-14), 165.6 (C-16), 171.3 (C-1), 211.0 (C-7).

(iii) ZEN-11,12-dihydrodiol. The numbering follows that of **Figure 4**. ^1H NMR (600 MHz, CD₃OD): δ 1.31 (m, 1 H, H-10), 1.33 (d, ^3J = 6.1 Hz, 3 H, 2-CH₃), 1.48 (m, 1 H, H-4), 1.63 (m, 1 H, H-4), 1,70 (m, 1 H, H-9), 1.85 (m, 2 H, H-5), 1.93 (m, 1 H, H-10), 2.04−2.14 (m, 3 H, H-6, H-8, H-9), 2.77 (ddd, ^3J = 11.6 Hz, ^3J = 5.6 Hz, ^2J = 3.1 Hz, 1 H, H-6), 3.03 (ddd, ^3J = 18.1 Hz, ^3J = 12.9 Hz, ^2J = 2.5 Hz, 1 H, H-8), 3.71 (dt, ^3J = 11.9 Hz, ^3J = 1.5 Hz, 1 H, H-11), 5.29 (dqd, ^3J = 11.2 Hz, ^3J = 6.1 Hz, ^3J = 1.6 Hz, 1 H, H-3), 5.32 (s, 1 H, H-12), 6.27 (d, ^4J = 2.5 Hz, 1 H, H-15), 6.85 (d, ^4J = 2.5 Hz, 1 H, H-13). ^{13}C NMR (150 MHz, CD₃OD): δ 13.2 (C-9), 20.3 (C-2), 22.4 (C-5), 31.0 (C-10), 34.6 (C-4), 35.6 (C-8), 43.4 (C-6), 70.1 (C-11), 72.1 (C-12), 72.8 (C-3), 101.3 (C-15), 101.9 (C-17), 108.8 (C-13), 149.1 (C-18), 162.5 (C-14), 165.4 (C-16), 171.5 (C-1), 213.7 (C-7).

(iv) P15 (cyclization product 1A). Some of the signals in the aliphatic region are obscured, and therefore the assignment was done by HMQC and HMBC. The numbering follows that of **Figure 4**. ^1H NMR (600 MHz, CD₃OD): δ 1.07 (d, 3 H, 2-CH₃), 1.15 (dtd, ^3J = 13.2 Hz, ^3J = 13.0 Hz, J = 4.3 Hz, 1 H, H-4), 1.39 (m, 1 H, H-6), 1.43 (m, 1 H, H-10), 1.46 (m, 1 H, H-8), 1.59 (m, 1 H, H-4), 1.61 (m, 2 H, H-5 + H-8), 1.63 (m, 1 H, H-9), 1.66 (m, 1 H, H-6), 1.69 (m, 1 H, H-10), 1.91 (qt, ^3J = 13.3 Hz, ^3J = 4.0 Hz, 1 H, H-9), 1.99 (qt, ^3J = 13.5 Hz, ^3J = 4.0 Hz, 1 H, H-5), 3.64 (dqd, ^3J = 11.4 Hz, ^3J = 6.1 Hz, ^3J = 1.9 Hz, 1 H, H-3), 3.74 (ddd, ^3J = 11.6 Hz, ^3J = 6.1 Hz, ^3J = 1.9 Hz, 1 H, H-11), 5.21 (d, ^3J = 6.2 Hz, 1 H, H-12), 6.33 (d, ^4J = 2 Hz, 1 H, H-15), 6.57 (d, ^4J = 2 Hz, 1 H, H-13). ^{13}C NMR (125 MHz, CD₃OD): δ 17.7 (C-9), 18.6 (C-5), 20.6 (C-2), 26.1 (C-10), 32.3 (C-4), 34.5 (C-6), 34.9 (C-8), 65.3 (C-3), 70.9 (C-11), 82.1 (C-12), 96.6 (C-7), 101.9 (C-13), 102.3 (C-15), 103.4 (C-17), 151.8 (C-18), 158.3 (C-16), 165.3 (C-14), 170.5 (C-1).

X-ray Crystallography. Data were collected on a Stoe-IPDS-II diffractometer using graphite monochromated Mo$_{Kα}$ radiation (λ = 0.71073 Å). The structure was determined by direct methods and refined by full matrix least-squares on F^2 (all data) (*14*). C and O atoms were refined with anisotropic temperature factors. H atoms could be refined with isotropic temperature factors.

Crystal data for CP-2: $C_{18}H_{22}O_6 \cdot H_2O$; M = 352.37 g/mol, orthorhombic, space group $P2_12_12_1$ (No. 19); Z = 4; a = 8.9234(4) Å, b = 10.5083(5) Å, c = 17.6970(7) Å; V = 1659.4(1) Å³; T = 200(2) K; $F(000)$ = 752; $ρ_{calcd}$ = 1.407 g/cm³ ; $μ(Mo Kα)$ = 0.108 mm^{-1}; 16246 reflections measured; 2519 independent reflections; $R_{(int)}$ = 0.0416 (323 parameters refined); goodness of fit on F^2 = 1.049; R_1 = 0.0284 [$I > 2σ(I)$]; wR_2 = 0.0700 (for all data); maximal residual electron density of 0.2 e/Å³. The supplementary crystallographic data for this structure can be obtained free of charge from The Cambridge Crystallographic Data Centre (www.ccdc.cam.ac.uk/data_request/cif) as entry CCDC-797016.

RESULTS AND DISCUSSION

Pattern of ZEN Congeners. ZEN and its congeners were produced by *F. graminearum* and found to reach a plateau phase between week 4 and 5. On day 40, the ZEN-related material was extracted and the extract analyzed by HPLC on a reversed-phase column. ZEN represented ~90% of the total peak area (at 280 nm), but several minor peaks eluting prior to ZEN were clearly detectable in the HPLC profile (data not shown). Using a small aliquot of the total extract, the fraction containing the polar constituents was separated from ZEN by preparative HPLC and subjected to LC-DAD-MS analysis (**Figure 3**). Sixteen of the peaks eluting earlier than ZEN from the reversed-phase column had a UV spectrum similar to that of ZEN, i.e., with three absorption bands at ~230, ~270, and ~310 nm. The $[M - H]^-$ ions of these compounds obtained upon ESI in the negative mode are also shown in **Figure 3**. Nine of the 16 peaks, i.e., P6−P10, P12, P13, P15, and P16, had a quasi-molecular ion at m/z 333 consistent with a monooxygenated ZEN. Thus, it could be assumed that some of the previously reported monohydroxylated congeners of ZEN (**Figure 2**) were indeed present in our fungal extract.

Identification of Novel ZEN Congeners. To separate sufficient amounts of the ZEN congeners for structure elucidation, the total fungal extract was separated on a preparative silica gel column into two fractions (see Materials and Methods). Analytical HPLC showed that the first fraction contained not only ZEN
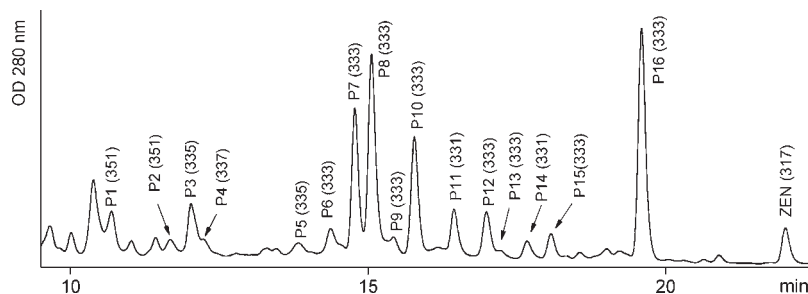
Figure 3. HPLC profile of the ZEN congeners together with their [M − H]⁻ ions as obtained by LC-DAD-ESI-MS analysis.
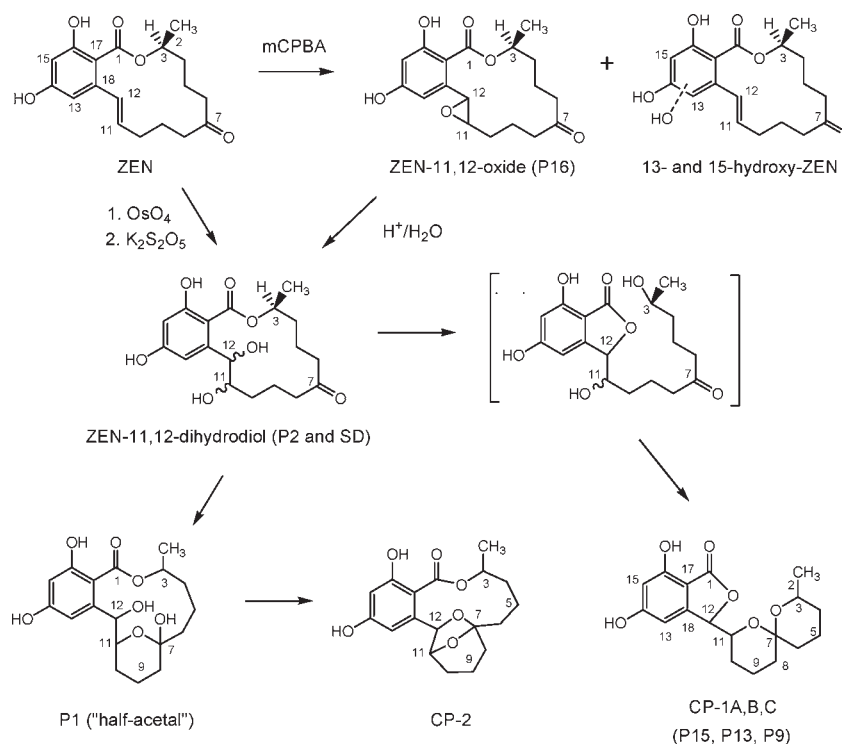

Figure 4. Chemical synthesis of ZEN-11,12-oxide and ZEN-11,12-dihydrodiol and formation of cyclization products (CP).

but also P15 and P16, whereas the second fraction contained P5−P14. The more polar compounds P1−P4 and some polar brown material did not elute from the silica gel under these conditions. From the first fraction, ZEN was crystallized in a benzene/hexane mixture and the mother liquor used to isolate P15 and P16 by preparative HPLC (see Materials and Methods). Approximately 10 mg of pure P16 and 11 mg of P15 were obtained and analyzed by NMR spectroscopy as well as tandem mass spectrometry (MS²).

Identification of P16. A comparison of the ¹H and ¹³C NMR spectra of P16 and ZEN (see Materials and Methods) revealed that the most notable difference was the chemical shift of the protons and carbon atoms at C-11 and C-12, which suggested the incorporation of one oxygen atom at these two positions. The suspected structure of a ZEN-11,12-oxide would also explain the similar HPLC retention times of P16 and ZEN (**Figure 3**). The MS² spectra of the [M − H]⁻ ions of P16 and ZEN exhibited a virtually complete disintegration of the [M − H]⁻ ions and a mass difference of 16 amu between the major fragment ions due to the presence of one additional oxygen atom in the P16 molecule (**Table 1**).

To confirm the putative structure of P16 as ZEN-11,12-oxide, ZEN was subjected to chemical epoxidation using *m*-chloroperbenzoic acid (mCPBA). As noted previously by Jensen et al. (*15*), the olefinic double bond of ZEN is quite resistant to epoxidation.

However, the profile obtained by LC-DAD-MS analysis of the reaction mixture after 5 h showed that, in addition to ∼55% unreacted ZEN, two peaks had formed that had [M − H]⁻ ions at *m/z* 333, indicative of monooxygenated ZENs. The less polar of these peaks, eluting at 19.4 min, had the same LC retention time and MS² spectrum of the [M − H]⁻ ion as P16 (**Table 1** and Materials and Methods), thus confirming the structure of ZEN-11,12-oxide (**Figure 4**). The more polar peak eluting at 17.8 min had a retention time and MS² spectrum of the [M − H]⁻ ion suggesting the structure of 13- and/or 15-hydroxy-ZEN (**Figure 4**). These products of monohydroxylation at the aromatic ring have recently been identified as major oxidative metabolites of ZEN in human hepatic microsomes, and the authentic compounds have been chemically synthesized (*12*). 13- and 15-hydroxy-ZEN do not separate under our HPLC conditions but differ significantly in their MS² spectrum of the [M − H]⁻ ion (*12*). According to its MS² spectrum (see Materials and Methods), the product formed from ZEN with mCPBA was a mixture of 13- and 15-hydroxy-ZEN. The formation of monohydroxylation products from aromatic systems by mCPBA has been reported for other compounds, e.g., for resorcinol diethers (*16*).

When the solution of the reaction products of ZEN with mCPBA was kept at ambient temperature for 1 month and

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Table 1. Characterization of ZEN and Some of Its Congeners and Cyclization Products (CP)

peak	LC t_R (min)	UV maxima (nm)	ESI-MS [M − H]⁻ (*m/z*)	ESI-MS² of [M − H]⁻ (*m/z*) (% relative intensity)	assigned structure
ZEN	22.0	236, 274, 314	317	317 (2), 299 (52), 273 (100), 261 (6), 203 (6), 175 (12), 161 (8), 149 (18)	ZEN
P16	19.4	226, 266, 306	333	333 (3), 315 (61), 305 (23), 289 (100), 277 (53), 271 (20), 261 (8), 247 (34), 246 (16), 245 (12), 235 (9), 233 (11), 203 (18), 191 (23), 175 (30), 165 (42), 164 (25), 147 (32)	ZEN-11,12-oxide
P15	18.0	218, 257, 292	333	333 (100), 315 (40), 289 (3), 271 (9), 247 (4), 191 (8)	CP-1A
P13	17.1	218, 257, 292	333	333 (100), 315 (31), 289 (3), 271 (8), 247 (4), 191 (30)	CP-1B
P9	15.4	218, 257, 293	333	333 (100), 315 (79), 289 (4), 271 (19), 247 (8), 203 (4)	CP-1C
P2	11.7	229, 265, 304	351	333 (8), 307 (3), 165 (100), 147 (4), 137 (5)	ZEN-11,12-dihydrodiol
P1	10.4	230, 266, 302	351	333 (4), 307 (3), 165 (100), 137 (7)	half-acetal of dihydrodiol
SD[a]	8.8	224, 255, 287	351	333 (8), 307 (3), 165 (100), 137 (5)	ZEN-11,12-dihydrodiol
CP-2	14.8	231, 281	333	333 (100), 315 (13), 289 (30), 191 (2), 165 (2), 149 (2)	CP-2

[a] Synthetic diol.

reanalyzed by LC-DAD-MS, the peak of ZEN-11,12-oxide was still present but the peak containing 13- and 15-hydroxy-ZEN had disappeared. This is consistent with the proposed structure, as 13-hydroxy-ZEN and 15-hydroxy-ZEN are unstable catechols prone to autoxidation (*12*).

Identification of P1, P2, P9, P13, and P15. Having established the chemical structure of P16 as a ZEN-11,12-oxide, we suspected that the congeners with [M − H]⁻ ions at *m/z* 351 (P1 and P2 in **Figure 3**) might represent ZEN-11,12-dihydrodiols. Therefore, P16 was treated with aqueous HCl to achieve hydrolysis of the epoxide. When the mixture was analyzed by LC-DAD-MS after 30 min, five new products were detected (**Figure 5A**). Two of them eluted early from the reverse-phase column at 10.7 and 11.7 min and had [M − H]⁻ ions at *m/z* 351, consistent with the structure of ZEN-11,12-dihydrodiols. The three remaining products had [M − H]⁻ ions at *m/z* 333 and eluted at 15.4, 17.1, and 18.1 min. The magnitudes of the peaks of P16 (epoxide) and of the product at 11.7 min (a dihydrodiol) had decreased after a hydrolysis time of 60 min and virtually disappeared after 90 min, whereas the other products were still present at the same ratio as after 30 min (**Figure 5B**).

The five products obtained from the acid-catalyzed hydrolysis of P16 were also detected in the fungal extract shown in **Figure 3**. The two putative dihydrodiols had retention times, [M − H]⁻ ions, and MS² spectra identical with those of fungal products P1 and P2, whereas the three other hydrolysis products were identical with P9, P13, and P15. The characteristic UV and MS data of these products are listed in **Table 1**.

To confirm the structures of P1 and P2, ZEN-11,12-dihydrodiol was chemically synthesized by reacting ZEN with osmium tetroxide, followed by reduction of the primary cyclic osmium ester with potassium disulfite (**Figure 4**). According to LC-DAD-MS analysis, ZEN was completely converted to two products, both of which had [M − H]⁻ ions at *m/z* 351 (**Figure 5C**). The major product eluting at 11.7 min was isolated by preparative HPLC and its chemical structure confirmed by ¹H and ¹³C NMR (for data, see Materials and Methods). Its HPLC retention time and MS² spectrum were identical with those of P2 (**Figure 3** and **Table 1**). The minor product, designated SD (synthetic diol), had the same MS² spectrum as P2 and P1 but eluted much earlier than P1 from the HPLC column (**Table 1**).

When an aliquot of the solution of the two synthetic ZEN-dihydrodiols in CHCl₃ (see Materials and Methods) was diluted with methanol and treated with NaBH₄, P2 and SD were quantitatively converted to four more polar products with [M − H]⁻ ions at *m/z* 353 (data not shown). It is known that the C-7 keto group of ZEN and its analogues is readily reduced by NaBH₄ to the respective alcohol, generating a mixture of the α- and

β-epimers (*17*). Thus, both P2 and SD contained a C-7 keto group.

When another aliquot of the solution of the two synthetic ZEN-dihydrodiols in CHCl₃ was stirred with aqueous HCl (see Materials and Methods), both P2 and SD disappeared and a product with an [M − H]⁻ ion at *m/z* 333 was formed (**Figure 5D**). This compound had the same HPLC retention time and MS² spectrum as one of the products obtained upon acidic hydrolysis of ZEN-11,12-oxide (**Figure 5A**, CP-1B) and P13 in the fungal extract (**Table 1**). Notably, it was not affected by treatment with NaBH₄, implying that the 7-keto group was lacking. Likewise, when the "aged" solution of acid-hydrolyzed P16 (**Figure 5B**) was treated with NaBH₄, none of the four compounds corresponding to fungal metabolites P1, P9, P13, and P15 was changed.

As mentioned above, the fungal product P15 was isolated in pure form by preparative HPLC from the mother liquor of the ZEN crystallization. When the ¹H and ¹³C NMR spectra of P15 and ZEN were compared (see Materials and Methods), it was obvious that the chemical structure of P15 was quite different from that of ZEN. In a previous paper on the chemical synthesis of ZEN-related compounds, it was noted that the 11,12-dihydrodiol of 14,16-*O,O*-dibenzyl-ZEN was unstable and easily rearranged to a cyclic spiro-acetal, particularly under acidic conditions (*15*). Assuming the same reaction for ZEN-11,12-dihydrodiol would lead to the structure of the cyclization product CP-1 depicted in **Figure 4**, which could plausibly be formed from ZEN-11,12-dihydrodiol through an acid-catalyzed transesterification leading to the cleavage of the macrocycle and subsequently to a new spiro-acetal at C-7, involving the hydroxyl groups at C-3 and C-11 (**Figure 4**). The dihydroisobenzofuranone structure could be supported by HMBC: H-12 exhibits a downfield shift (δ_H 5.21) and HMBC signals to C-1, C-10, C-11, C-13, C-14, C-17, and C-18. The spirocyclic structure and the bond from C-2 to C-12 were established by analysis of 2D NMR data, including HMBC and HMQC spectra. NOESY correlations and the analysis of ¹H−¹H coupling constants revealed a rigid conformation of the spirocyclic region, showing that both tetrahydropyran rings have a chair conformation. The axial orientation of H-11 (δ_H 3.74) was indicated by a large ¹H−¹H coupling ($J = 11.6$ Hz), and therefore, the dihydroisobenzofuranone sits in an equatorial position. A similar large ¹H−¹H coupling ($J = 11.4$ Hz) showed an axial orientation of the H-3 (δ_H 3.64) and thus an equatorial position of the methyl group (C-2). Because of the similarity of both rings of the spirocyclic structure, protons H-5 and H-9 and protons H-6 and H-8 showed a similar chemical shift, and the assignment contains some uncertainty. Therefore, the configuration of the quaternary acetal carbon and the relative configuration of the spirocyclic region could not be determined.
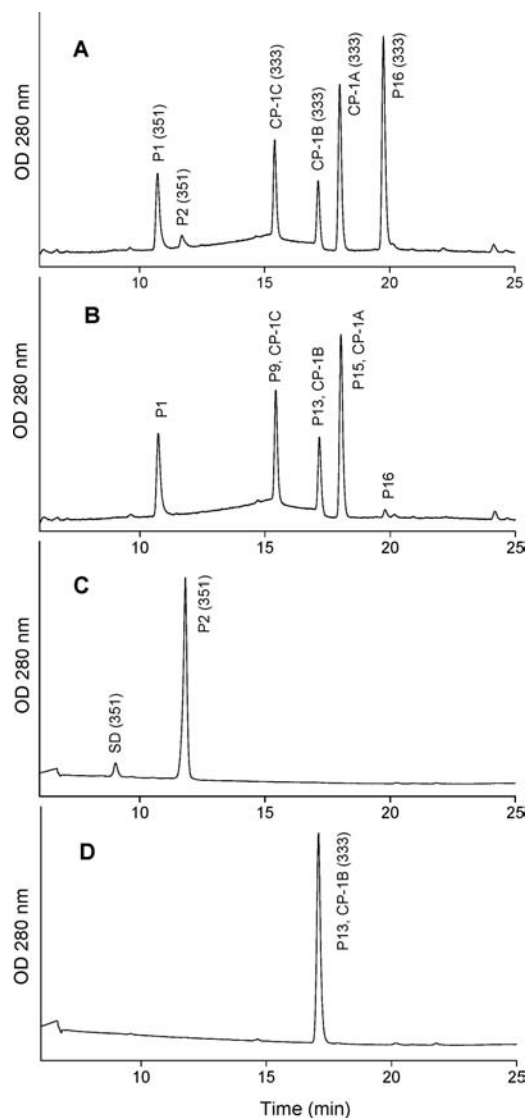
Figure 5. HPLC profile and $[M - H]^-$ ions of the products of acid hydrolysis of P16 (**A**) after 30 min and (**B**) after 90 min as well as the products obtained by reaction of ZEN with OsO_4 (**C**) before and (**D**) after treatment with acid. See the text for the designation of the peaks.

Because P9, P13, and P15 have identical UV and MS^2 spectra, it is assumed that these three compounds represent stereoisomeric forms, designated CP-1A, CP-1B, and CP-1C, respectively (**Table 1**). The fact that the $[M - H]^-$ ions of CP-1A, CP-1B, and CP-1C at m/z 333 remain the most prevalent ions in their MS^2 spectra indicates that the spiro-acetal ring system is very stable against collision-induced fragmentation. Also, the absence of the free 7-keto group in these CP-1 type products readily explains their inertness to $NaBH_4$.

As one of the products of acidic hydrolysis of P16, viz. P1 (**Figure 5A**), also proved to be inert to $NaBH_4$ but has an $[M - H]^-$ ion at m/z 351 (see above), the structure of a half-acetal of a ZEN-11,12-dihydrodiol is proposed for P1 (**Figure 4**).

In an old preparation of synthetic ZEN-11,12-dihydrodiols, evaporated to dryness over time, the formation of crystals was observed. X-ray diffraction analysis revealed yet another cyclization product (**Figure 6**) designated CP-2, which represents an acetal arising from the reaction of the keto group at C-7 with the two hydroxyl groups at C-11 and C-12 (**Figure 4**). In this product, the original lactone group is preserved, and no spiro compound is
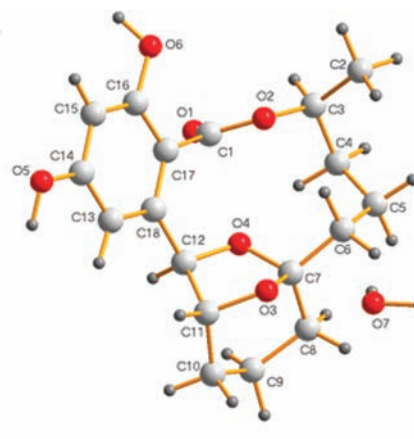

Figure 6. Structure of cyclization product CP-2 as obtained by X-ray diffraction analysis.

formed. CP-2 is also very refractory against collision-induced fragmentation of its $[M - H]^-$ ion (**Table 1**).

In conclusion, we report for the first time that an aliphatic epoxide, the corresponding dihydrodiol, and three spiro-acetals arising from the dihydrodiol are among the ZEN congeners formed by *F. graminearum*. Although the evidence for the gross chemical structures of these novel mycotoxins is unequivocal, their stereochemistry is yet unknown and needs further clarification. Moreover, it remains to be shown whether the dihydrodiol is a true metabolite of the fungus or a product of chemical hydrolysis of the epoxide. Likewise, the three cyclization products P9, P13, and P15 may be artifacts arising from P16 or P2.

Confirmation of Previously Reported ZEN Congeners. From the ethyl acetate fraction of the silica gel chromatography (see above), P6−P8 and P10 (**Figure 3**) were isolated in small amounts by repeated analytical HPLC. LC-DAD-MS analysis of the collected compounds showed that their purity was >90%, but their amounts were not sufficient for 2D NMR spectroscopy. Comparison of their MS^2 spectra (**Table 2**) showed that the spectra of P6 and P8 were identical, as were the spectra of P7 and P10.

P7 and P10 could be identified by comparison with an authentic 5-hydroxy-ZAN standard after chemical hydrogenation (see Materials and Methods) using PtO_2 as a catalyst, which reduces the olefinic double bond but not the 7-keto group. The product resulting from the catalytical hydrogenation of P10 had a HPLC retention time and MS^2 spectrum identical with those of the standard (**Table 2**). Hydrogenated P7 also had an identical MS^2 spectrum but different retention time. The stereochemistry of the standard, i.e., whether the 5-hydroxy group is α- or β-oriented, is not known. However, as under our HPLC conditions the β-isomers of ZEL and ZAL are eluting earlier than the α-isomers, we tentatively propose that P7 is 5β-hydroxy-ZEN and P10 is 5α-hydroxy-ZEN, which have been reported previously as congeners of ZEN in cultures of *F. graminearum* (5−8).

Another previously reported stereoisomeric pair of monohydroxylated ZEN consisted of 10α- and 10β-hydroxy-ZEN (11). To probe whether P6 and P8 might have the structures of these allylic hydroxyl compounds, a selective oxidation reaction was used: It is known that manganese dioxide oxidizes α,β-unsaturated alcohols more readily than saturated alcohols to the respective carbonyl compounds (18). When this oxidation was conducted with a mixture of P7 (5β-hydroxy-ZEN) and P8, only P8 but not P7 was oxidized, suggesting that P8 is indeed 10-hydroxy-ZEN. According to the elution sequence, P6 might be 10β-hydroxy-ZEN and P8 might be 10α-hydroxy-ZEN. As the

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Table 2. Characterization of ZEN Congeners P6–P8, P10, and P11

peak	LC t_R (min)	UV maxima (nm)	ESI-MS [M − H]⁻ (m/z)	ESI-MS² of [M − H]⁻ (m/z) (% relative intensity)	assigned structure
P6	14.4	237, 270, 313	333	333 (2), 315 (72), 289 (100), 271 (43), 261 (8), 247 (10), 191 (30), 163 (13), 161 (27), 147 (15), 123 (10)	10β-hydroxy-ZEN
P7	14.7	238, 273, 313	333	315 (71), 297 (20), 289 (11), 271 (42), 263 (26), 245 (100), 219 (11), 175 (10), 161 (6), 149 (13)	5β-hydroxy-ZEN
P8	15.1	236, 272, 313	333	333 (2), 315 (66), 289 (100), 271 (41), 261 (9), 247 (11), 203 (8), 191 (26), 163 (12), 161 (27), 147 (13), 123 (10)	10α-hydroxy-ZEN
P10	15.7	238, 274, 313	333	315 (71), 297 (23), 289 (14), 271 (44), 263 (23), 245 (100), 219 (11), 175 (7), 161 (7), 149 (13)	5α-hydroxy-ZEN
P11	16.5	218, 248, 286	331	331 (2), 313 (75), 287 (100), 269 (26), 243 (6), 233 (17), 217 (18), 191 (12), 175 (12), 163 (7), 161 (6), 149 (5)	10-keto-ZEN

product obtained from the oxidation of P8 with manganese dioxide had the same retention time and MS² spectrum as P11, this congener is probably 10-keto-ZEN (**Table 2**).

Conclusion. In addition to confirming the formation of some previously reported monohydroxylated congeners of ZEN, i.e., the α- and β-epimers of 5- and 10-hydroxy-ZEN, several novel congeners have been identified in this study of the metabolites of a *F. graminearum* strain. The common characteristic of these novel metabolites is the involvement of the aliphatic double bond of ZEN: Both an 11,12-epoxide (P16) and an 11,12-dihydrodiol (P2) were unambiguously identified by chromatographic and mass spectrometric comparison with synthetic standards. The structures of both compounds were also supported by ¹H and ¹³C NMR spectroscopy. The formation of ZEN-11,12-oxide by *Fusarium* fungi is not really surprising, because aliphatic epoxides have also been identified with other macrocyclic β-resorcylic acid lactones of fungal origin, e.g., hypothemycin, aigialomycin A and B, and several other hypothemycin analogues (*19, 20*). Moreover, other *Fusarium* toxins, e.g., trichothecenes, have long been known to contain epoxide groups (*20, 21*). For trichothecenes, the epoxide function appears to be very important for their toxicity (*22–24*). Therefore, it should prove to be interesting to compare the cytotoxicity of ZEN-11,12-oxide with that of ZEN. Other interesting questions would be whether the high estrogenic activity of ZEN is retained in ZEN-11,12-oxide and how this epoxide is metabolized in the mammalian organism.

The novel ZEN-11,12-dihydrodiol P2 and its half-acetal P1 are possibly fungal metabolites but may also be artifacts arising through chemical hydrolysis of the ZEN-11,12-oxide. The dihydrodiol of ZEN is unstable under acidic conditions, which was previously observed by Jensen et al. (*15*) with a synthetic dihydrodiol obtained from an O-benzylated derivative of ZEN. The structure of the resulting cyclization product CP-1 (**Figure 4**), originally proposed by Jensen et al. (*15*), has been confirmed in our study using NMR spectroscopy. Analogous products related to aigialomycins and containing a spiroacetal structure have recently been identified as aigialospirol and its analogues from the mangrove fungus *Aigialus parvus* (*25*). The stereochemistry of the two dihydrodiols and three CP-1s observed as fungal products in our study remains to be clarified. Further studies are also required to elucidate the structures of P3–P5, which represent minor congeners of ZEN (**Figure 3**). Their UV spectra (data not shown) imply that the olefinic double bond is lacking, which would mean that P3 and P5 are monohydroxy-ZANs and P4 is a monohydroxy-ZAL, according to their [M − H]⁻ ions.

The total quantity of ZEN congeners produced in our fungal culture was only ∼10% of the amount of ZEN. However, it is known that the pattern of mycotoxins and their amounts may vary considerably, depending on the particular strain of the fungus as well as the temperature, moisture, soil, host plant, and other factors. The future analysis of food items for ZEN should therefore include the identification of the ZEN congeners to obtain a realistic estimate of the overall exposure of consumers to this type of mycotoxin.

ABBREVIATIONS USED

COSY, correlated spectroscopy; CP, cyclization product; mCPBA, *m*-chloroperbenzoic acid; DAD, diode array detector; ESI, electrospray ionization; HMBC, heteronuclear multiple-bond correlation; HMQC, heteronuclear multiple-quantum correlation; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography and mass spectrometry; NOESY, two-dimensional nuclear Overhauser effect spectroscopy; SD, synthetic diol; TIC, total ion current; TLC, thin layer chromatography; ZAL, zearalanol; ZAN, zearalanone; ZEL, zearalenol; ZEN, zearalenone.

LITERATURE CITED

(1) Zinedine, A.; Soriano, J. M.; Moltó, J. C.; Mañes, J. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food Chem. Toxicol.* **2007**, *45*, 1–18.

(2) Bennett, J. W.; Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* **2003**, *16*, 497–516.

(3) Stob, M.; Baldwin, R. S.; Tuite, J.; Andrews, F. N.; Gillette, K. G. Isolation of an anabolic uterotrophic compound from corn infected with *Gibberella zeae*. *Nature* **1962**, *196*, 1318.

(4) Urry, W. H.; Wehrmeister, H. L.; Hodge, E. B.; Hidy, P. H. The structure of zearalenone. *Tetrahedron Lett.* **1966**, *7*, 3109–3114.

(5) Bolliger, G.; Tamm, C. Vier neue Metabolite von *Giberella zeae*: 5-Formyl-zearalenon, 7′-Dehydrozearalenon, 8′-Hydroxy- und 8′-*epi*-Hydroxy-zearalenon. *Helv. Chim. Acta* **1972**, *55*, 3030–3048.

(6) Jackson, R. A.; Fenton, S. W.; Mirocha, C. J.; Davis, G. Characterization of two isomers of 8′-hydroxyzearalenone and other derivatives of zearalenone. *J. Agric. Food Chem.* **1974**, *22*, 1015–1019.

(7) Stipanovic, R. D.; Schroeder, H. W. Zearalenol and 8′-hydroxyzearalenone from *Fusarium roseum*. *Mycopathologia* **1975**, *57*, 77–78.

(8) Hagler, W. M.; Mirocha, C. J.; Pathre, S. V.; Behrens, J. C. Identification of the naturally occurring isomer of zearalenol produced by *Fusarium roseum* 'Gibbosum' in rice culture. *Appl. Environ. Microbiol.* **1979**, *37*, 849–853.

(9) Richardson, K. E.; Hagler, W. M.; Mirocha, C. J. Production of zearalenone, α- and β-zearalenol, and α- and β-zearalanol by *Fusarium* spp. in rice culture. *J. Agric. Food Chem.* **1985**, *33*, 862–866.

(10) Steele, J. A.; Mirocha, C. J.; Pathre, S. V. Metabolism of zearalenone by *Fusarium roseum* graminearum. *J. Agric. Food Chem.* **1976**, *24*, 89−97.

(11) Pathre, S. V.; Fenton, S. W.; Mirocha, C. J. 3′-Hydroxyzearalenones, two new metabolites produced by *Fusarium roseum*. *J. Agric. Food Chem.* **1980**, *28*, 421−424.

(12) Pfeiffer, E.; Hildebrand, A.; Damm, G.; Rapp, A.; Cramer, B.; Humpf, H.-U.; Metzler, M. Aromatic hydroxylation is a major metabolic pathway of the mycotoxin zearalenone in vitro. *Mol. Nutr. Food Res.* **2009**, *53*, 1123−1133.

(13) Yang, X.; Khong, T. T.; Chen, L.; Choi, H. D.; Kang, J. S.; Son, B. W. 8′-Hydroxyzearalanone and 2′-hydroxyzearalanol: Resorcylic acid lactone derivatives from the marine-derived fungus *Penicillium* sp. *Chem. Pharm. Bull.* **2008**, *56*, 1355−1356.

(14) Sheldrick, G. M. *SHELX-97, Program for the Solution and Refinement of Crystal Structures*; Universität Göttingen: Göttingen, Germany, 1997.

(15) Jensen, N. P.; Brown, R. D.; Schmitt, S. M.; Windholz, T. B.; Patchett, A. A. Chemical modifications of zearalenone. I. *J. Org. Chem.* **1972**, *37*, 1639−1647.

(16) Srebnik, M.; Mechoulam, R. A facile method for regiospecific hydroxylation of resorcinol diethers. *Synthesis* **1983**, 1046−1048.

(17) Krenn, P.; Berthiller, F.; Schweiger, W.; Hametner, C.; Ludwig, R.; Adam, G.; Krska, R.; Schuhmacher, R. Production of zearalenone-4-glucoside, α-zearalenol-4-glucoside and β-zearalenol-4-glucoside. *Mycotoxin Res.* **2007**, *23*, 180−184.

(18) Bharucha, K. R. Oxidation by manganese dioxide of some unsaturated alcohols. *J. Chem. Soc.* **1956**, 2446−2447.

(19) Wee, J. L.; Sundermann, K.; Licari, P.; Galazzo, J. Cytotoxic hypothemycin analogues from *Hypomyces subiculosus*. *J. Nat. Prod.* **2006**, *69*, 1456−1459.

(20) Winssinger, N.; Barluenga, S. Chemistry and biology of resorcylic acid lactones. *Chem. Commun.* **2007**, 22−36.

(21) Ueno, Y. Trichothecene mycotoxins: Mycology, chemistry, and toxicology. *Adv. Nutr. Res.* **1989**, *3*, 301−353.

(22) Rocha, O.; Ansari, K.; Doohan, F. M. Effects of trichothecene mycotoxins on eukaryotic cells: A review. *Food Addit. Contam.* **2005**, *22*, 369−378.

(23) Sundstrøl Eriksen, G.; Pettersson, H.; Lundh, T. Comparative cytotoxicity of deoxynivalenol, nivalenol, theit acetylated derivatives and de-epoxy metabolites. *Food Chem. Toxicol.* **2004**, *42*, 619−624.

(24) Pestka, J. J.; Smolinski, A. T. Deoxynivalenol: Toxicology and potential effects on humans. *J. Toxicol. Environ. Health, Part B* **2005**, *8*, 39−69.

(25) Isaka, M.; Yangchum, A.; Intamas, S.; Kocharin, K.; Jones, E. B. G.; Kongsaeree, P.; Prabpai, S. Aigialomycins and related polyketide metabolites from the mangrove fungus *Aigialus parvus* BCC 5311. *Tetrahedron* **2009**, *65*, 4396−4403.