Short Communication

Structure of a Novel Metabolite from Deoxynivalenol, a Trichothecene Mycotoxin, in Animals

Takumi YOSHIZAWA, Hiroaki TAKEDA and Toshinori OHI

Department of Food Science, Faculty of Agriculture, Kagawa University, Kagawa 761-07, Japan

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Deoxynivalenol (DON), 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one, is a toxic metabolite such *Fusarium* spp. as *F. graminearum*[1] and *F. culmorum*.[2] It is commonly found together with nivalenol in wheat and barley grains in Japan,[3] and is also one of the most important trichothecene mycotoxins occurring naturally in several cereals and animal feed in other countries.[4] As a result of the consumption of these contaminated agricultural products, animals, even man, are suffering from sublethal toxicoses including emesis, diarrhea and feed refusal.[5] However, the metabolic fate of the toxin is still unknown. In this paper, we describe the elimination of DON in rats and the structural elucidation of a novel metabolite found in the excreta.

DON was dissolved in 10% aqueous ethanol and intubated to Wistar male rats weighing 240～245 g at a single dose of 6 mg/kg. Urine and feces samples were collected every 12 hr for 4 days. For estimating the distribution of DON and its metabolite(s) in the tissues and biological fluids, the rats received a single oral dose of 8～11 mg of DON/kg.

An individual urine sample was introduced successively into an Amberlite XAD-2 column (1 × 14 cm, eluted with acetone), and a Florisil column (1 cm i.d., 5 g, eluted with chloroform–methanol, 9 : 1). A feces sample was extracted three times with 50% aqueous methanol followed by centrifugation. The aqueous layer was defatted with *n*-hexane, concentrated and chromatographed over the same columns. A plasma sample, after dilution with two volumes of water, was chromatographed over an XAD-2 column. Each liver tissue was homogenized with water (50 ml) and denatured in boiling water followed by centrifugation. The supernatant was defatted and then chromatographed in the same way as the feces sample. Trichothecene metabolites in these extracts were converted into trimethylsilyl ethers (TMS) by reacting with a mixture of trimethylsilylimidazole–bis(trimethylsilyl)-acetamide–trimethylchlorosilane (3 : 3 : 2), and quantitated by GLC with an electron capture detector (2% OV-17 on Gas Chrom Q, at 190°C, nivalenol-TMS as an internal standard). DON was extrapolated to quantify its metabolite(s).

In addition to DON (t_R, 4.0 min), an unknown metabolite designated as DOM-1 (t_R, 2.4 min), was found in the rat excreta. Urinary and fecal elimination of both metabolites were completed by 72 hr: 4.5 and 4.4% of the administered dose for DON and DOM-1, respectively, in the urine; 0.3 and 5.6% in the feces. Maximum elimination was reached at the following times after dosing: DON at 12 hr in the urine and the feces, DOM-1 at 36 and 48 hr in the urine and the feces, respectively. Furthermore, both metabolites were also detected in the plasma and the liver at the following levels (ppb) at 12 hr after dosing: 43 and 63 for DON, and 24 and 16 for DOM-1, respectively.

By GC-MS of the TMS of DOM-1, its molecular ion was found at *m/z* 496.2477 (calcd for $C_{24}H_{44}O_5Si_3$, MW 496.2493), suggesting the elimination of one oxygen atom and the retention of three hydroxyl groups at the C-3, C-7 and C-15 positions. Other fragments at *m/z* 481 (M−15), 406 (M−90), 391 (M−105), 376, 361 and 309 were also shifted by 16 mass units (one oxygen atom) as compared with corresponding ions in the TMS of DON.

The new metabolite was isolated from the extract of the rat urine as follows: a) the

extract was dissolved in water, applied to a Sep-Pak C_{18} cartridge (Waters Associates Inc., Mass.) and eluted successively with 10, 20 and 30% aqueous methanol; b) the eluate in the last fraction was further purified by HPLC (SS-10-ODS column, 4.6×250 mm, eluted with 30% aqueous methanol). The metabolite was negative to the color reaction on a TLC plate after treating with 4-(*p*-nitrobenzyl)-pyridine,[6] suggesting the absence of the 12,13-epoxy function in the metabolite.

The PMR of DOM-1 taken in acetone-d_6 plus D_2O was as follows: (90 MHz, δ); 1.38 (3H, C-14, s), 1.78 (3H, C-16, q), 3.74 (2H, C-15, s), 4.1 to 4.15 (2H, C-2 and C-4), 4.0 to 4.3 (1H, C-3), 4.99 (1H, C-11, d, $J=6$ Hz), and 6.56 (1H, C-10, dd, $J=1.5$ and 6 Hz). Instead of a singlet resonance at δ 3.03 due to methylene protons of the epoxy ring in DON, doublet resonances were observed at δ 4.92 and 5.09 (each 1H, $J=0.99$ Hz) in DOM-1, which were assigned to terminal methylene protons at the C-13 position. According to the formation of this double bond, a singlet signal (δ, 1.09) of methyl protons at C-14 in DON was shifted to the low magnetic field, whereas a methine proton at δ 4.81 (C-7) was shifted to δ 4.58 due to magnetic anisotropy. The CMR of the metabolite was as follows: (δ, acetone-d_6 plus D_2O); 15.31 (C-16), 20.02 (C-14), 45.12 (C-4), 49.45 and 53.45 (C-5 and C-6), 61.32 (C-15), 69.78 (C-3), 70.66 (C-11), 75.16 (C-7), 81.97 (C-2), 135.16 (C-9), 139.99 (C-10), and 202.03 (C-8, in methanol-d_4). Signals at δ 66.42 (C-12, s) and 47.50 (C-13, t) in DON were shifted to δ 155.39 and 106.38, respectively, in the metabolite. Chemical shifts of both carbons and protons at the C-12 and C-13 positions of DOM-1 were similar to those of verrucarin K[7] with a trichothec-9,12-diene skeleton. Furthermore, by treating with *m*-chloroperbenzoic acid in chloroform, the metabolite was quantitatively converted into the parent toxin.

Based upon these data, 3α,7α,15-trihydroxy-trichothec-9,12-dien-8-one (Fig. 1) is proposed for the chemical structure of the newly found metabolite, DOM-1. In view of the metabolism of xenobiotics, it is noteworthy that the data presented here may include a direct deoxygenation (de-epoxydation) at an oxide ring to form a double bond.

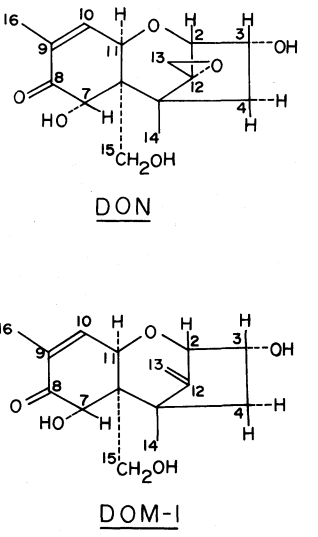

FIG. 1. Structures of Deoxynivalenol (DON) and Its Metabolite (DOM-1).

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