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Synthesis, Characterization, and Occurrence in Bread and Cereal Products of an Isomer of 4-Deoxynivalenol (Vomitoxin)

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Acetylation of 4-deoxynivalenol (DON, vomitoxin) with acetic anhydride/pyridine gave mixtures of di- and triacetyl derivatives, which on further refluxing with acetic anhydride were transformed into an isomer. NMR, MS, IR, and UV spectral data of the latter indicated it to be 3,8,15-triacetoxy-12,13-epoxytrichothec-8-en-7-one. On hydrolysis, it gave a compound isomeric with 4-deoxynivalenol, to which the structure 3,8,15-trihydroxy-12,13-epoxytrichothec-8-en-7-one (isoDON) was assigned. Thermal decomposition of DON under nitrogen at 160 °C for 1 h resulted in approximately 65% decomposition and the formation of five products, the Me_3Si derivative of one having an MS identical with that of Me_3Si-isoDON. Samples of white and whole meal bread baked from fortified and naturally DON contaminated wheat when analyzed by GC/single ion monitoring showed that isoDON was formed to the extent of 3–13% of the DON present; levels were higher in the crust than in the crumbs. No isoDON was found in products made from uncontaminated wheat. IsoDON was also detected in processed wheat-based breakfast cereal made from grain, naturally contaminated with DON at levels ranging from 0.35 to 0.75 mg/kg DON.

Fungal infection of grains (i.e., barley, corn, and wheat) by mycotoxigenic *Fusarium* spp. is a common phenomenon in countries with temperate climates. Occasional, environmental factors favor epidemic development of the fungi, which may result in mycotoxin formation and contamination of food for human and animal consumption. In 1980, such an outbreak occurred in Canada in the Ontario soft white winter wheat crop. The predominant mycotoxin was identified as the trichothecene, 4-deoxynivalenol (DON, vomitoxin, 3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one), present at levels averaging 1 mg/kg, with a maximum of 8.5 mg/kg (Trenholm et al., 1981). A maximum tolerance level of 0.3 mg/kg for uncleaned Ontario soft white winter wheat was introduced in Canada in 1982, but this has since been increased to 2 mg/kg based on estimated losses during further processing and revised toxicological data.

Milling studies with hard red spring wheat naturally contaminated with DON indicated that the mycotoxin was differentially distributed throughout the kernel, with relatively higher levels in the dockage, outer bran fractions, and shorts and lower levels in the inner flour fractions (Scott et al., 1983; Young et al., 1984). DON was not destroyed on processing naturally contaminated Quebec hard spring wheat into flour and baking into bread (El-Banna et al., 1983; Scott et al., 1983), although levels were slightly reduced. Similar results were reported with the milling of contaminated Ontario Soft white winter wheat; further processing of this wheat into a variety of baked products resulted in reductions of DON up to 35% (Young et al., 1984).

In the present baking study with DON-contaminated flour, the conversion of DON to an isomer is reported. This isomer is identical with a compound isolated during acetylation studies with DON based on spectral characteristics. A structure is assigned to the isomer of DON.

MATERIALS AND METHODS

Materials. 4-Deoxynivalenol (DON) was prepared biosynthetically from liquid cultures of *Fusarium roseum* (ATCC 28114) (Greenhalgh et al., 1984). The mixed silylating reagent (Trisil TBT) was purchased from Pierce Chemical Co., Rockford, IL. White winter wheat flour, naturally contaminated with DON (ca. 0.5 mg/kg), was supplied by Health and Welfare, Canada. DON-contaminated wheat samples together with corresponding samples of a wheat-based breakfast cereal were obtained from a Canadian breakfast cereal manufacturer.

Thermal Decomposition of DON. DON (0.5 mg) was sealed under nitrogen in a 1-mL glass vial and heated for 1 h at 160 or 200 °C. After cooling to room temperature, the contents of the vial were dissolved in 10% methanol in $CHCl_3$, and an aliquot was taken and subsequently derivatized.

Bread Manufacture. Loaves of bread were produced in the laboratory by using pilot-scale equipment and were prepared both from naturally DON contaminated flour

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and from fortified flour (0.1 and 3 mg/kg DON) by dissolving the DON in methanol and adding to the water prior to mixing the dough. Doughs were hand mixed by using the following recipe: flour (840 g), yeast (18 g), salt (15 g), fat (6 g), water (506 mL), ascorbic acid (0.025 g), and glutex (10% potassium bromate) (0.25 g). Each dough was mechanically worked in a Morton Z-blade mixer, proved for 10 min at 30 °C, and divided into three equal loaves. After proving for a further 50 min, the loaves were baked for 25 min at 240 °C. The cooled loaves were divided roughly into crust and crumb, homogenized into bread crumbs, and stored at −18 °C prior to analysis.

Extraction and Cleanup. The procedure of Scott et al. (1981) was modified to allow for the water content of the bread by reducing the volume of water used in the preliminary water/methanol extraction. To check the recovery, each batch of four DON-contaminated samples included one control bread sample (flour or wheat as appropriate) fortified with DON (0.2 mg/kg).

Derivatization. The dried extract (4 g equiv of cereal) in a 1-mL glass vial was treated with 0.1 mL of mixed silylating agent and after being allowed to stand for a few min was analyzed directly by gas chromatography/mass spectrometry (GC/MS).

Equipment. A Carlo Erba 4160 GC fitted with capillary columns was interfaced with either of two MS systems, one a VG 7070H and the other a VG 12000 quadrupole instrument. Chromatography utilized a 25 m × 0.22 mm i.d. fused silica CP SIL 5 CB column operating with helium carrier gas (0.75 bar). Standards and heat-degraded material were analyzed by split injection (25:1) (1 μL), whereas food extracts were analyzed in the splitless mode, the injector being purged with helium at 20 mL/min, 40 s after injection. In both cases, the injector temperature was 250 °C and the column temperature was held initially at 150 °C for 1 min, then programmed at 5°C/min to 220 °C, and again held isothermally for 5 min.

Both MS were operated in the electron impact mode (EI) (70-eV electron energy: 200-μA trap current) with a source temperature of 200 °C, the accelerating voltage for the VG 7070H being 4 kV. The VG 7070H was scanned from m/z 600–20 at 1 s/decade whereas the 12000 quadruple was scanned from m/z 40–600 in 1 s with 0.7-s interscan delay. Single ion monitoring (SIM) on the VG 7070H was by field-controlled magnet current set at m/z 512 [molecular ion of $(Me_3Si)_3$-DON], and the source and collector slit widths were adjusted to give flat-topped peaks with sufficient resolution to separate unit masses at m/z 512. Multiple ion detection (MID) on the 12000 quadrupole instrument was under computer control with a 100-ms dwell time on each of the ions, m/z 512, 497, and 422.

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 250 and 62.5 MHz, respectively, on a Bruker WM 250 NMR spectrometer. Chemical shifts are referenced to $CDCl_3$ at 7.24 and 77.0 ppm, respectively, for ^1H and ^{13}C and are reported as ppm relative to Me_4Si. All compounds were dissolved in $CDCl_3$ for analysis. ^1H spectra were accumulated by using 16K data points, a 3-kHz spectral window, 60° (4 μs) pulses, and a 5-s repetition rate. Resolution was enhanced by Gaussian line shape transformation. ^1H assignments were confirmed by $^1H/^1H$ correlation spectroscopy [COSY (Bax and Freeman, 1981)] employing a (90°–D–t_1–45°–D–acquisition) pulse sequence with pulse phasing for N-type selection. D was set at 0 to emphasize short-range coupling or at 0.25 s for long-range couplings (1 Hz), which are not resolvable in a normal 1D spectrum. One-hundred forty-eight 1K spectra of 16 scans each were accumulated by using 90° pulses of 6-μs and 5-s recycle time. Zero filling in the f_1 dimension was used to give a final data matrix of 1K × 512W, resulting in a resolution of 0.3 Hz. ^{13}C spectra were accumulated by using 16K data points, a 15-kHz spectral window, 45° (6 μs) pulses, and a 2-s recycle. ^{13}C assignments and multiplicities of all spectral resonances were confirmed with $1/J$ (8 ms) spin-echo spectra (Blackwell et al., 1984).

Acetylation of DON. Deoxynivalenol (125 mg) or the product after acetylation with acetic anhydride/pyridine at room temperature was refluxed in acetic anhydride (10 mL) with stirring for 6 and 2 h, respectively. The reaction mixture was cooled and then neutralized by decanting into a cold saturated solution of sodium bicarbonate. The neutral solution was extracted with chloroform (2 × 60 mL) and the extract concentrated under vacuum. The residue was further purified by preparative thin-layer chromatography (TLC) on silica gel plates developed with ethyl acetate/hexane (1/1). A minor compound, R_f 0.68 (23 mg), was crystallized from ethanol: mp 154–156 °C (lit. mp 156–157 °C); MS 422.1592, calcd for $C_{21}H_{26}O_9$ 422.1574 (Yoshizawa and Morooka, 1973; Blight and Grove, 1974). The MS and NMR data are identical with those reported for 3,7,15-triacetoxy-12,13-epoxytrichothec-9-en-8-one. The major component, R_f 0.47 (98 mg), was also recrystallized from ethanol: mp 110–112 °C; MS 422.1586, calcd for $C_{21}H_{26}O_9$ 422.1574; IR (Nujol) 1754, 1740, 1728, 1672, and 1652 cm^{-1}; UV λ_{max} 248 nm; ^1H NMR δ 1.26 (3 H, H-14), 1.86 (3 H, H-16), 2.01 (3 H, Ac-CH_3), 2.09 (3 H, Ac-CH_3), 2.13 (2 H, H-4,m), 2.20 (3 H, Ac-CH_3), 2.55, 2.96 (2 H, H-10, J_{ab} = 18.9 Hz, $J_{10,11}$ = 2.2, 3.6 Hz), 2.76, 2.96 (2 H, H-13, J_{ab} = 3.8 Hz), 3.75 (1 H, H-2, $J_{2,3}$ = 4.3 Hz), 4.12, 4.38 (2 H, H-15, J_{ab} = 11.8 Hz), 4.43 (1 H, H-11, $J_{10,11}$ = 2.2, 3.6 Hz), 5.14 (1 H, H-3, $J_{2,3}$ = 4.3 Hz, $J_{3,4}$ = 10.1, 4.6 Hz); ^{13}C NMR δ 13.9 (C-14), 17.8 (C-16), 20.2, 20.8, 20.9 [3 CH_3 (Ac)], 35.0 (C-10), 41.0 (C-4), 45.6 (C-5), 50.0 (C-13), 54.5 (C-6), 64.4 (C-15), 65.1 (C-12), 70.7 (C-11), 71.1 (C-3), 78.9 (C-2), 141.1 (C-9), 141.9 (C-8), 168.1, 170.0, 170.3 [C=O (Ac)], 190.0 (C-7) (assignments may be reversed for 141.1 and 141.9). These data are compatible with the structure 3,8,15-triacetoxy-12,13-epoxytrichothec-8-en-7-one (I, isoAcDON).

Hydrolysis of IsoAcDON. 3,8,15-Triacetoxy-12,13-epoxytrichothec-8-en-7-one (70 mg) in absolute ethanol (5 mL) was treated for 30 min at room temperature with 0.1 M sodium ethoxide (3 mL). The reaction mixture was passed through Amberlite IR-120 ($^+$H) ion-exchange resin and the ethanol removed under vacuum. The residue was purified by preparative TLC (ethyl acetate) and gave a compound, R_f 0.53 (29 mg), as an amorphous solid: MS 296.1265, calcd for $C_{15}H_{20}O_6$, 296.1261; UV λ_{max} 277 nm; ^1H NMR δ 1.30 (3 H, H-14), 1.9 (3 H, H-16, $J_{16,10b}$ = 1.9 Hz), 2.04, 2.26 (2 H, H-4, J_{ab} = 14.7 Hz, $J_{3,4}$ = 10.8, 4.4 Hz), 2.47, 2.95 (2 H, H-10, J_{ab} = 19.0 Hz, $J_{10,11}$ = 2.2, 4.0 Hz, $J_{16,10b}$ = 1.9 Hz), 2.79, 2.85 (2 H, H-13, J_{ab} = 4.3 Hz), 3.45 (1 H, H-2, $J_{2,3}$ = 4.4 Hz), 3.62, 3.90 (2 H, H-15, J_{ab} = 11.4 Hz), 4.45 (1 H, H-3, $J_{2,3}$ = 4.4 Hz, $J_{3,4}$ = 10.8, 4.4 Hz), 4.69 (1 H, H-11, $J_{11,10}$ = 2.2, 4.0 Hz), 6.12 (1 H, HO-8). ^{13}C NMR δ 14.3 (C-14), 17.0 (C-16), 34.1 (C-10), 43.9 (C-4), 46.7 (C-5), 49.4 (C-13), 57.1 (C-6) (tentative assignment), 64.0 (C-15), 67.2 (C-12), (tentative assignment), 69.1 (C-3), 71.3 (C-11), 80.8 (C-2), 126.7 (C-8), 196.7 (C-7). These data are compatible with the structure 3,8,15-trihydroxy-12,13-epoxytrichothecen-8-en-7-one (II, isoDON).

RESULTS AND DISCUSSION

Acetylation Studies. Acetylation of DON with acetic anhydride and pyridine in our hands yielded mixtures of the di- and triacetyl derivatives, and to complete the re-

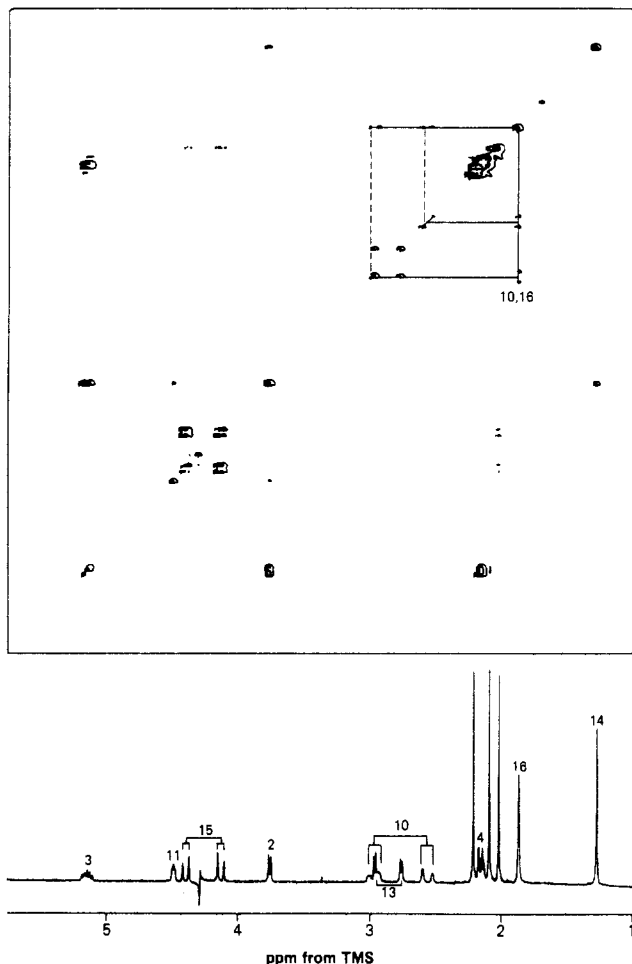

Figure 1. 250-MHz $^1H/^1H$ correlation NMR spectrum (COSY) for 3,8,15-triacetoxy-12,13-epoxytrichothec-9-en-7-one. The delay (D) in the pulse sequence (90°–D–t_1-45°-D-acquire) has been set to emphasize long-range couplings (1–2 Hz). The normal 1D spectrum is shown below the plot on the same scale.

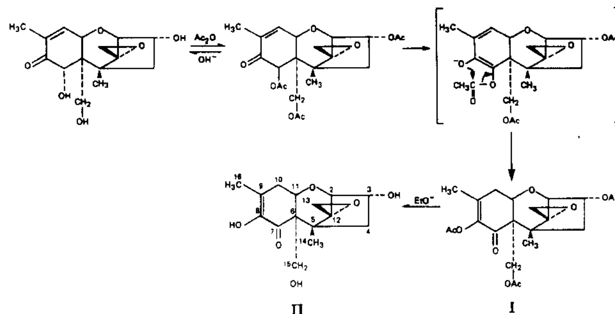

Figure 2. Proposed mechanism for the formation of iso-4-deoxynivalenol (II) from 4-deoxynivalenol (DON) via triacetyldeoxynivalenol and isotriacetyldeoxynivalenol (I).

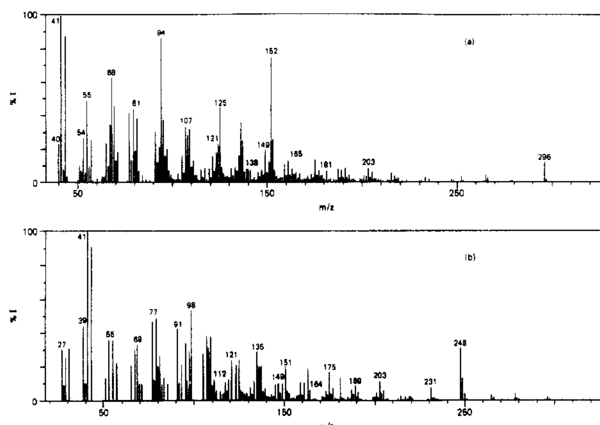

Figure 3. Electron impact mass spectra of (a) iso-4-deoxynivalenol and (b) 4-deoxynivalenol. Samples analyzed by direct insertion probe using a VG 12000 quadruple, scanning from m/z 600 to 40 in 1 s, and temperature programming at the maximum rate at 250 °C.

action, the mixtures were further refluxed with acetic anhydride to afford a single product. This product was an isomer of triacetylDON and had different IR, UV, MS, and NMR spectral characteristics. Comparison of the ^1H NMR with that of triacetylDON indicated the absence of resonances at 6.6 ppm (H-10) and 6.05 ppm (H-7) and those assigned for H-11 and H-13 had moved upfield from 4.70 to 4.43 ppm and from 2.97 and 3.10 to 2.77 and 2.96 ppm, respectively; the H-14 methyl resonance had moved downfield 0.92 ppm to 1.26 ppm. An additional quartet was observed at 2.96, 2.55 ppm. A $^1H/^1H$ correlation (COSY) spectrum established coupling between this quartet and H-11. A second COSY spectrum, in which long-range couplings (1–2 Hz) are emphasized, is shown in Figure 1. The ^1H spectrum appears along the diagonal and can be compared to the 1D spectrum below the 2D plot. Off-diagonal resonances indicate long-range coupling between the quartet (2.96, 2.55 ppm) and the C-16 methyl protons, which is not resolved in the normal high-resolution spectrum. These data confirmed the presence of a methylene group at C-10. In comparison with the ^{13}C NMR of DON, the C-7 CHOH had disappeared and the new CH_2 resonance at 35 ppm was assigned to C-10, the double bond resonance was shifted downfield and assigned to C-8, C-9, and the keto resonance at 190 ppm was assigned to C-7. These data indicate the structure to be 3,8,15-triacetoxy-12,13-epoxytrichothec-8-en-7-one (I).

Hydrolysis of I, under the same reaction conditions that converted triacetylDON to DON, resulted in a product (II) isomeric with DON. Capillary GC of the HFB derivative of II showed the same retention time as for DON, whereas those of the Me_3Si derivatives differed. The ^1H and ^{13}C NMR of II were compared with those of I. The quartet at 2.47, 2.95 ppm (2 H) for H-10 was still present and a new resonance (6.12 ppm, 1 H) appeared, which was assigned to the enol OH proton at C-8. These data indicate the structure to be 3,8,15-trihydroxy-12,13-epoxytrichothec-8-en-7-one (isoDON, II). A proposed mechanism for the rearrangement of triacetylDON via an enol intermediate and its subsequent hydrolysis to give II is shown in Figure 2.

The electron impact MS of isoDON and DON are shown in Figure 3. In DON, the molecular ion (m/z 296) is of low intensity and the ion m/z 248 (M – 48) corresponds to loss of water and formaldehyde with rearrangement occurring through proton transfer. Vesonder et al. (1973) suggested that these losses are consistent with a trichothecene A-ring containing a ketone (C-8), hydroxyl (C-7), and hydroxymethyl (C-6) groups. The altered structure of isoDON markedly affects the fragmentation pattern; with the M – 48 elimination being no longer facile, the M – 48 ion is essentially absent. Other differences in the spectra include the presence of intense ions, m/z 152 and m/z 94, which are absent in DON, and result from further significant fragmentation and rearrangement.

The GC/MS of Me_3Si-isoDON and Me_3Si-DON confirm the presence of three hydroxyl moieties with the increase in molecular weight from 296 to 512. Comparison of the spectra of the Me_3Si derivatives shows an enhanced ion m/z 497 for isoDON, presumably due to loss of a methyl group and the absence of an ion (M – 90) at m/z 422 due to the loss of $(CH_3)_3SiOH$ group, which suggests the de-

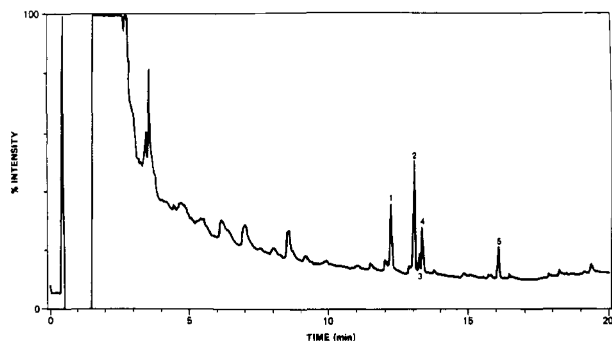

Figure 4. Total ion current chromatogram for the products of thermal decomposition (160 °C for 1 h) of 4-deoxynivalenol. Sample analyzed as Me_3Si derivatives on VG 7070H by splitless injection onto a capillary column (CP SIL 5), temperature programmed from 150 °C, isothermal for 1 min, and then programmed at 5 °C/min to 220 °C. Spectra were obtained by scanning at 1 s/decade from m/z 650.

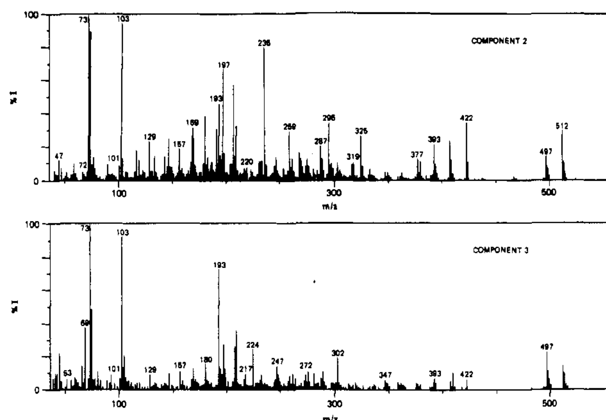

Figure 5. Electron impact spectra of Me_3Si derivatives of components 2 and 3 from thermally decomposed 4-deoxynivalenol. Component 2 corresponds to authentic Me_3Si-DON and component 3 to Me_3Si-isoDON; GC/MS conditions were identical with those in Figure 3.

rivatized hydroxyls in isoDON to be more stable than those in DON. Other notable differences in the spectra are the absence of m/z 235 in Me_2Si-isoDON and the presence of m/z 193 as a major fragment ion.

Thermal Decomposition. The thermal decomposition of DON was examined, by Me_3Si derivatization of the products and subsequent analysis by MS. Single ion monitoring (m/z 512) showed that a loss of approximately 65% of DON occurred after heating for 1 h at 160 °C in a nitrogen atmosphere and almost 95% at 200 °C, although in the latter case some charring occurred. A total ion chromatogram for the heat degraded material is shown in Figure 4 with components 1–5 being DON derived. Examination of compounds 1, 4, and 5 by TLC using a variety of visualizing agents indicated the absence of an epoxy moiety, and their characterization will be the subject of a further publication. The retention time of compound 2 corresponded that of Me_3Si-DON, that of compound 3 corresponded to that of Me_3Si-isoDON; the mass spectra (Figure 5) were identical with those of standards. Me_3Si-isoDON was characterized by a molecular ion (m/z 512), an enhanced ion at m/z 497 (M − 15), reduced intensity of the ions from m/z 393 to 422, the absence of m/z 235, and an intense ion at m/z 193. The characterization of Me_3Si-isoDON shows unequivocally that the isomer can be formed by heat-induced rearrangement.

Analysis of Foods Subjected to Thermal Processing. Control samples of bread crust and crumb of loaves baked in the laboratory were analyzed by GC/SIM (m/z 512) and showed no DON present. The recovery form crust and crumb samples fortified with DON (0.2 mg/kg) was 75% with a single sharp peak for Me_3Si-DON and no evidence for the presence of Me_3Si-isoDON.

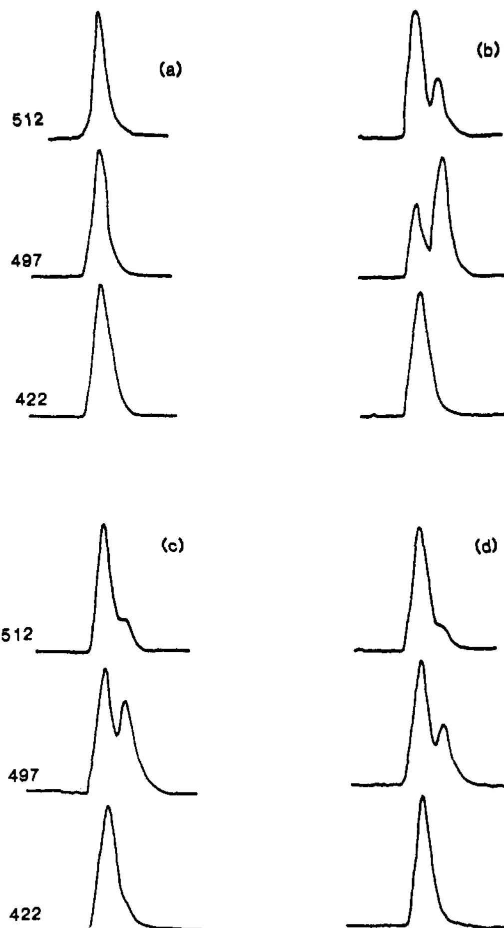

Figure 6. Multiple ion detection chromatograms. Traces obtained on a VG 12000 quadruple MS instrument switching under computer control between m/z 512, 497, and 422 with 100-ms dwell time/mass. Chromatographic conditions were as for Figure 4, but with splitless injection. Selected regions only for small time windows of the chromatograms are illustrated for Me_3Si derivatives of (a) unheated DON, (b) heat-degraded DON, (c) extract from the crust of whole meal bread baked with naturally contaminated flour, and (d) extract from a breakfast cereal manufactured from DON-contaminated wheat.

However, in crust and crumb samples of bread (white and whole meal) baked from naturally DON contaminated and fortified flour, an additional peak at m/z 512 was detected. The full MS spectrum corresponded to that of isoDON formed in the model thermal degradation study and the synthetic sample. By use of m/z 512 for quantification, an estimated 3–13% of the isomer was shown to be present in the samples. The amount of isoDON was greater in the crust than in the crumbs, which is consistent with the greater thermal treatment. The relative increase of m/z 497 to m/z 512 intensity provides a good means of monitoring isomerization, and multiple ion detection (MID) was employed for the ions at m/z 512, 497, and 422 (M − 90). The MID traces of a limited region of the capillary column chromatograms are displayed in Figure 6. It shows the absence of isoDON in Me_3Si-derivatized unheated DON (Figure 6a). In the model system for heat-degraded DON, the isomer appears as a second peak in the m/z 512 trace and as a major component for m/z 497 (Figure 6b). The ion m/z 422 is essentially absent from the isoDON spectrum, which corresponds to that found with the heat-degraded material. The results for a sample

of crust of whole meal bread show the barely discernible traces of isoDON at m/z 512, but it is clearly evident although not completely resolved at m/z 497 (Figure 6c).

Both natural wheat and flour were analyzed for DON and in no cases was isoDON detected; it occurred only in samples that had been heat treated. Identical analytical procedures were used in an extensive survey of barley samples for DON (Gilbert et al., 1983) and for confirmation of positive DON results in wheat and corn samples from different countries (Osborne and Willis, 1984). In neither case was any evidence found for isoDON, although only 35 samples out of a total of 360 contained DON levels >0.1 mg/kg.

Samples of wheat-based breakfast cereal made from three different batches of naturally DON contaminated wheat at levels ranging from 0.35 to 0.75 mg/kg were analyzed. The manufacturing process involved pressure cooking at 45 psi, partial drying, milling, flaking, and finally toasting of the product at 175–180 °C. Analyses of this breakfast cereal showed that significant amounts of DON survived processing. As with the bread samples, there is an indication from the MID traces of the presence of isoDON, this being most evident in the trace for m/z 497 (Figure 6d).

In summary, DON undergoes isomerization, which can be induced either chemically or thermally to form an isomer, isoDON. Chemically, an enol intermediate is proposed, followed by acyl migration from C-8 to C-7 and double bond migration to the C-8, C-9 position. Thermally, a similar rearrangement can be induced as has been demonstrated synthetically and by its presence in bread, especially the crust, and wheat-based cereal.

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Structure of a Metabolic Derivative of T-2 Toxin (TC-6) Based on Mass Spectrometry

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A new metabolite of T-2 toxin in cows named TC-6 has been tentatively identified as 3′-hydroxy-7-hydroxy-HT-2 toxin. The new metabolite is related to TC-3 (3′-hydroxy-HT-2 toxin) and like TC-3 forms two isomers when reacted with trifluoroacetic acid anhydride. Its only difference from TC-3 is a hydroxyl group tentatively assigned to the C-7 position. This metabolite is a product of T-2 metabolism in the cow and can be found in feces and urine.

T-2 toxin [4β,15-diacetoxy-8-[(3-methylbutyryl)oxy]-3-hydroxy-12,13-epoxytrichothec-9-ene] is a trichothecene mycotoxin produced by various species of *Fusarium* but predominantly by *Fusarium tricinctum* or *Fusarium sporotrichioides*. These two species are thought to be the same by some taxonomists but classified separately by others. The distribution of this toxin in animals has been studied by Chi et al. (1978) in chicks, Robison et al. (1979a,b) in bovine and porcine milk, Robison et al. (1979a,b) in swine, Yoshizawa et all. (1980) in chickens, and Yoshizawa et al. (1981) in a lactating cow.

The products of metabolism of T-2 in the cow have been reported as HT-2, neosolaniol, 4-deacetylneosolaniol, and unkown derivatives called TC-1, TC-3, TC-5, TC-6, TC-7, and TC-8 (Yoshizawa et al., 1981). Similarly, in the chicken, the metabolic products were described as HT-2, neosolaniol, TB-1, TB-3, TB-4, TB-5, TB-6, TB-7, and TB-8 (Yoshizawa et al., 1980). Presently we are not certain which of the unknown chicken metabolites (TB series) correspond to the bovine metabolites (TC series) although we feel they are identical or closely related. Recently, TC-1 and TC-3, found in the cow, were identified as 3′-hydroxy-T-2 toxin and 3′-hydroxy-HT-2 toxin, respectively (Yoshizawa et al., 1982). We have obtained mass spectral evidence for the identification of TC-6, and the presentation of this data is the subject of this paper.

In order to substantiate the evidence used to elucidate the structure of TC-6 (Figure 1), it is necessary to review the reactivity of TC-1 and TC-3 in a chromatographic system (Pawlosky et al., 1984). Both TC-1 and TC-3 react with silylating reagents to form the fully silylated derivatives that resolve on a SE-54 (DB-5) bonded phase capillary column. In methane chemical ionization mass spectrometry, the $M^+ + 1$ ions 657 for TC-3 and 627 for TC_1 are formed. However, when TC-1 and TC-3 are reacted with trifluoroacetic acid anhydride, two isomers are

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