Enzyme-Linked Immunosorbent Assay Employing Monoclonal Antibody Specific for Deoxynivalenol (Vomitoxin) and Several Analogues[1]

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A monoclonal antibody was prepared against deoxynivalenol (DON, vomitoxin), a trichothecene mycotoxin occurring in grain contaminated with *Gibberella zeae* (anamorph = *Fusarium graminearum*), and incorporated into competitive direct and indirect enzyme-linked immunosorbent assays (ELISAs). DON antibodies were secreted by hybridomas derived from mice inoculated with DON conjugated to bovine serum albumin. Conjugation of DON to carrier proteins was facilitated by conversion of DON to 3-*O*-hemisuccinyl-DON after protection of two of the three available hydroxyls with a cyclic boronate ester. DON was detectable at 10–250 ng/assay (0.2–5.0 μg/mL) for direct ELISA and 10–150 ng/assay (0.2–2.0 μg/mL) for indirect ELISA. The monoclonal antibody cross-reacts with 3-acetyl-DON, 3-*O*-hemisuccinyl-DON, DON, 12,13-deepoxy-DON, nivalenol, and fusarenone X (in order of decreasing affinity) but has low affinity for 15-acetyl-DON and T-2 toxin.

Deoxynivalenol (DON, vomitoxin) is one of the sesquiterpene mycotoxins classified as 12,13-epoxytrichothecenes (Mirocha et al, 1977). It occurs naturally in infected corn (Hart et al., 1982; Mirocha et al., 1976), small grains (Hart and Braselton, 1983; Neish and Cohen, 1981), and mixed feeds (Mirocha et al., 1976). DON is produced by *Gibberella zeae* (Schw.) Petch (anamorph = *Fusarium graminearum* Schw.) and is associated with emesis and feed refusal in swine (Mirocha et al., 1976); it inhibits protein synthesis (Thompson and Wannemacher, 1986) and impairs human lymphocyte blastogenesis (Forsell and Pestka, 1985). In vivo effects of DON include skin irritation, hemorrhaging, hematological changes, radiomimetic effects, and immunosuppression (Forsell et al., 1986; Pestka et al., 1987; Ueno, 1983).

A rapid, simple assay method for DON that does not require extensive laboratory equipment would be beneficial for routine screening of commodities intended for consumption by animals and humans and in the diagnosis of DON-related toxicoses in production animals. Detection methods for DON (Pathre and Mirocha, 1977; Scott, 1982), including gas chromatography, gas chromatography–mass spectrometry, high-pressure liquid chromatography, and thin-layer chromatography, involve considerable sample preparation, are time-consuming, and require technical expertise. Enzyme-linked immunosorbent assays (ELISA) employing polyclonal (Gendloff et al., 1984; Pestka et al., 1980; Ram et al., 1986; Warner et al., 1986) or monoclonal (Dixon et al., 1987; Gendloff et al., 1987) antibodies have been developed to detect other mycotoxins. These assays require little sample preparation, are rapid and relatively simple to execute, yet are sensitive. In addition, enzyme immunoassays lack the health hazards associated with radioimmunoassays. Monoclonal antibodies have important advantages over polyclonal antibodies. They may be extremely specific, even if the immunizing antigen was not pure, and offer a theoretically unlimited supply of homogeneous reagent (Goding, 1980).

Elicitation of antibodies with affinity for the native DON molecule has been elusive. Small molecules such as DON will not elicit an immune response without attachment to larger, immunogenic molecules. Since DON cannot be directly conjugated to carrier proteins, chemical groups capable of covalent linkage to protein must be introduced onto the molecule. This allows for some versatility in the orientation of the antigen (DON) for presentation to the immune system. Introduction of a carboxyl group for protein conjugation can most easily be accomplished at the C8-keto position by preparing DON 8-*O*-carboxymethyl oxime. A similar approach was used to produce antibodies against aflatoxin B_1 (Chu et al., 1977). An alternate method for carboxylation involves esterification of a hydroxyl using a cyclic acid anhydride (Erlanger et al., 1957). The presence of three hydroxyls on the DON molecule, however, might result in the addition of multiple carboxyls to DON using standard methods, leading to undesirable cross-linking of carrier proteins and masking of antigenic determinants peculiar to DON. This problem can be solved by protecting two of the hydroxyls during derivatization to carboxylated DON. Phenylboronate esters of 1,3-diols have been used as protecting agents in organic synthesis (Ferrier and Prasad, 1965; Perun et al., 1974; Yurkevich et al., 1969). These studies suggested protecting the C7- and C15-hydroxyls (a 1,3-diol) of DON by formation of a cyclic boronate ester prior to esterification with a cyclic acid anhydride We successfully used this approach to derivatize DON exclusively at C3.

We report here the production of monoclonal antibodies that can be used to detect DON and several analogues in direct and indirect enzyme immunoassays. The approach described may be applicable to other molecules with similar problems of immunogenicity.

MATERIALS AND METHODS

Materials. Deoxynivalenol (DON) and 15-acetyldeoxynivalenol were produced in culture and purified by the method of Witt et al. (1985) and Pestka et al. (1986), respectively. Fusarenone X (FX) and nivalenol were supplied by Romer Laboratories (Washington, MO 63090) and 3-acetyldeoxynivalenol (3-Ac-DON) and 12,13-deepoxydeoxynivalenol (DOM-1) by Steven P. Swanson (Department of Veterinary Biosciences, University of Illinois, Urbana 61810). Sources: *N*-hydroxysuccinimide, Aldrich Chemical Co. (Milwaukee, WI 53233); 1-butylboronic acid, Alltech Associates, Inc. (Deerfield, IL 60015); Adsorbosil silica gel (200/435 mesh), Anspec (Ann Arbor, MI 48107);

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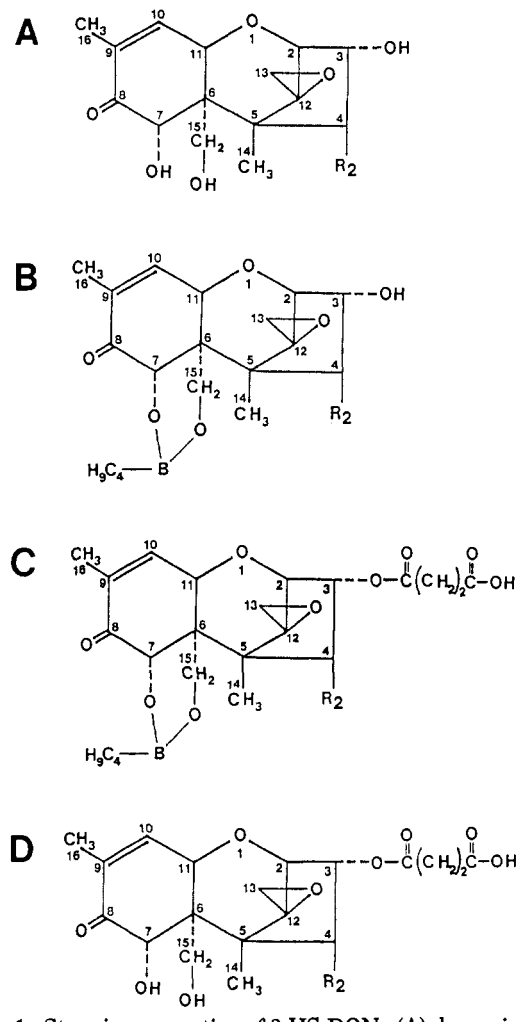


Figure 1. Steps in preparation of 3-HS-DON: (A) deoxynivalenol (DON); (B) 7,15-*O*-(butylboronyl)-DON; (C) 3-*O*-hemisuccinyl-7,15-*O*-(butylboronyl)-DON; (D) 3-*O*-hemisuccinyl-DON (3-HS-DON).

goat anti-mouse immunoglobulin conjugated to horseradish peroxidase (GAM-HRP), Cooper Biomedical (Malvern, PA 19355); silica gel G TLC plates (20 × 20 cm), Fisher Scientific Co. (Pittsburg, PA 15219); 2,2'-azinobis(3-ethylbenzothiazolinesulfonicacid (ABTS), bovine serum albumin (BSA), carboxymethoxylamine ((aminooxy)acetic acid), 1,3-dicyclohexylcarbodiimide, glutaric anhydride, horseradish peroxidase (HRP), 4-(*p*-nitrobenzyl)pyridine (NBP), ovalbumin (OA), succinic anhydride, and T-2 toxin, Sigma Chemical Co. (St. Louis, MO 63178); LHP-K silica gel TLC plates (10 × 10 cm), Whatman (Clifton, NJ 07014). Myeloma cell line P3/NS1/1-Ag4-1 (NS-1) was obtained from American Type Culture Collection, Rockville, MD 20852.

Derivatization of DON. 3-*O*-Hemisuccinyl-DON (3-HS-DON) was prepared by protection of the C7- and C15-hydroxyls with a cyclic boronate ester, esterification at the C3-position, and then removal of the boronate ester. The reaction sequence is outlined in Figure 1. In a 2-mL reaction vial, 68 mg of 1-butaneboronic acid was added to 20 mg of DON dissolved in 200 μL of pyridine. The mixture was stirred overnight at room temperature and yielded 7,15-*O*-(butylboronyl)-DON (compound B, Figure 1). Then, 800 μL of 1.7 M succinic anhydride (in pyridine) was added with stirring. The reaction vial was sealed and the mixture stirred for 90 min in a boiling water bath, yielding 3-*O*-hemisuccinyl-7,15-*O*-(butylboronyl)-DON (compound C, Figure 1). The pyridine was evaporated

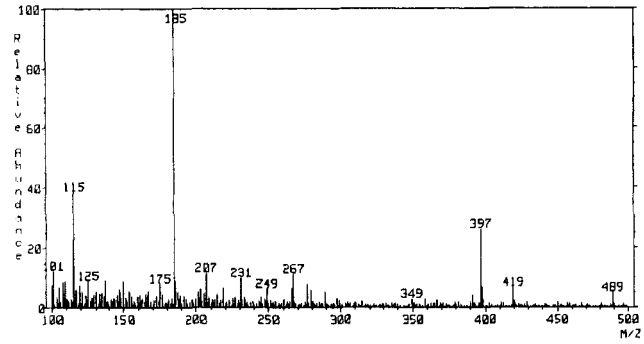


Figure 2. Positive-ion FAB-MS (6-kV xenon atom beam) for 3-*O*-hemisuccinyldeoxynivalenol run in a glycerol matrix, obtained on a JEOL HX-110 HF mass spectrometer.

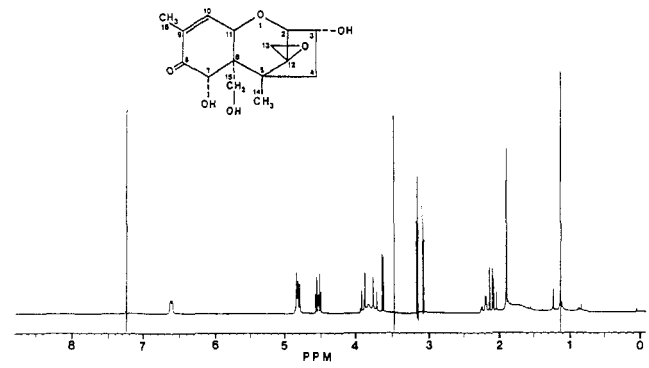


Figure 3. ^1H NMR spectrum for deoxynivalenol in $CDCl_3$, obtained on a Bruker WM-250 (250-MHz) FT spectrometer.

under a stream of N_2 at 100 °C and the residue dissolved in a small amount of methanol. This solution was air-dried onto 1 g of silica gel and was carefully layered as a slurry in benzene onto a silica gel column (25 × 300 mm) packed in benzene. The column was washed with 100 mL of benzene, and then excess succinic anhydride was eluted with benzene–ethyl acetate–acetic acid (80:20:1), at a flow rate of 7 mL/min until no nonvolatile material was present in the eluate (approximately 700 mL). Derivatized DON (3-*O*-hemisuccinyl-DON; compound D, Figure 1) was eluted with benzene–ethyl acetate–acetic acid (70:30:1); 8-mL fractions were monitored by silica gel TLC. Unreacted DON and hemisuccinyl derivatives were visualized with NBP (Takitani et al., 1979). Fractions containing a compound at R_f 0.61 (3-*O*-hemisuccinyl-DON), which appeared blue after treatment with NBP, were pooled and evaporated to dryness under reduced pressure in a rotary evaporator at 50 °C. Additional benzene was added as needed to remove the last traces of acetic acid as an azeotrope. The residue was taken up in 5 mL of water. Chloroform (5 mL) was added to the aqueous solution and the mixture shaken vigorously. The aqueous phase was removed, and the organic phase was extracted with 5 mL of water, four times. The aqueous phases were pooled and evaporated to dryness under reduced pressure in a rotary evaporator at 50 °C. The residue (3-HS-DON) was dissolved in ethyl acetate. After TLC on Whatman LHP-K silica gel (chloroform–methanol, 1:1), 3-HS-DON gave a single charred spot (R_f 0.61) when sprayed with 30% H_2SO_4 and heated at 100 °C. The yield from 20 mg of DON was 15 mg DON-equivalents of 3-HS-DON, as estimated by TLC visualized with NBP and compared with DON standards. The presence of specific chemical groups on 3-HS-DON was tested by treating TLC plates with selective visualizing reagents (see Results and Discussion).

Spectral data for 3-HS-DON were as follows: positive-ion FAB-MS (Figure 2) m/z 115 (glycerol + Na), 185

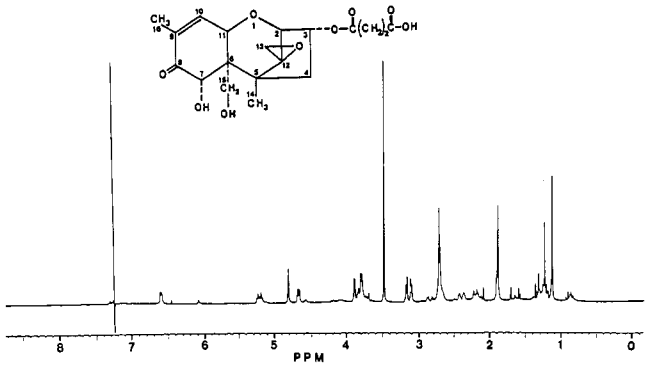


Figure 4. ^1H NMR spectrum for 3-*O*-hemisuccinyldeoxynivalenol in $CDCl_3$, obtained on a Bruker WM-250 (250-MHz) FT spectrometer.

([glycerol]$_2$), 207 ([glycerol]$_2$ + Na), 397 (3-HS-DON), 419 (3-HS-DON + Na), 489 (3-HS-DON + glycerol); ^1H NMR (Figure 3) δ 1.15 (3 H, H-14), 1.89 (3 H, H-16), 2.20 (2 H, H-4), 3.11, 3.17 (2 H, H-13), 3.49 (1 H, H-2), 3.81, 3.90 (2 H, H-15), 4.68 (1 H, H-11), 4.83 (1 H, H-7), 5.21 (1 H, H-3), 6.61 (1 H, H-10).

Spectral data for underivatized DON were as follows: positive-ion FAB-MS m/z 297; ^1H NMR (Figure 4) δ 1.14 (3 H, H-14), 1.91 (3 H, H16), 2.10 (2 H, H-4), 3.09, 3.14 (2 H, H-13), 3.62 (1 H, H-2), 3.77, 3.89 (2 H, H-15), 4.55 (1 H, H-3), 4.81 (1 H, H-11), 4.85 (1 H, H-7), 6.62 (1 H, H-10).

Some hemisuccinyl-DON was also prepared by direct reaction with succinic anhydride, without protection by the cyclic boronate ester (HS-DON mixture). The 3-*O*-hemiglutarate derivative of DON (3-HG-DON) was prepared as was 3-HS-DON, substituting glutaric anhydride for succinic anhydride. DON 8-*O*-carboxymethyl oxime (DON-CMO) was synthesized by the method of Chu et al. (1977) as for aflatoxin B_1 oxime.

DON–Protein Conjugates. 3-HS-DON, 3-HG-DON, and DON-CMO were conjugated to BSA (bovine serum albumin, fatty acid free, Sigma), OA (ovalbumin, fraction V, Sigma), and HRP (horseradish peroxidase, fraction VI, Sigma) through an activated *N*-hydroxysuccinimide ester (Bauminger et al., 1973). Conjugation ratios (moles of DON/mole of protein) were estimated from the number of unreacted amino groups (Habeeb, 1966). Total protein was determined by the method of Bradford (1976). Conjugation ratios were typically 5–10 for DON–OA conjugates and 20–30 for DON–BSA conjugates (conjugation ratios for DON–HRP were not determined).

Immunization of Mice. Six-week-old BALB/c female mice were injected intraperitoneally or subcutaneously with either 3-HS-DON–BSA, 3-HG-DON–BSA, or DON-CMO–BSA. Initial intraperitoneal inoculations were 100–250 μg of conjugate in 0.5 mL of saline–Freund's complete adjuvant (1:1), followed 2 weeks later by 100–250 μg of conjugate in 0.5 mL of saline–Freund's incomplete adjuvant (1:1) (Herbert, 1978). Subsequent intraperitoneal booster inoculations followed at 2-week intervals and consisted of 100–250 μg of conjugate in 0.5 mL of saline. Subcutaneous inoculations were made in the shoulder region with 250–500 μg of conjugate in 0.5 mL of saline, boosted at 2–9-week intervals with a similar dosage. Mice were bled 1 week after each booster inoculation via the retroorbital venous plexus (Herbert, 1978). Antisera were screened for anti-DON activity with the indirect ELISA described below.

Hybridoma Production. Hybridoma cell lines were produced from mice exhibiting anti-DON activity by poly(ethylene glycol) fusion of spleen cells with NS-1 myeloma cells as described previously (Dixon et al., 1987). Hybridoma colony supernatants were screened for anti-DON antibody activity in the indirect ELISA described below. Selected hybridomas with anti-DON activity were stored in fetal bovine serum–dimethyl sulfoxide (9:1) under liquid N_2. Reagent monoclonal antibodies were purified from ascites fluid by ammonium sulfate precipitation (Dixon et al., 1987).

Indirect ELISA. Microtiter plates (96-well Immulon I, Dynatech Laboratories, Inc., Alexandria, VA 22314) were coated with DON–OA or OA (control), and the assay was performed as described previously (Dixon et al., 1987). Absorbance at 405 nm was determined for each well with an EIA Reader Model EL 308 (Bio-Tek, Winooski, VT 05404). Antiserum was considered to contain DON-specific antibody (anti-DON) if binding of antibody to the solid-phase DON–OA (detected by absorbance greater than for solid-phase OA control) was inhibited by free DON.

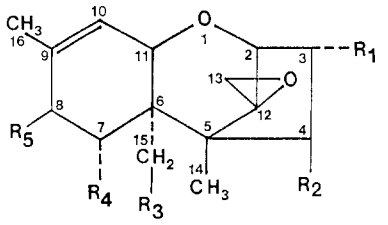
Direct ELISA. Direct ELISA was performed by a modification (Warner et al., 1986) of the procedure described previously by Pestka et al. (1980). Optimum concentrations of antibody and DON–HRP were determined experimentally with serial dilutions of these reagents. Briefly, 125 μL of antibody (3 μg/mL) in sodium phosphate buffer, pH 7.5, normal saline (PBS) containing OA (fraction II, 1 μg/mL) was dispensed into each well (Immulon 2 Removawell strips, Dynatech). The antibody was dried onto the wells in a forced-air oven at 50 °C. DON–HRP (1.35 μg/mL in PBS containing 1% BSA) was mixed with an equal volume of DON or analogue in water, and 100 μL of this mixture was immediately added to each well. After incubation at 37 °C for 40 min, the wells were washed eight times with PBS–0.5% Tween 20 and 100 μL of ABTS substrate (400 μM ABTS, 0.009% H_2O_2, 45 mM sodium citrate buffer, pH 4.0) was added. The reaction was stopped with 100 μL of 300 mM citric acid containing 15 mM sodium azide and the absorbance at 405 nm determined for each well.

ELISA of Spiked Corn Extract. Corn kernels testing negative for DON by TLC were ground in a Model 2A Romer Mill (Romer Laboratories, Washington, MO 63090) and extracted with five volumes (w/v) of 70% methanol for 60 min, with shaking. The solids were pelleted by centrifugation, and the supernatant was decanted. The extract was reduced to 20% of its volume in a rotary evaporator at 50 °C. Aliquots of this extract (now aqueous) were spiked with DON dissolved in water to achieve the desired concentrations. The spiked extracts were assayed by direct ELISA.

RESULTS AND DISCUSSION

Analysis of DON Esterified without Protection. DON reacted with succinic anhydride without protection of C7- and C15-hydroxyls yielded at least three compounds (R_f 0.33, 0.60, and 0.68) detected by TLC on Whatman LHP-K silica gel (chloroform–methanol, 1:1) visualized with NBP; the slowest migrating compound (R_f 0.33) gave the most intense spot of the three. Positive-ion FAB-MS had peaks at m/z 297 (DON), 397 (hemisuccinyl-DON), and 497 ([hemisuccinyl]$_2$-DON) in the approximate ratio of 1:1:4. It is not certain, however, whether the lower MW peaks were actually present in the derivative mixture or were fragments formed in the mass spectrometer by sequential loss of hemisuccinates, since DON (R_f 0.84) was not detected by TLC. The highest MW species (m/z 497) was in greatest abundance and could correspond to the most intense spot on TLC (R_f 0.33). We believe the compounds at R_f 0.60 and 0.68 were isomers of hemisuccinyl-DON with R_f 0.60 corresponding to 3-HS-DON (R_f 0.61) described below. No peak for (hemisuccinyl)$_3$-

Table I. Structures of Deoxynivalenol (DON) and Related Compounds



compound	R_1	R_2	R_3	R_4	R_5
deoxynivalenol	OH	H	OH	OH	=O
nivalenol	OH	OH	OH	OH	=O
fusarenone X	OH	OAc[a]	OH	OH	=O
3-Ac-DON	OAc	H	OH	OH	=O
3-HS-DON	OHS[b]	H	OH	OH	=O
15-Ac-DON	OH	H	OAc	OH	=O
T-2 toxin	OH	OAc	OAc	H	OIp[c]

[a] OAc = $-OOCCH_3$. [b] OHS = $-OOC(CH_2)_2COOH$. [c] OIp = $-OOCCH_2CH(CH_3)_2$.

DON (M + H^+ 597) was seen in the FAB-MS. Initial attempts at chromatographically separating the several derivatives in the mixture were unsuccessful, and an alternate method of obtaining a single hemisuccinate derivative of DON was devised.

DON Esterified with Protection. Production and Characterization of 3-HS-DON. By protecting C7 and C15 during esterification with succinic anhydride, a single derivative, 3-HS-DON, was synthesized (Figure 1). Although phenylboronate derivatives of 1,3-diols have been described previously (Ferrier and Prasad, 1965; Perun et al., 1974; Yurkevich et al., 1969), a phenylboronate derivative of DON could not be isolated. This may have been due to steric hindrance by the large, rigid ring, since the butylboronate ester formed readily. We were careful to confirm the desired derivatization because of the problems associated with carboxylation at multiple positions.

On silica gel TLC plates developed in chloroform–methanol (1:1), 3-HS-DON (R_f 0.61) migrated more slowly than did DON (R_f 0.84). Visualization of TLC plates with various reagents demonstrated the retention of specific chemical groups on the 3-HS-DON molecule. This was important because in order to elicit antibodies with affinity to DON, the DON molecule should be unmodified, except for addition of the hemisuccinyl required for protein conjugation. 3-HS-DON turned blue when treated with NBP, as did underivatized DON, indicating that the 12,13-epoxide was intact (Takitani, 1979). In contrast, DOM-1 (12,13-deepoxy-DON) did not turn blue when treated with NBP. After treatment with aluminum chloride (Baxter et al., 1983), a reagent for visualizing trichothecenes possessing the 8-keto moiety, DON and 3-HS-DON both fluoresced under long-wave ultraviolet light. This suggested the ketone at C8 was still present in the hemisuccinyl derivative, since T-2 toxin (Table I), lacking the C8-ketone, did not fluoresce after aluminum chloride treatment. The expected increase in acidity by the introduction of a carboxyl following conversion of DON to 3-HS-DON was demonstrated by spraying TLC plates with 0.04% bromcresol purple (pK_{ind} 6.1) in 50% ethanol. The pH indicator turned yellow (acid) in the presence of 3-HS-DON, but not in the presence of DON.

Additional evidence for the presence of a carboxyl group on the derivative was the increased migration of 3-HS-DON upon protonation during TLC. DON and 3-HS-DON had R_f values of 0.76 and 0.43, respectively, when silica gel TLC plates were developed in chloroform–methanol (75:25). However, when the developing solvent was acidified, as chloroform–methanol–acetic acid (75:15:10), the migration of 3-HS-DON increased (R_f 0.86), while the R_f of DON was unchanged. This enhanced migration rate suggests an acid changing from an ionized to an un-ionized state.

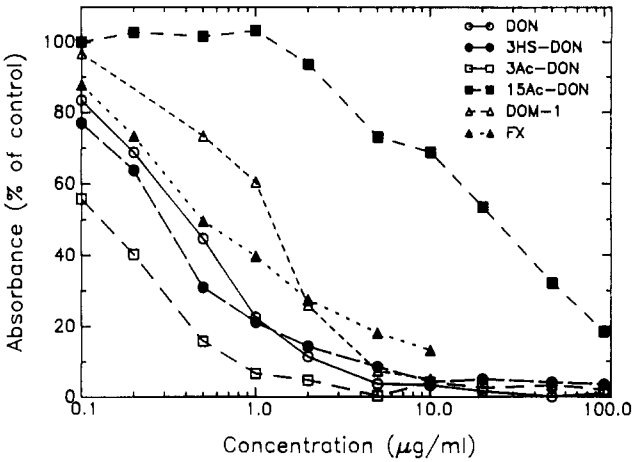


Figure 5. Cross-reactivity of deoxynivalenol (DON) analogues in an indirect ELISA utilizing a monoclonal antibody elicited by 3-*O*-hemisuccinyldeoxynivalenol (3-HS-DON) conjugated to bovine serum albumin. Structures of these compounds shown in Table I.

The FAB-MS spectrum of 3-HS-DON (Figure 2) confirmed the expected mass for M + H^+ of m/z 397, i.e., 297 for DON plus 100 for a single hemisuccinate. Our ^1H NMR spectrum for underivatized DON (Figure 3) agrees with a previously published spectrum for DON (Cole and Cox, 1981). The ^1H NMR spectrum for 3-HS-DON (Figure 4) shows the expected shift of H3 (4.55–5.21 ppm), which is similar to the H3 peak in the spectrum of 3-Ac-DON published previously (Cole and Cox, 1981). The protons at positions 7 and 15 were unchanged, indicating these positions did not undergo esterification. These data are consistent with 3-*O*-hemisuccinyldeoxynivalenol (3-HS-DON).

Hybridoma. Although antisera produced in response to all DON–BSA conjugates did bind to solid-phase DON–OA in an indirect ELISA, only 3-HS-DON–BSA elicited antisera in which antibody binding to solid-phase DON–OA was inhibited by free DON. The hybridoma line secreting DON-specific antibody was derived from a mouse injected subcutaneously, with 500 μg of DON–BSA in normal saline, boosted with 500 μg of DON–BSA (subcutaneous injection) on week 4 and with 250 μg of DON–BSA on weeks 10, 12, 21, and 29. The antibody-secreting hybridoma line selected for enzyme immunoassays had a probability of monoclonality of 0.95 after two subclonings (Coller and Coller, 1983).

Characterization of Monoclonal Antibody. The monoclonal antibody (aDON-1) used for ELISA was IgG_1 with κ-light chain, determined with use of a mouse Ig subclass identification kit (Boehringer Mannheim Biochemicals, Indianapolis, IN 46250).

The specificity of aDON-1 was determined in the indirect ELISA using 3-HS-DON–OA as the solid-phase antigen. The antibody, aDON-1, had a higher affinity for 3-O-acylated DON (3-acetyl-DON, and 3-HS-DON) than for DON, while fusarenone X and nivalenol (differing from DON at C4) reacted somewhat less than DON and 15-acetyl-DON and T-2 toxin cross-reacted poorly (Figure 5; Table I; results for nivalenol and T-2 toxin not shown). Although aDON-1 had affinity for 12,13-deepoxy-DON (DOM-1; Figure 5), the concentration of DOM-1 inhibiting

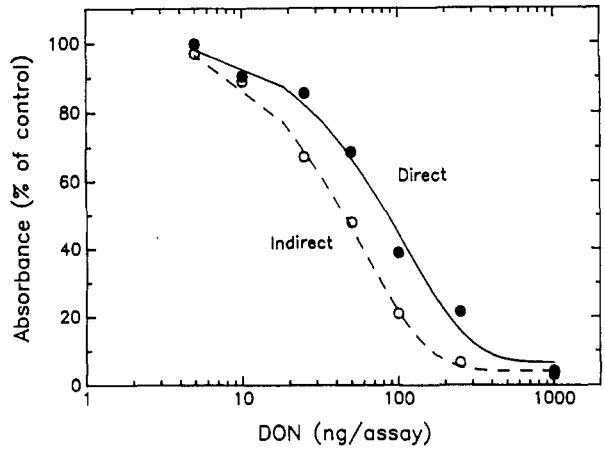


Figure 6. Standard curves for deoxynivalenol by both direct and indirect ELISA. For regression lines of direct and indirect assay, r^2 = 0.989 and 0.999, respectively.

50% of color development in the indirect ELISA was nearly 3 times the concentration of DON required for the same level of inhibition.

The competitive ELISAs utilizing aDON-1 detected DON at 10–250 ng/assay (0.2–5.0 μg/mL) for direct ELISA and 10–150 ng/assay (0.2–2.0 μg/mL) for indirect ELISA (Figure 6). This range is useful as the Food and Drug Administration has issued a "level of concern" of 2 μg of DON/g of whole grain, and DON is regulated at these levels in Canada (Kuiper-Goodman, 1985). The direct ELISA had a wider range than the indirect ELISA. Therefore, changes in DON concentration in the indirect ELISA resulted in more dramatic changes in absorbance, enhancing quantification. The direct ELISA is more desirable for routine screening, however, since incubation with the second antibody–conjugate is unnecessary. It was necessary for samples to be aqueous (1% methanol for T-2 toxin), as even 10% methanol interfered with the assay. Sensitivity of direct ELISA was similar for DON in spiked corn extracts and in water. However, color development in the ELISA was slower when spiked corn extracts were assayed. Assay of corn samples, therefore, requires that DON standards be prepared in extracts of DON-free corn.

Summary. Previous attempts at producing antibodies against DON have been unsuccessful (Zhang et al., 1986). We found that proper orientation of DON on the carrier protein was critical to antibody production. Antibodies elicited by our DON 8-*O*-carboxymethyl oxime derivative, and most antibodies elicited by 3-HS-DON, did bind the appropriate DON–protein conjugate, but competitive inhibition of this binding by free DON was not detected. Increasing the length of the bridge (with the hemiglutarate derivative) did not overcome this problem. The higher affinity of these antibodies for DON–protein conjugates than for free DON suggests the recognition of not only DON, but also the bridge region (e.g., succinyl) and possibly part of the protein (e.g., lysine side chain) by these antibodies. However, hybridoma technology enabled us to select a clone producing a monoclonal antibody (aDON-1) that was competitively inhibited from binding a DON–protein conjugate by free DON, i.e., and antibody with high affinity for underivatized DON. Evidence for recognition of the bridge region is the high affinity of aDON-1, elicited by immunization with 3-HS-DON, for DON analogues containing esters at C3, 3-HS-DON and 3-Ac-DON (Figure 5). The low affinity of aDON-1 for 15-Ac-DON suggests that much of the DON molecule is recognized. By using the activated ester conjugation procedure, we avoided undesirable cross-reactivity previously demonstrated with a mixed-anhydride method (Gendloff et al., 1986). It is not clear why DON does not elicit useful antibodies as readily as, for example, T-2 toxin (Gendloff et al., 1987; Hunter et al., 1985). The reason may be that T-2 toxin is more highly substituted than DON (Table I).

An antibody has recently been developed against 3,7,15-Ac_3-DON in another laboratory (Zhang et al., 1986). However, the radioimmunoassay for DON utilizing this antibody requires acetylation of DON in contaminated grain samples (Xu et al., 1986). This acetylation reaction, including several subsequent cleanup steps, is unnecessary with the anti-DON antibody (aDON-1) described here. The antibody, aDON-1, and ELISA described herein will be useful in the rapid and simple routine screening for DON in contaminated grain samples. This antibody may also be useful for studying DON metabolism by the fungus and for diagnostic detection of DON and its metabolites in tissue and biological samples of animals exposed to this mycotoxin.

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Registry No. T-2 toxin, 21259-20-1; DON, 51481-10-8; 15-acetyl-DON, 88337-96-6; FX, 23255-69-8; 3-Ac-DON, 50722-38-8; DOM-1, 113507-96-3; nivalenol, 23282-20-4.

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