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# Note

## Heptafluorobutyrylation of trichothecenes using a solid-phase catalyst[a]

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Non-macrocyclic trichothecenes known to be produced by species of *Fusarium* and certain other fungal genera now number over 80 (ref. 1). However, only a few have been detected as naturally occurring contaminants of grains and other agricultural commodities[2]. Deoxynivalenol (DON) and nivalenol (NIV), which are type B trichothecenes possessing a conjugated 8-carbonyl group (I, Fig. 1), and the type A trichothecenes T-2 toxin (T-2), HT-2 toxin (HT-2) and 4,15-diacetoxyscirpenol (DAS), which lack the 8-carbonyl group (II, Fig. 1), are the main ones found.

Gas chromatography (GC) with electron-capture detection (ECD) or mass spectrometric (MS) detection has been used by many laboratories for the determination of trichothecenes in grains and feeds, with heptafluorobutyrylation and trimethylsilylation being the most commonly used derivatization procedures[3,4]. Heptafluorobutyrate (HFB) derivatives of trichothecenes were first applied to the determination of T-2 and DAS, which react very readily at room temperature with heptafluorobutyrylimidazole (HFBI)[5], and then to DON[6] which requires heating with this reagent for 1 h at 60°C. HFB derivatives of related trichothecenes, including HT-2, verrucarol, 4- and 15-acetoxyscirpendiol (monoacetoxyscirpenol, MAS), NIV and fusarenon X, have also been formed with HFBI[6–12]. We have, however, recently noted deterioration of one brand of HFBI: after storage of the distilled reagent for one month at −8°C, the capillary GC background due to reagent blank showed a marked increase.

A second reagent used for heptafluorobutyrylation of trichothecenes is heptafluorobutyric anhydride (HFBA) with 4-dimethylaminopyridine (4-DMAP) or trimethylamine as catalysts dissolved in an organic solvent[13–15]. Derivatization of DON proceeds faster at 60°C than with HFBI[13]. Polymer-bound 4-(*N*-benzyl-*N*-methylamino)pyridine has been used as a catalyst for acetylation of linalool with acetic anhydride[16]. The convenience of an insoluble solid catalyst encouraged us to investigate its use in heptafluorobutyrylation of trichothecenes with HFBA.

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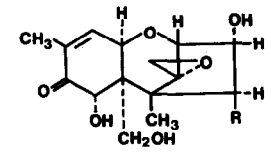
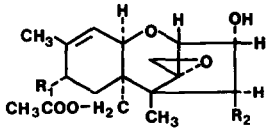


I



II

Fig. 1. Chemical structures of nivalenol (NIV) (I, R = OH), deoxynivalenol (DON) (I, R = H), 15-acetoxyscirpendiol (15-MAS) (II, $R_1$ = H; $R_2$ = OH), 4,15-diacetoxyscirpenol (DAS) (II, $R_1$ = H; $R_2$ = $OCOCH_3$), HT-2 toxin (HT-2) [II, $R_1$ = $(CH_3)_2CHCH_2COO$; $R_2$ = OH] and T-2 toxin (T-2) [II, $R_1$ = $(CH_3)_2CHCH_2COO$; $R_2$ = $OCOCH_3$].

## EXPERIMENTAL

### *Standards and reagents*

DON, 3-acetyl-DON and 3,15-diacetyl-DON were from the Plant Research Centre (Agriculture Canada, Ottawa, Canada), NIV was purchased from Wako Chemicals (Dallas, TX, U.S.A.) and HT-2 and DAS were purchased from Makor Chemicals (Jerusalem, Israel); T-2 was from the U.S. Department of Agriculture (Peoria, IL, U.S.A.) and 4- and 15-MAS were from C.P. Gorst-Allman (Council for Scientific and Industrial Research, South Africa). Stock solutions of trichothecene standards were prepared in chloroform–methanol (3:1) and stored in the freezer. HFBA was purchased from Pierce (Rockford, IL, U.S.A.) and "dimethylaminopyridine" on polystyrene [polymer-bound 4-(*N*-benzyl-*N*-methylamino)pyridine, 1.6 mmol "DMAP" per g resin] from Fluka (Buchs, Switzerland). Testosterone was from U.S.P.C. (Rockville, MD, U.S.A.); testosterone bis-HFB was prepared using HFBA and acetone as previously described[17].

### *Derivatization*

Stock solutions of trichothecenes were evaporated under nitrogen in a 4-ml screw-cap vial to give 1 μg of each trichothecene. HFBA (50 μl) and toluene–acetonitrile (80:20) (450 μl) were added, the vial was capped (PTFE-lined insert) and the contents were mixed for 30 s on a vortex mixer. A 10-mg amount of "DMAP" on polystyrene was added and the capped vial heated in a sand bath for 2 h at 90°C. After cooling to room temperature, a 40-μl aliquot of the liquid phase was evaporated to dryness under nitrogen in a 1.8-ml screw-cap vial, and 1.6 ml *N*-hexane, containing 50 ng/ml testosterone bis-HFB[17] as internal quantitation standard, was added. The reaction was also studied at 22° and 60°C and with only 25 μl HFBA.

### *Gas chromatography*

Capillary GC was carried out on Varian Model 3700 or Model 3400 gas chro-

matographs equipped with on-column capillary injectors (either at 23°C or programmed from 50 to 200°C), 1 m × 0.5 mm I.D. deactivated fused-silica retention gap, make-up gas conversion adapter, J & W Scientific DB-1701 fused-silica capillary column (15 m × 0.33 mm I.D., 0.25 μm film thickness) and $^{63}$Ni electron-capture detector (at 300°C). Column temperature programmes were: Varian 3700: 70°C (2 min), 30°C/min to 175°C (2 min), 2°C/min to 220°C (10 min); Varian 3400: 50°C (1 min), 50°C/min to 175°C (5 min), 3°C/min to 220°C (10 min). The carrier gas was helium (*ca.* 2 ml/min) and the make-up gas was nitrogen (*ca.* 27 ml/min). Attenuation was $64 \cdot 10^{-12}$ A/mV. Chromatograms were recorded and peaks integrated on a Spectra-Physics SP4270 computing integrator or Shimadzu C-R6A Chromatopac data processor; chart speed was 5 mm/min.

*Gas chromatography–mass spectrometry*

A VG Analytical 7070 EQ mass spectrometer was operated in the electron-impact mode at 70 eV and interfaced with a Varian 6000 gas chromatograph, equipped with an on-column injector and a J & W Scientific DB-5 column (30 m × 0.25 mm I.D., 0.25 μm film thickness); the temperature was programmed from 70°C (1 min) at 50°C/min to 170°C (5 min), then at 3 or 5°C/min to 220°C.

## RESULTS AND DISCUSSION

Use of polymer-bound "DMAP" as an insoluble solid catalyst for derivatization of trichothecenes with HFBA has the advantage that no aqueous washing step is necessary. An aliquot of derivatizing solution is taken leaving behind the solid catalyst, and the excess reagent and solvent are readily removed by evaporation. By comparison, washing with aqueous sodium bicarbonate solution, water or phosphate buffer is required with HFBA/4-DMAP[13–15] or HFBI[5–12]. The time course of the reaction at 90°C for six trichothecenes with HFBA/polymer-bound "DMAP" is shown in Fig. 2. As expected from the literature on heptafluorobutyrylation with HFBI, the type B trichothecenes NIV and DON react more slowly than type A trichothecenes. A reaction time of 90 min was considered necessary to ensure complete derivatization of all the trichothecenes under the conditions described in the Experimental section. Initial studies established that a toluene/acetonitrile ratio of 80:20 afforded better miscibility of the HFBA than a 95:5 ratio. Use of 25 μl HFBA instead of 50 μl resulted in slower reaction of NIV and DON (data not shown).

Partial heptafluorobutyrylation of NIV and DON was evident at lower temperatures and shorter reaction times. The chromatogram of a 60°C/20 min reaction mixture obtained using the polymer-bound catalyst (Fig. 3) indicates longer retention times for NIV tris-HFB and DON bis-HFB in relation to those of the fully derivatized trichothecenes (NIV tetrakis-HFB and DON tris-HFB). By comparison, such conditions of temperature and time (60°C/20 min)[13] give complete derivatization of DON and NIV with 4-DMAP as catalyst. After 1 min at room temperature (with the polymer-bound catalyst), NIV tetrakis-HFB and DON tris-HFB were not yet formed and these mild reaction conditions were used to prepare NIV tris-HFB and DON bis-HFB for GC–MS confirmation (Fig. 4). These partial derivatives ($M^+ = 900$ and 688, respectively) have not been previously reported. It is presumed that the unreacted hydroxyl group is the 7-hydroxyl group, which would be expected to be more

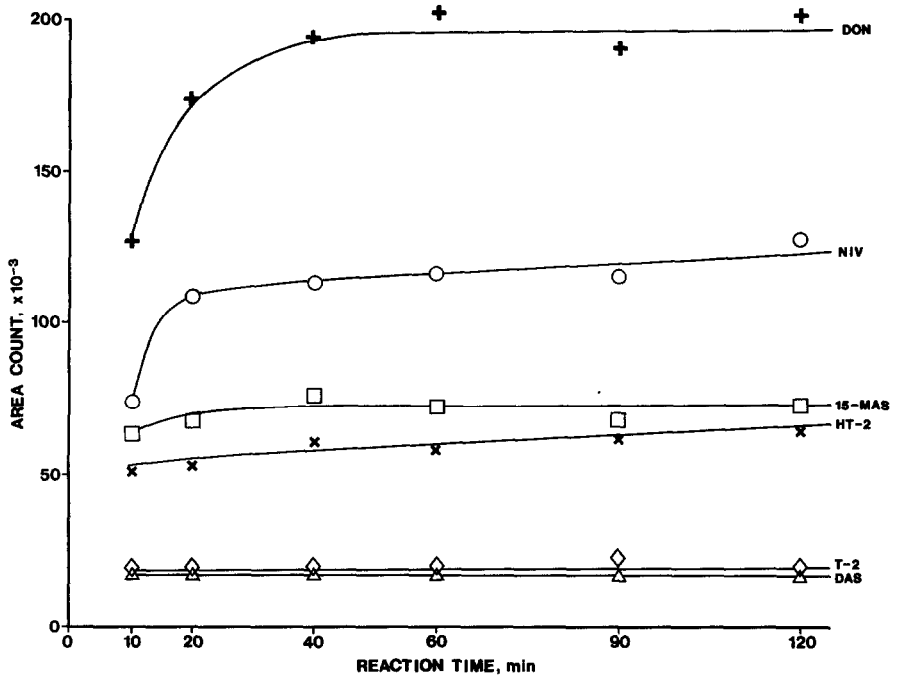



Fig. 2. Rates of reaction of trichothecenes (DON, NIV, 15-MAS, HT-2, T-2 and DAS) with HFBA (50 $\mu$l) in the presence of polymer-bound "DMAP" at 90°C.

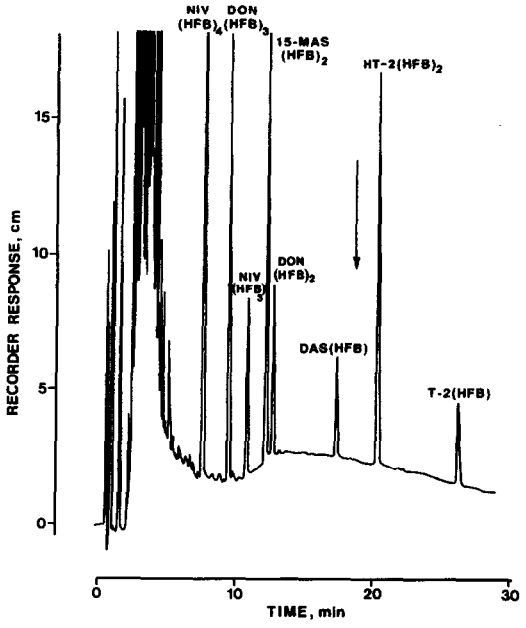



Fig. 3. Gas chromatogram (Varian 3400) of trichothecene HFB derivatives, showing incomplete derivatization of NIV and DON as indicated by presence of NIV tris-HFB and DON bis-HFB. Other marked peaks are fully derivatized trichothecenes: NIV tetrakis-HFB, DON tris-HFB, 15-MAS bis-HFB, DAS HFB, HT-2 bis-HFB and T-2 HFB. Arrow shows retention time of testosterone bis-HFB internal standard. Reaction with HFBA/polymer-bound "DMAP" at 60°C after 20 min.

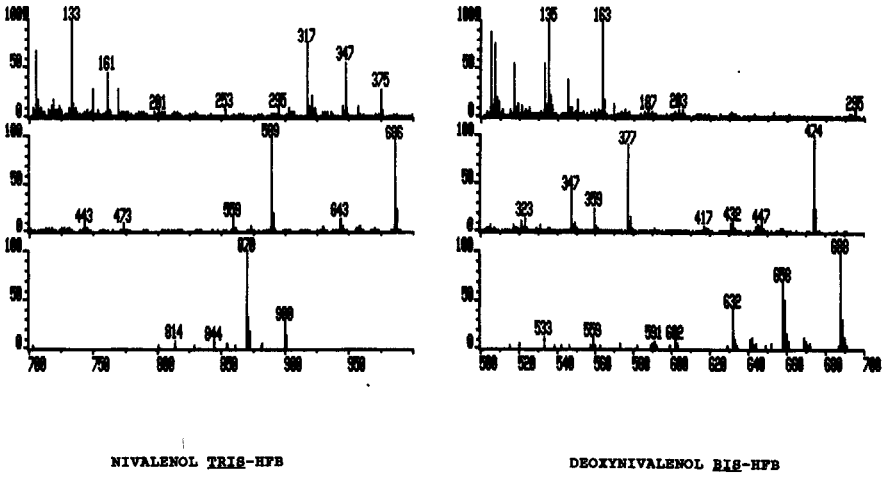



Fig. 4. Mass spectra of NIV tris-HFB and DON bis-HFB separated by GC following derivatization with HFBA/polymer-bound "DMAP" at 22°C for 1 min.

difficult to derivatize than the 3- and 15-hydroxyl groups on account of the possibilities of steric hindrance and hydrogen bonding to the 8-carbonyl group. In a comparative reaction model, the 7-hydroxyl group in DON or 3-acetyl-DON does not react under partial acetylation conditions with a limited amount of acetic anhydride[18,19]. In further support of this less reactive 7-hydroxyl group, we found that (like DON) 3-acetyl-DON and 3,15-diacetyl-DON formed only minor amounts of the fully reacted HFB derivative after 10 min at room temperature. Incomplete trifluoroacetylation and trimethylsilylation of NIV and DON have also been observed[20–22]. NIV tris-trimethylsilyl contained an unreacted 7-hydroxyl group based on nuclear magnetic resonance spectroscopic evidence[21]. However, formation and identification by GC–MS of two DON bis-trimethylsilyl derivatives[20], at least one of which must have a derivatized 7-hydroxyl group, indicates that regiospecificity of trimethylsilylation of DON may differ from that of acylation.

Several types of internal standards have been used in GC of trichothecenes[3,5,8,10,11,14,15]; some have been added to the sample before extraction, others before derivatization and others after derivatization (as a derivatized internal standard or an underivatized compound such as methoxychlor). After examination of a number of steroid heptafluorobutyrates, we propose testosterone bis-HFB as a post-derivatization internal standard for trichothecene HFB derivatives to correct for instrumental variations. Its retention time is conveniently between those of DAS and HT-2 HFB derivatives (Fig. 3), it contains fluorine rather than chlorine, whose ECD responses may not vary proportionately, and it can be prepared[17] and stored as a stock solution in hexane for at least 2 weeks at room temperature. GC–MS ($M^+ = 680$) confirmed its identity.

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