Preparation of the *Fusarium* toxin, nivalenol, by oxidation of the putative biosynthetic precursor, 7-deoxynivalenol

Kenneth C. Ehrlich
Southern Regional Research Center, U.S. Department of Agriculture, P.O. Box 19687, New Orleans, LA 70179, USA

*Key words:* *Fusarium* toxins, trichothecenes, nivalenol, 7-deoxynivalenol, free radical oxidation, biosynthesis

**Abstract**

Nivalenol is a toxic trichothecene metabolite which is produced by a number of different *Fusarium* species. However, the nature of its immediate biosynthetic precursor is not known. Oxidation of 7-deoxynivalenol(3α,4β,15-trihydroxy-12,13-epoxytrichothec-9-ene-8-one) to nivalenol occurred with reagents known to react by a free radical pathway, such as hydrogen peroxide-ferrous ion-ascorbic acid or lead tetracetate, but not with electrophilic reagents requiring prior formation of the enol. These results suggest that 7-deoxynivalenol or an acetylated derivative could be the biosynthetic precursor of nivalenol.

**Introduction**

Nivalenol (Nv) is a highly toxic trichothecene metabolite produced by several different *Fusarium* species including *F. nivale* [1], *F. roseum* [2], and *F. crookwellense* [3]. It has been found as a natural contaminant of moldy wheat [1], barley [2], and corn [4]. Trichothecenes containing the 7-hydroxy-8-oxo functionality are common fungal metabolites of contaminated grains, for example, 4-deoxynivalenol (4-DON, vomitoxin), 3-acetyl-4-DON, Nv, and fusarenone X (Fus X) [2,5]. 7-Deoxynivalenol (7-DON) has been identified in fermentation cultures of *F. graminearum* on corn [6]. It is possible that 7-DON is a biosynthetic precursor of Nv. The present study reports reactions of 7-DON and Ac-7-DON with several different oxidizing agents in an attempt to examine whether or not 7-DON could serve as a biosynthetic precursor of nivalenol.

**Materials and methods**

Preparation of 7-deoxynivalenol

Ac-7-DON (Fig. 1) was prepared from T-2 toxin by selenium dioxide oxidation in glacial acetic acid at 80 °C for 72 h as described by Bamburg, et al. [7] except that 2,2′-azobis[2-methylpropiionitrile] (AIBN) (Kodak) was added as a catalyst [8]. The yield of Ac-7-DON was 49% if recovered starting material was taken into account. After deacetylation in 1 M NaOH in methanol, 7-DON was recovered by passage through a column of Dowex 50 × 8 (H+ form) and lyophilization. The yield was 98%. TLC, Rf (methylene chloride/methanol 93 : 7), 0.18. Infrared (cm⁻¹) 1640, 1240, 975 m.p., 285 °C (dec); lit. 275–280 (dec) [9]. Based on the nmr spectrum of Ac-7-DON prepared by reacetylation of 7-DON, the hydrolysis product was >95% pure.
Fig. 1. Structure of trichothecenes related to nivalenol. Nv, nivalenol, R₁ = R₂ = R₃ = R₄ = OH; 4-DON, 4-deoxy-
nivalenol (vomitoxin) R₁ = R₃ = R₄ = OH, R₂ = H; Ac-4-
DON, 3-acetyl-4-deoxynivalenol, R₁ = OAc, R₂ = H, 
R₃ = R₄ = OH; fus-x, fusarenone-X, R₂ = OAc, 
R₁ = R₂ = R₃ = R₄ = OH; 7-DON, 7-deoxynivalenol, 
R₁ = R₂ = R₃ = OH, R₄ = H; Ac-7-DON, triacetyl-7-deoxy-
nivalenol, R₁ = R₂ = R₃ = OAc, R₄ = H; Ac-Nv, tetraace-
tylnivalenol, R₁ = R₂ = R₃ = R₄ = OAc.

Enol oxidation reactions

The general scheme for hydroxylation of carbonyl 
compounds described by Hassner, et al. [10] was 
followed. This involved, first, converting Ac-7-
DON to its silyl enol ether and, second, oxidizing 
the enol with m-chloroperbenzoic acid. To pre-
pare the silyl enol ether, Ac-7-DON was treated 
with triethylamine in dimethylformamide follow-
ed by addition of trimethylsilyl chloride. In a 
second attempt to prepare the silyl enol ether, 
Ac-7-DON was treated with the non-nucleo-
philic base, sodium hydride, followed by addition 
of trimethylsilyl chloride was performed accord-
ing to the procedure of Hudrlik and Takacs [11].

Oxidation of Ac-7-DON with lithium 
diisopropylamide (Aldrich) followed by diperoxo-
oxohexamethylyphosphoramidomolybdenum (VI) 
pyridine (MoOPh), following conditions de-
scribed by Vedejs, et al. [12] for preparation of 
α-hydroxyketones, was attempted. For treatment 
with MoOPh, 0.4 mmole of Ac-7-DON was 
added to 0.3 mmoles of lithium diisopropylamide 
(0.6 M in tetrahydrofuran) at −78 °C under nitro-
gen. After 10 min, 0.6 mmole of MoOPh was 
introduced and the reaction allowed to warm to 
−35 °C for 15 min. The expected color change 
was not observed. After further warming at −20 °C 
the reaction was terminated by addition of 5 ml of a 
saturated solution of sodium sulfite and products partitioned between ethyl ether and water. Thin
layer chromatography (TLC) involved elution on 
silica gel plates with 2:1 toluene-ethyl acetate 
followed by spraying with aluminum chloride and heating to 80 °C for 5 min.

Chemical hydroxylation reactions

Hydroxylation of 7-DON was performed using 
the conditions described by Udenfriend, et al. 
[13]. 7-DON (0.1 mmole) was added to 20 mg 
ascorbic acid (0.1 mmole), Na₂ EDTA 
(0.05 mmole), and 3 mg FeSO₄·7H₂O in 1 ml of 
100 mM phosphate buffer pH 5.5. Hydrogen 
peroxide (0.15 mmol) was added as a 30% 
solution (final concentration, 0.3%). The reaction 
was supplemented with additional ascorbic acid 
(0.025 mmole) every 16 hr and continued for 5 
days. At intervals aliquots were withdrawn, 
acetylated in pyridine: acetic anhydride (1:1), 
and after removal of pyridine and acetic anhy-
dride by evaporation at low pressure, the presence 
of Ac-Nv was assayed by TLC (2:1 toluene-ethyl 
acetate) on silica gel. A component staining 
yellow (blue fluorescence under long wavelength 
UV) with aluminum chloride like an 8-oxo-tri-
chothecene migrated with the 
same 
Rf as authen-
tic Ac-Nv (R e = 0.45) M + ion, m/e 480, nmr, 
ppm, (CDCl₃) 0.85 (3H,s), 1.88 (3H,d,J = 1.5), 
2.70 and 3.13 (1H,d,J = 4), 4.03 (1H,d,J = 5) 
4.28 and 4.67 (each 1H,d,J = 12), 4.70 (1H, 
J = 4), 5.25 (1H,dd,J = 5.3), 5.87 (1H,dd,J = 3), 
6.09 (1H,s), 6.62 (1H,dd,J = 6, 1.5); mp(hexane-
7-DON was converted to Nv in approximately 
20% yield.

Nv was also prepared from Ac-7-DON by 
reaction with lead tetraacetate in glacial acetic 
acid at 90 °C. Ac-7-DON(80 μmoles) was heated 
in 2 ml glacial acetic acid with 250 mmoles lead 
tetraacetate (Aldrich) in the presence of 1% AIBN. 
The reaction was performed under an argon 
atmosphere for 18 hr at 90 °C. Conversion to 
Ac-Nv was approximately 25% under these condi-
tions and 10% if the AIBN was omitted. Only 
Ac-Nv and starting material were recovered after 
this treatment.