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Synthesis of stable isotope labeled 3-acetyldeoxynivalenol

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Stable isotope labeled 3-acetyldeoxynivalenol (3-AcDON) was synthesized in excellent yield from deoxynivalenol as starting material. This is the first synthesis of a stable isotope labeled type-B trichothecene suitable as internal standard for HPLC-MS/MS or GC-MS analysis of trichothecene mycotoxins. The isotopic purity of the 3-d_3-AcDON was determined to be 94.9%.

Keywords: Acetyldeoxynivalenol / Analysis / Deoxynivalenol / Stable isotope / Synthesis / Trichothecenes

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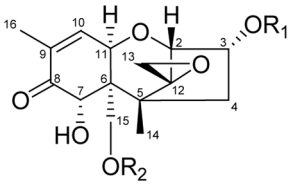
1 Introduction

Mycotoxins are secondary metabolites produced by several fungi during their growth period. Due to their various toxic effects, these fungal metabolites pose a potentially serious health risk to both animals and humans. So far, more than 400 different mycotoxins are known, of which about 100 are produced by moulds of the *Fusarium* genus such as *Fusarium verticillioides*, *Fusarium culmorum* or *Fusarium graminearum*. They infect the crops in the field affecting yield and quality of cereal products. The main toxins produced by *Fusarium* moulds are fumonisins, zearalenone, and the so-called trichothecenes [1, 2].

Trichothecenes are a large group of sesquiterpenes sharing the same basic chemical structure, a 12,13-epoxytrichothec-9-ene ring system [1]. The group is subdivided into type A trichothecenes such as diacetoxyscirpenol or T2-toxin and type B trichothecenes such as nivalenol or deoxynivalenol (DON **1**; Fig. 1), the difference being a keto substitution at C-8 in the latter case. Considering its occurrence and mean concentration, DON appears to be the most important mycotoxin in cereal commodities [2]. Growth and production profiles indicate that 3-acetyldeoxynivalenol (3-AcDON **2**; Fig. 1) [3] and 15-acetyldeoxynivalenol (15-AcDON **3**; Fig. 1) are the biosynthetic precursors of DON, depending on the particular *Fusarium* chemotype. While 15-AcDON occurs mainly in North America and the UK, the 3-AcDON producing strains are prevalent in Europe and Asia [3, 4]. In the course of mould growth, the



1: R_1 = H, R_2 = H

2: R_1 = Ac, R_2 = H

3. R_1 = H, R_2 = Ac

Figure 1. Structures of deoxynivalenol **1**, 3-acetyldeoxynivalenol **2**, and 15-acetyldeoxynivalenol **3**.

acetylated derivatives are hydrolyzed to DON. However, significant amounts of 3- and 15-AcDON can also occur concomitantly [5].

Multiple methods are available for the detection and quantitation of trichothecenes, such as TLC, HPLC with UV or fluorescence detection, and GC with electron-capture detection (ECD) or flame ionization detection (FID) [2]. However, these methods are not very specific or require a derivatization step prior to analysis.

Thus, GC and especially HPLC coupled with mass spectrometric detection have recently become the methods of choice. To assure high accuracy and reproducibility of the quantitative results, either performing laborious matrix calibration [6] or using suitable internal standards is mandatory. Currently, structural analogs of the trichothecenes such as neosolaniol [7], verrucarol [8, 9] or deepoxy-DON [10] are common as internal standards. However, stable isotope labeled analytes would be the ideal standards for mass spectrometric detection, as they compensate matrix suppression in HPLC-MS analysis and have a relative response factor close to 1.

In this paper, we report the synthesis of a stable isotope labeled type B trichothecene, namely, 3-AcDON, which is

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useful as internal standard for GC-MS or HPLC-MS/MS analysis of trichothecenes.

2 Materials and methods

All solvents and reagents were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (Deisenhofen, Germany) in gradient or reagent grade quality. Water for HPLC separation was purified with a Milli-Q Gradient A10 (Millipore, Schwalbach, Germany) system.

2.1 DON-7,15-phenylboronic acid ester (4)

A solution of DON (200 mg, 0.68 mmol, 1 eq.) and phenylboronic acid (92 mg, 0.76 mmol, 1.1 eq.) in acetone (10 mL) was refluxed for 4 h over molecular sieve (4 Å). The solution was filtered and the solvent evaporated under reduced pressure [11]. The residue was analyzed by ^{1}H-NMR for a complete conversion and, if necessary, refluxed for two more hours under the same conditions. ^{1}H-NMR (Bruker DPX-400 MHz, $CDCl_3$) δ 1.09 (3H, s, H-14), 1.91 (3H, b, H-16), 2.04 (1H, dd, J_{AB} = 15.0 Hz, $J_{4A,3}$ = 3.8 Hz, H-4A), 2.19 (1H, dd, J_{AB} = 15.0 Hz, $J_{4B,3}$ = 10.8 Hz, H-4B), 3.15 (1H, d, J_{AB} = 4.0 Hz, H-13A), 3.17 (1H, d, J_{AB} = 4.0 Hz, H-13B), 3.66 (1H, d, $J_{2,3}$ = 4.5 Hz, H-2), 3.89 (1H, d, J_{AB} = 12.6 Hz, H-15A), 4.10 (1H, dd, J_{AB} = 12.6 Hz, $J_{15B,15\text{-}OH}$ = 2.2 Hz, H-15B), 4.55 (1H, d, $J_{11,10}$ = 5.8 Hz, H-11), 4.61 (1H, m, $J_{3,2}$ = 4.5 Hz, $J_{3,4A}$ = 3.8 Hz, $J_{3,4B}$ = 10.8 Hz, H-3), 5.22 (1H, d, $J_{7,7\text{-}OH}$ = 2.2 Hz, H-7), 6.55 (1H, dd, $J_{10,16}$ = 1.5 Hz, $J_{10,11}$ = 5.8 Hz, H-10). The reaction mixture still contained free phenylboronic acid, but further purification was not necessary prior to the next step.

2.2 DON-3-d_3-acetyl-7,15-phenylboronic acid ester (5)

The crude product of Section 2.1, DON-7,15-phenylboronic acid ester (260 mg, 0.68 mmol, 1 eq.), was dissolved in dry pyridine (10 mL) under nitrogen. The solution was cooled in an ice bath (0°C) and d_3-acetyl chloride (75 µL, 83 mg, 1.02 mmol, 1.5 eq., 99% D) was added rapidly. After stirring for 1 h, the mixture was poured into 100 mL ice water to deactivate excess acetyl chloride. For cleanup, the solution was acidified with concentrated hydrochloric acid and extracted three times with 30 mL chloroform each. The combined organic layers were dried over sodium sulfate, yielding 272 mg crude product.

2.3 3-d_3-Acetyldeoxynivalenol (6)

3-d_3-AcDON-7,15-phenylboronic acid ester was dissolved in acetone (10 mL), water (5 mL), and TCA (200 µL). For transesterification, 200 mg mannitol was added and the solution stirred for 1 h on a water bath (40°C). Then 5 mL of saturated sodium hydrogen carbonate solution was added and the mixture was extracted three times with 20 mL chloroform each. The combined organic layers were dried over sodium sulfate and purified *via* preparative RP-18 HPLC (Knauer Eurospher 100, 250 × 16 mm, 5 µm, gradient H_2O + MeOH, 70:30–15:85 in 50 min) yielding 180 mg 3-d_3-AcDON (79% total yield). Exact mass (Bruker Micro-TOF): *m/z* 364.1468, calculated for $C_{17}H_{19}D_3O_7 + Na^+$: 364.1446. ESI-MS (API 4000 QTRAP): negative mode: products of *m/z* 340 $[M - H]^-$, MS/MS (–16 V): *m/z* 310 (100), 173 (55), 340 (47), 247 (26), 217 (17), 309 (11), 191 (10), positive mode: products of *m/z* 342 $[M + H]^+$, MS/MS (17 V): *m/z* 231 (100), 342 (90), 203 (45), 201 (31), 294 (27), 213 (22), 219 (18). EI-MS (HP5973): trimethylsilylated 3-d_3-AcDON: (70 V) *m/z* 73 (100), 46 (26), 103 (18), 75 (14), 193 (11), 380 (11), 395 (10), 191 (10), 147 (9). ^{1}H-NMR: (Bruker DPX-400 MHz, $CDCl_3$) δ 1.17 (3H, s, H-14), 1.69 (1H, dd, $J_{15OH,15A}$ = 4.6 Hz, $J_{15OH,15B}$ = 5.5 Hz, OH-15), 1.91 (3H, b, $J_{16,10}$ = 1.5 Hz, H-16), 2.17 (1H, dd, $J_{4A,3}$ = 11.3 Hz, J_{AB} = 15.1 Hz, H-4A), 2.38 (1H, dd, $J_{4B,3}$ = 4.4 Hz, J_{AB} = 15.1 Hz, H-4B), 3.12 (1H, d, J_{AB} = 4.3 Hz, H-13A), 3.19 (1H, d, J_{AB} = 4.3 Hz, H-13B), 3.79 (1H, dd, J_{AB} = 11.7 Hz, $J_{15A,15OH}$ = 4.6 Hz, H-15A), 3.81 (1H, $J_{7OH,7}$ = 1.8 Hz, OH-7), 3.89 (1H, dd, J_{AB} = 11.7 Hz, $J_{15B,15OH}$ = 5.5 Hz, H-15B), 3.92 (1H, d, $J_{2,3}$ = 4.4 Hz, H-2), 4.70 (1H, d, $J_{11,10}$ = 5.9 Hz, H-11), 4.84 (1H, d, $J_{7,7OH}$ = 1.8 Hz, H-7), 5.23 (1H, m, $J_{3,2}$ = 4.4 Hz, $J_{3,4A}$ = 11.3 Hz, $J_{3,4B}$ = 4.4 Hz, H-3), 6.61 (1H, m, $J_{10,16}$ = 1.5 Hz, $J_{10,11}$ = 5.9 Hz, H-10).

3 Results and discussion

Starting material for the synthesis was DON, which was prepared according to a procedure previously described by Altpeter [12]. Briefly, rice was inoculated in baby food jars with *F. graminearum* (DSMZ 4528) and kept at 28°C for 12 days, followed by extraction with methanol/water (70:30), further cleanup, and hydrolysis of 3-AcDON to DON [12]. d_3-Acetyldeoxynivalenol (3-d_3-AcDON) was prepared in a three-step synthesis according to Scheme 1. First, the 7- and 15-hydroxy groups of DON were protected as cyclic phenylboronic acid esters. In the second step the hydroxyl group in position 3 was acetylated with d_3-acetyl chloride. The synthesis was completed by hydrolysis of the phenylboronic acid ester followed by purification with preparative HPLC on an RP-18 column. The overall yield was 79%, purity was >95%. The isotopic purity was determined to be 94.9% by LC-MS/MS, with 5.1% 3-AcDON containing two deuterium atoms. Figure 3 shows the HPLC-ESI-MS/MS spectra of 3-AcDON compared to those of 3-d_3-AcDON in the negative (left spectra) and positive (right spectra) ionization mode.

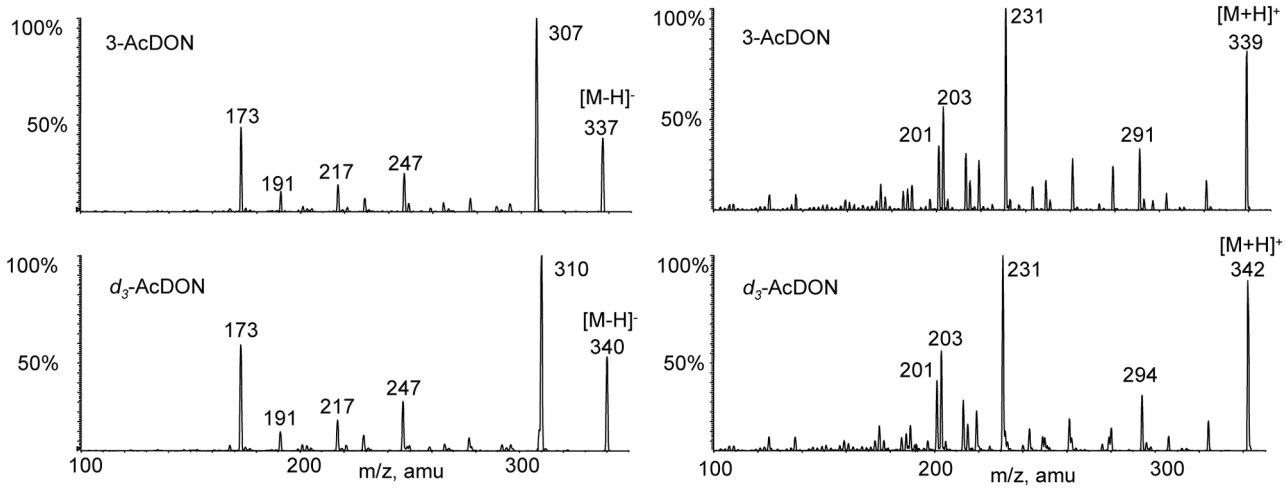
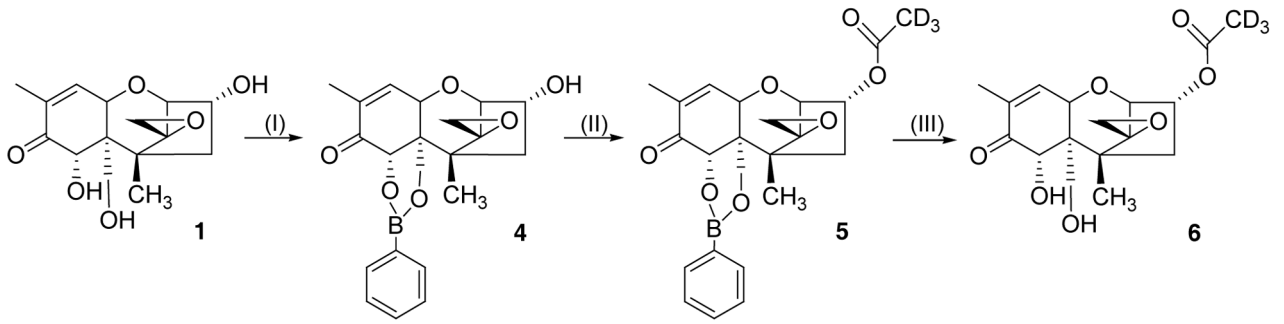


Figure 2. ESI-MS product ion spectra of 3-AcDON and d_3-3-AcDON (collision energy –16 and 17 V, respectively).



Scheme 1. Synthesis of 3-d_3-AcDON. Reagents and conditions: (I) 1.1 eq. phenylboronic acid, acetone, reflux, 4 h, (II) 1.5 eq. d_3-acetyl chloride, pyridine, 0°C, 1 h, (III) mannitol, H_2O, acetone, TFA, 40°C, 1 h.

4 Concluding remarks

d_3-Acetyldeoxynivalenol was prepared from DON as starting material in excellent yield and high isotopic purity. This is the first synthesis of a stable isotope labeled type B trichothecene suitable as internal standard for HPLC or GC analysis coupled with mass spectrometric detection.

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