Heat-induced isomers of acetylated derivatives of deoxynivalenol and nivalenol

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Summary

As a part of a study on the heat stability of deoxynivalenol (DON) and nivalenol (NIV), the decomposition patterns of both compounds were determined by ELISA using a monoclonal antibody which recognizes the partially acetylated derivatives of DON and NIV (3,15-diacetyl-DON and 3,4,15-triacetyl-NIV, respectively). In that investigation it was found that heat-induced derivatives of the toxins have a stronger cross-reactivity with the antibody. Considering the nature of the antibody, it was speculated that a structural change due to the rearrangement of the A-ring in the trichothecenes probably occurred. To corroborate this hypothesis, DON and NIV were heated in acetic anhydride to determine which compounds are formed during acetylation. Two new compounds were isolated from the reaction mixture: 3,8,15-triacetoxy-12,13-epoxytrichothec-8-en-7-one (TAisoDON) and 3,4,8,15-tetraacetoxy-12,13-epoxytrichothec-8-en-7-one (TeAisoNIV), indicating that during heating at least part of the decrease in DON and NIV level is due to isomerization.

Key words: deoxynivalenol, nivalenol, heating, isomerization, HPLC, ELISA.

Introduction

With the common occurrence of deoxynivalenol (DON) and nivalenol (NIV) in cereals, it is important not only to understand the factors that lead to their occurrence, but also to determine management strategies to minimize their occurrence in food products. Several investigations suggest that, depending on the conditions in which the treatment is performed, the elimination of trichothecenes from contaminated cereals is possible; however, information on the decomposition products that remained in the commodities is insufficient. In an investigation carried out in our laboratory it was found that the heat-induced derivatives of DON and NIV have stronger cross-reactivity with a monoclonal antibody which recognizes the partially acetylated derivatives: 3,15-diacetyl-DON...
(DADON) and 3,4,15-triacetyl-NIV (TANIV). Considering the nature of the antibody, it was speculated that a structural change due to the rearrangement of the A-ring in the trichothecene nucleus probably occurred. Therefore, as an attempt to corroborate this hypothesis, DON and NIV were heated in acetic anhydride to determine which compounds are formed during acetylation. In addition, decomposition patterns of DADON and TANIV were determined by HPLC and ELISA to find out whether or not the isomerization also occurs with the acetylated derivatives.

Materials and Methods

Heating treatments  

a) Dry stage: Standard toxins were heated according to the method reported by Yumbe-Guevara and Yoshizawa.  
b) Reaction with acetic anhydride: DON and NIV standards were heated in acetic anhydride at 135 °C for 2 h (10 mg toxin/ml acetic anhydride) in screw cap test tubes (5 ml) sealed with Teflon® tape to avoid solvent evaporation. Reaction mixtures were evaporated to dryness and redissolved in methanol prior to GC-MS, HPLC and ELISA analyses.

Heat-induced compounds purification  

Unknown compounds (A and B) were purified by preparative silica gel TLC with ethylacetate-hexane (1:1, v/v).

Analysis  

GC-MS, HPLC and ELISA determinations were conducted according to the method reported by Yumbe-Guevara and Yoshizawa. For the acetylated compounds it was necessary to modify the HPLC conditions using H2O:CH3OH:CH3CN (48:50:2, v/v/v) as mobile phase at a flow rate of 0.75 ml/min. NMR spectra were taken in CDCl3 solution with tetramethylsilane as an internal standard using a JEOL JNM-A400 spectrometer; 1H and 13C NMR spectra were recorded at 400 and 100 MHz, respectively.

Results and Discussion

During heating, DON, NIV, DADON and TANIV were decomposed with the extent of reduction dependent upon temperature and processing time. At 200 °C, the half decomposition times were 6, 6, 16 and 5 min for DON, NIV, DADON and TANIV, respectively.

For all toxins, the reduction pattern as determined by ELISA was the same as HPLC when the heating temperature was above 170 °C. However, at 160 °C the toxin level as determined by ELISA increased in the first 30 min of heating in contrast to the HPLC analysis, for which a slight decomposition was observed (Fig. 1).

Taking into account that the antibody used can recognize specifically the partially acetylated derivatives DADON and TANIV, it was suggested that a structural change due to the rearrangement of the A-ring in the trichothecene nucleus probably occurred. To determine which compounds are formed during acetylation, DON and NIV were
Fig. 1. Comparison of DON, NIV, DADON and TANIV decomposition patterns at 160 °C as determined by HPLC (-----) and ELISA (------).
heated in acetic anhydride. As determined by GC-MS, the DON mixture consisted of 10 % DADON, 34 % 3,7,15-triacetyl-DON (TADON) and 56 % of an unknown compound (A). In the case of NIV the reaction mixture consisted of 14 % 3,4,7,15-tetraacetyl-NIV (TeANIV) and 86 % of an unknown compound (B). The $\lambda_{max}$ of TADON and TeANIV was determined to be 228 nm while that of the unknown compounds shifted to 248 nm. The mass spectra of A and B presented a representative ion at m/z 136 and neither compound showed the TMS characteristic ion at m/z 73, indicating the absence of hydroxyl groups that can be etherified (Fig. 2). The NMR data were compatible with the structure of 3,8,15-triacetoxy-12,13-epoxytrichothec-8-en-7-one (TAisoDON) and 3,4,8,15-tetraacetoxy-12,13-epoxytrichothec-8-en-7-one (TeAisoNIV), respectively (Fig. 3).

IsoDON and isoNIV were obtained by hydrolysis (2N NaOH, 3 h) of TAisoDON and TeAisoNIV, respectively. For isoDON, the characteristic ion m/z 235 of DON shifts to m/z 193. For isoNIV the molecular ion ($M^+$) and $M-15$ (m/z 600 and 585, respectively) are considerably enhanced compared with those in the NIV mass spectrum (Fig. 4). For both compounds, the $\lambda_{max}$ shifts from 220 nm in the parent toxins to 277 nm in the products.

Considering that the C-7 hydroxyl group is resistant to acetylation and that the fully acetylated compound (TADON or TeANIV) should be almost inert, we propose that isomerization occurs when the compound is partially acetylated (DADON or TANIV). Further processing of the sample yielded a mixture of the parent and the isomer toxin, both fully acetylated.

Subsequent studies to assess the toxicity of these heat-induced compounds are necessary to determine if heating is a suitable decontamination procedure for cereals contaminated with *Fusarium* mycotoxins.

References

Fig. 2. Mass spectra of TADON, TeANIV and their respective heat-induced isomers (TAisoDON and TeAisoNIV).
Fig. 3. NMR spectra and chemical structures of the heat-induced isomers TAisoDON (A) and TeAisoNIV (B).
Fig. 4. Mass spectra of DON, NIV and their respective heat-induced isomers (isoDON and isoNIV).