Journal of Luminescence 188 (2017) 551–557

Contents lists available at ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Analysis of the photophysical properties of zearalenone using density functional theory

Michael Appell[a,*], Lijuan C. Wang[a], Wayne B. Bosma[b,*]

[a] Mycotoxin Prevention and Applied Microbiology Research, United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL 61604, USA
[b] Mund-Lagowski Department of Chemistry and Biochemistry, Bradley University, 1501 W. Bradley Ave., Peoria, IL 61625, USA

ARTICLE INFO

Keywords:
Food safety
Mycotoxin
Excited state
Detection
Spectroscopy
DFT

ABSTRACT

The intrinsic photophysical properties of the resorcylic acid moiety of zearalenone offer a convenient label free method to determine zearalenone levels in contaminated agricultural products. Steady-state fluorescence and density functional methods were applied to investigate the role of structural chemistry on zearalenone detection. Geometry optimization calculations using the B3LYP density functional identified a tautomeric form of zearalenone. Excited state geometries for zearalenone and a tautomeric form were obtained by MNDO semi-empirical optimizations. Steady-state fluorescence studies suggest that fluorescence quenching at neutral pH is associated with water interactions. Time-dependent density functional and ground state calculations indicate that the anionic and dianionic forms of zearalenone possess lower band gaps, excitation energies, and the lowest unoccupied molecular orbitals are positioned over the non-fluorophoric portion of zearalenone. These results suggest that deprotonation of one or more of the phenolic hydroxyls diminishes the intensity of the fluorescence emission of zearalenone.

1. Introduction

Zearalenone is a common occurring estrogenic mycotoxin produced by several *Fusarium* species and a natural contaminate of corn and other cereal grains [1]. Swine are especially sensitive to the estrogenic effects of zearalenone, and this toxin has been shown to bind to the estrogenic receptors and inhibit ovulation [2,3]. In fact, exposure to zearalenone has been shown to be associated with hyperestrogenism in swine [4]. Several methods have been developed to monitor zearalenone levels in food and feed products, including many liquid chromatography based methods utilizing the intrinsic fluorescence of zearalenone as a convenient method of detection and to determine toxin levels [5].

Influences of solvent and environment on the fluorescence intensity of zearalenone and its analogs have been observed [6]. Under certain conditions, the fluorescence intensity of zearalenone is quenched by water, however, this phenomenon is not observed in many other common protic/aprotic and polar/apolar solvents [7,8]. Water molecules bound to solutes can have complex properties compared to bulk water [9], and the intermolecular interactions of water have influence over the excited state properties as observed through density functional theory, time-dependent density functional theory, and semi-empirical theoretical studies [10–13]. However, pH and other factors have shown to influence of zearalenone fluorescence intensity and maxima position [8]. The contributions of chemical structure, bond order, and deprotonation on the photophyscial properties of zearalenone are not clear. First principle calculations offer a means to define the contributions of chemical structure to photophysical properties and excited states [14–16].

The goal of this study is to elucidate the contributions of chemical structures to the ground state and excited state properties of zearalenone. We investigate in detail the role of chemical structure on zearalenone fluorescence using steady-state fluorescence under various conditions, as well as, density functional quantum chemical methods and semi-empirical methods. It is found that a stable tautomeric form of zearalenone exhibits similar fluorescence properties as zearalenone. The transition of the hydrogen bond interactions associated with the resorcylic ring hydroxyls and the carbonyl exhibit a modest influence on the molecular orbitals and properties (see Fig. 1). However, the anionic and dianionic forms of the zearalenone and its tautomeric form possess very different molecular orbital and electronic properties as

---

* Corresponding authors.
E-mail addresses: michael.appell@ars.usda.gov (M. Appell), bosma@bradley.edu (W.B. Bosma).

http://dx.doi.org/10.1016/j.jlumin.2017.04.062
Received 22 September 2016; Received in revised form 20 April 2017; Accepted 27 April 2017

0022-2313/ Published by Elsevier B.V.

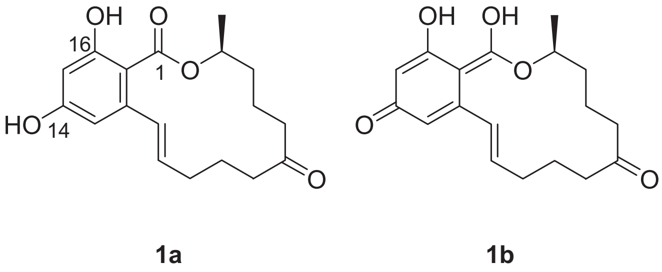

Fig. 1. Structures of zearalenone (**1a**) and tautomeric form (**1b**).

compared to the neutral forms. We explore the ground state versus excited state properties and identify several important relationships that explain the role of chemical structure on the fluorescence quenching of zearalenone.

2. Experimental

2.1. Steady-state fluorescence

Acetonitrile, methanol, sodium phosphate monobasic, sodium phosphate dibasic, 2-(hydroxypropyl)-*β*-cyclodextrin, zearalenone and aflatoxin B_1 were purchased from Sigma Aldrich (St. Louis, MO). Buffer was prepared at 100 mM. Zearalenone standard solutions were prepared as 1 mg mL^{-1} in acetonitrile. Aflatoxin B_1 standard solutions were prepared as 0.1 mg mL^{-1} in acetonitrile. Fluorescence measurements were performed on a Cary Varian Fluorescence Spectrophotometer using 1.0 cm quartz cell (3 mL volume). The slit widths for the excitation and emission were set at 5 nm. Excitation of zearalenone was at 275 nm and excitation of aflatoxin B_1 was at 365 nm. Spectra were recorded between 250 and 350 nm (excitation spectra) and 400–600 nm (emission spectra).

2.2. Computational chemistry

The conformation of zearalenone observed by nuclear magnetic resonance spectroscopy was used as the initial structure for optimization and to build tautomeric forms [17]. Initial structures of zearalenone, its tautomers, and anionic/dianionic forms were built using HyperChem Professional v.8.0.10 (Gainesville, FL, USA) and coordinates were transferred with Openbabel v 2.3.0. The geometry optimizations were carried out using the B3LYP hybrid density functional method. The 6–13G*, 6–31G**, and 6–311 + +G** basis sets were used for geometry optimizations with the default settings as implemented in Spartan ’16 v1.0.0 (Irvine, CA, USA). Excited state calculations were conducted using time-dependent B3LYP density functional at the 6–311 + +G** level on geometries obtained by optimization using the MNDO (Modified Neglect of Diatomic Overlap) method. Natural charges on the chemical structures investigates were obtained by Natural Bond Orbital analysis of the electronic structures following density functional optimization.

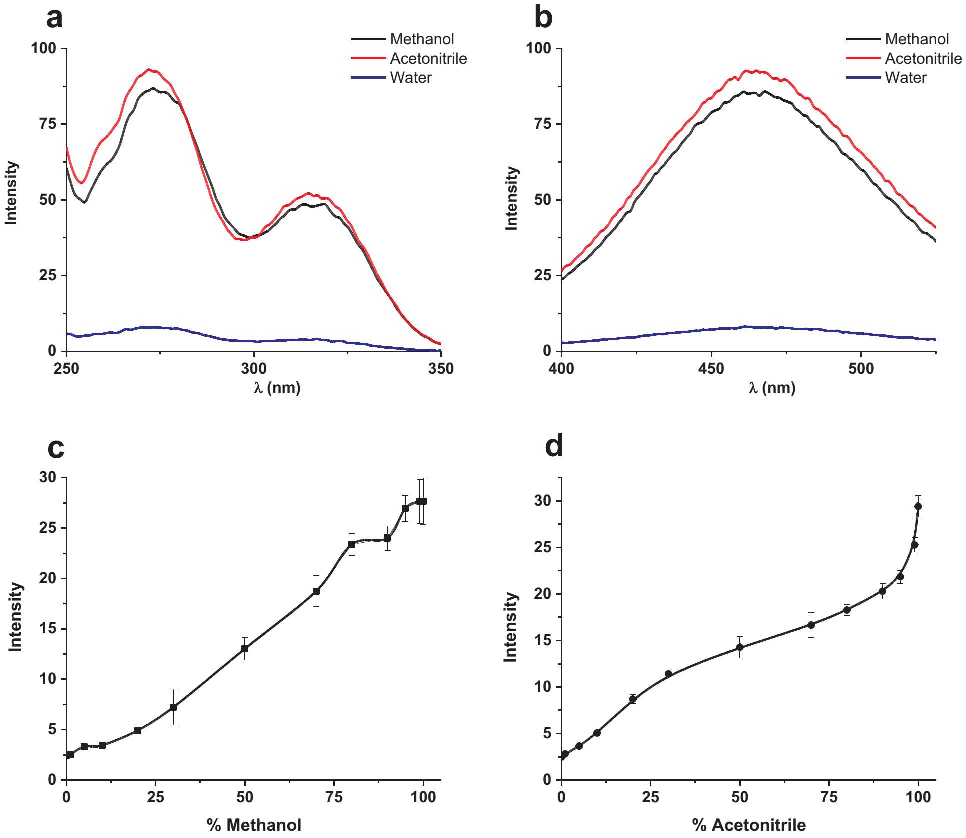

Fig. 2. Experimental studies on the influence of methanol, acetonitrile, and water on the fluorescence excitation (2a, λ_{em} 460 nm) and emission (2b, λ_{ex} 275 nm) spectra of zearalenone (1.0×10^{-5} M). Influence of mixtures of % methanol (2c) and % acetonitrile (2d) on the steady state fluorescence intensity of zearalenone in water (3.0×10^{-6} M, λ_{ex} 275 nm, λ_{em} 460 nm).