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Theoretical insight into the stabilization of triazole fungicides via their interactions with dications

Jana Jaklová Dytrtová[a,*], Jindřich Fanfrlík[a], Renáta Norková[a,b], Michal Jakl[c], Pavel Hobza[a]

[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo náměstí 2, 166 10 Prague 6, Czech Republic
[b] Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic
[c] Department of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague - Suchdol, Czech Republic

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ABSTRACT

The frequent use of pesticides in crop protection leads to their accumulation in soil as well as to the formation of their complexes with ions present in soil. The formation of such complexes can change the mobility of both the pesticides and the ions as well as their ability to penetrate biological membranes and enter biota. The problem of triazole-fungicide complexation with dications is demonstrated on the case of zinc and cadmium ions (M). Complexation with tebuconazole (**1**) has been studied using electrospray ionization mass spectrometry and quantum mechanical DFT-based computations. These experiments have revealed that the deprotonation of **1** leads to the stabilization of the complexes formed, which has been confirmed by our computations. In terms of stability, two types of complexes have been described: (i) a less stable complex (approx. 125 kJ mol^{-1}) with neutral **1** $[M(\mathbf{1})_{2,3,4}]^{2+}$ and $[MCl(\mathbf{1})_{1,2}]^{+}$, and (ii) a more stable complex (approx. 220 kJ mol^{-1}) with deprotonated **1** $[M(\mathbf{1})_n(\mathbf{1}\text{-H})]^{+}$ ($n = 0,1$). The high stability of the complexes with deprotonated **1** is given by their stabilization via a metal interaction with the oxygen atom or phenyl ring. The structure of $[M(\mathbf{1}\text{-H})]^{+}$ is very compact when compared to the structure of parent **1**.

1. Introduction

The ability of ions to pass through biomembranes is influenced by the presence of potential complex formers in the soil [1–3]. Some of these ions may be important macronutrients (e.g., Ca, Mg), essential elements (e.g., Zn, Cu) [4,5] as well as hazardous metals (e.g., Cd, Pb) [6]. Therefore, besides the inherent contamination of the environment with pesticides themselves; the application of pesticides may also cause some additional risks: (i) changes in nutrient availability and (ii) changes in the mobility of hazardous metals.

Tebuconazole ((*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)-pentane-3-ol; **1**, Fig. 1) is a fungicide widely used in crop protection (the second most frequent (10.3% w/w) among fungicides and forming almost 2.5% w/w of all active substances applied in Czech agriculture) [7]. The triazole ring in its structure predetermines the potential ability of **1** (as well as of other triazoles) to create complexes with cations. The explanation based on the structure and stability of these complexes is incomplete.

The ability of **1** as well as other 1,2,4-triazole compounds to create complexes with d-block elements arises from the coordination between the central metal cation and 4-N of the 1,2,4-triazole ring [8]. The ability of 4-N to create electrophilic/ionic coordination bonds has been probed by X-ray crystallography [8] and IR and ^{1}H NMR spectroscopy [9]. Previous works using ESI-MS (electrospray ionization mass spectrometry) have described **1** complexes with cadmium iodide [6] and copper chloride [4], copper cations [5], silver cations [5], and others. The complexes differ mainly in stability as well as in stoichiometry and fragmentation patterns. More information on the metal complexes with **1** is essential for the full understanding of the ability of 1,2,4-triazoles to couple with metals and the potential risk to soil ecology.

This work provides a systematic study of the stoichiometry and stability of Zn(II) and Cd(II) complexes with **1** under ESI conditions, which is completed with information from previous studies with others dications. Deuteration is used to discover the deprotonation abilities of **1** and the stability of the deprotonated complexes (**1**-H). Moreover, the calculations focusing on the deprotonation of the Zn(**1**) complex complement the experimental results, providing a strong basis for further studies.

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* Corresponding author at: Institute of Organic Chemistry and Biochemistry AS CR, v.v.i. Mass Spectrometry, Flemingovo náměstí 2, 16610 Prague 6, Czech Republic. Tel.: +420 220 183 347; fax: +420 220 183 462.
E-mail address: dytrtova@uochb.cas.cz (J. Jaklová Dytrtová).

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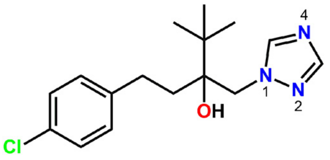

Fig. 1. Tebuconazole structure; 1-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)-pentan-3-ol.

2. Experimental

2.1. Mass spectrometry

Our mass-spectrometry studies were performed using a Finnigan LCQ Advantage ion-trap mass spectrometer (ThermoFinnigan, USA) fitted with an electrospray ion source operated in the positive-ion and negative-ion mode [10]. The sample solutions were continuously (0.7 mL h^{-1}) fed into the ion source through a silica capillary. Nitrogen was used as the nebulizer gas. The operating conditions were set as follows: a spray voltage of 5.0 kV, a capillary voltage of 0–10 V and a tube lens offset of −5 to 10 V, a heated capillary temperature of 225 °C, a sheath-gas flow rate, and auxiliary-gas flow rate of 10–50 arbitrary units. For the determination of the complex stability (appearance energy, AE), collision-induced dissociation (CID) experiments [11] were used.

The sample solutions were prepared at the desired concentrations from a tebuconazole (Sigma–Aldrich, Czech Republic) stock solution (10^{-2} mol L^{-1}) in methanol (Maneko, Czech Republic) and $ZnCl_2$ or $CdCl_2$ (Sigma–Aldrich, Czech Republic) stock solution (10^{-2} mol L^{-1}) in pure water (>18 MΩ). Deuterated tebuconazole was prepared by dissolution of solid tebuconazole (10^{-6} mol L^{-1}) in deuterated methanol (Sigma–Aldrich, Czech Republic); the solution was sonicated for 30 min and then measured.

2.2. Calculations

The density functional theory (DFT) method with empirical dispersion correction (D) [12,13] within the resolution of identity approximation (RI) RI-DFT-D3/TPSS/TZVPP was used for single-point calculations and gradient optimizations. The cheap and fast RI-DFT-D/BLYP/SVP method was used for molecular dynamics (MD) simulations. First, $[Zn(\mathbf{1})]^{2+}$ was built and geometrically optimized using 1 ps MD, followed by gradient optimization. We used the L-BFGS algorithm (convergence criteria: an energy change of 0.0025 kJ mol^{-1} and the maximal gradient of 0.50 kJ mol^{-1} $Å^{-1}$) for gradient optimization and the Berendsen thermostat with a 1 fs time step for MD. To obtain the $[Zn(\mathbf{1}\text{-H})]^{+}$, single protons of $[Zn(\mathbf{1})]^{2+}$ were removed. The most stable $[Zn(\mathbf{1}\text{-H})]^{+}$ complexes (within 20 kJ mol^{-1}) were further relaxed by 1 ps MD and gradient optimization. The calculations were performed using the Turbomole6.4 program [14].

3. Results

3.1. The mass spectrometric approach

Fig. 2 shows the ESI-MS spectrum of the tebuconazole (**1**) and cadmium-chloride mixture sprayed from a methanol/water (v/v 1/1) solution. The complexes are divided into three groups: (i) double-charged **1**/Cd complexes with the general formula $\mathbf{[Cd(1)_{\mathit{x}}]}^{2+}$, where *x* is 2,3, or 4; (ii) single-charged **1**/Cd complexes with Cl^{-} as a counter ion originating from the metal-salt precursor of the general formula $\mathbf{[CdCl(1)_{\mathit{y}}]}^{+}$, where *y* is 1,2, or 3; and (iii) single-charged **1**/Cd complexes with deprotonated **1** appearing as $\mathbf{[Cd(1)_{\mathit{z}}(1\text{-H})]}^{+}$, where *z* is 0 or 1. Protonated **1** $(\mathbf{1} + H)^{+}$ is also present in the spectrum.

The Zn(II) complexes mimic the Cd(II) complexes as follows (Fig. 3): (i) double-charged **1**/Zn complexes with the general formula $\mathbf{[Zn(1)_{\mathit{x}}]}^{2+}$, where *x* is 2,3, or 4; (ii) single-charged **1**/Zn complexes with Cl^{-} as a counter ion originating from the metal-salt precursor of the general formula $\mathbf{[ZnCl(1)_{\mathit{y}}]}^{+}$, where *y* is 1,2, or 3; and (iii) single-charged **1**/Zn complexes with deprotonated **1** appearing as $\mathbf{[Zn(1)_{\mathit{z}}(1\text{-H})]}^{+}$, where *z* is 0 or 1. Protonated **1** $(\mathbf{1} + H)^{+}$ is also present in the spectrum. Additionally, one Zn cluster, $[Zn_2Cl_3(\mathbf{1})_3]^{+}$, was also observed.

In general, the appearance energies (AEs) of the Zn(II) complexes are higher than the AEs of analogous complexes with Cd(II) (Table 1, Fig. 4). The fragmentation pattern of the complex analogs follows

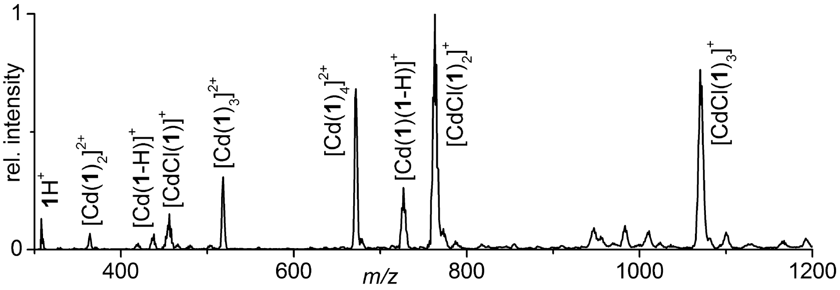

Fig. 2. The ESI-MS mass spectrum of the equimolar mixture of tebuconazole (**1**) and cadmium chloride (both 5×10^{-5} mol L^{-1}) in methanol/water (1/1) as a solvent.

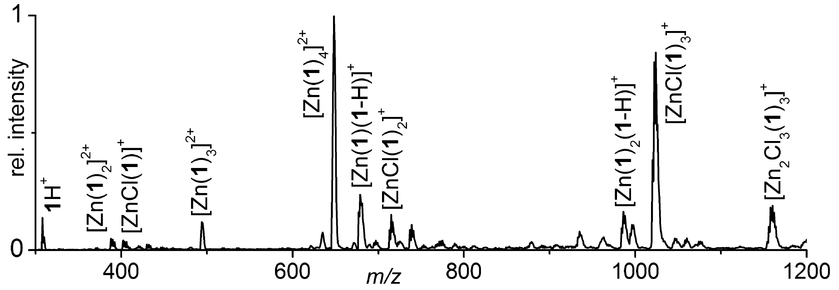

Fig. 3. The ESI-MS mass spectrum of the equimolar mixture of tebuconazole (**1**) and zinc chloride (both 5×10^{-5} mol L^{-1}) in methanol/water (1/1) as a solvent.