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On the photodegradation of azithromycin, erythromycin and tylosin and their transformation products – A kinetic study

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ABSTRACT

In this study, the photo-induced degradation of azithromycin (**Azi**), erythromycin (**Ery**) and tylosin (**Tyl**) was investigated. The three macrolides are regularly found in different kinds of water, and are thus considered a potential environmental risk. In search of efficient ways for elimination, the compounds were systematically irradiated with a polychromatic UVC light source emitting 185, 254 and discretely further up to 580 nm. Due to their specific structural features, the macrolides possess different optical absorption characteristics leading to photodegradation pathways with dissimilar kinetic properties. Hence, the photodegradation products and their kinetics were analyzed using high-performance liquid chromatography (HPLC) coupled to high-resolution time-of-flight mass spectrometry. Among the degradants, *i.e.* the products formed during UV irradiation, both sugar moieties and lactone macrocycles were observed. Applying a first order kinetic model, the half-life of **Azi** was determined as 1.0–3.7 min, that of **Ery** as 1.0–14.2 min depending on the reaction conditions. The two compounds possess much lower absorption cross-sections than **Tyl**, in particular at 254 nm. Their half-lives appeared at least three times higher than that of **Tyl** that has $t_{1/2}$ of 0.2–1.0 min. Based on quantum efficiency considerations, it was assumed that the degradation of **Ery** and **Azi** was mainly due to hydroxyl radical formation, which originated from water irradiation below 200 nm, whereas **Tyl** experienced predominantly photo induced degradation. The photodegradants of **Azi** exhibited half-lives of over 200 min, whereas most of the photo-products of **Ery** and **Tyl** showed half-lives of less than 10 min. Photodegradation processes were investigated at pH 3, pH 7 and pH 9 and in the presence of hydrogen peroxide. Both the educts and the photo-products were degraded more rapidly under neutral and acid conditions.

1. Introduction

The widespread use of antibiotics has transformed a once unique and powerful pharmaceutical weapon into an environmental risk supposedly affecting human health in the long term. Antibiotically active compounds are used primarily in human and veterinary medicine. Not only are they administered for bactericidal purposes and for prevention of infections in humans but also for food conservation and for treatment of companion animals and livestock. Among other antibiotics, the macrolides azithromycin (**Azi**), erythromycin (**Ery**) and tylosin (**Tyl**) were found in groundwater, surface water, slurry and soil (Fatta-Kassinos et al., 2011; Hirsch et al., 1998; Jacobsen et al., 2004; Kümmerer, 2009; Sacher and Thomas, 2001; Solomon et al., 2009). The highest concentration of **Ery** in effluents was reported between 200 and 6000 ng/L, followed by surface water amounting to 10–1700 ng/L. In groundwater **Ery** was observed at levels of 50 ng/L. The observation of **Azi** in surface water was reported as high as 3 ng/L and 69 ng/L in effluents, whereas a concentration of **Tyl** in surface water amounted to 0.72 ng/L (Christian et al., 2003; López-Serna et al., 2011; Prieto-Rodriguez et al., 2012). In groundwater, levels of **Azi** were found in the order of 257 ng/L, while no observations of **Tyl** were reported (López-Serna et al., 2013). When the active ingredient of any drug is not fully absorbed and distributed in the human or animal body after administration, the compound is excreted and channeled into the wastewater system. Conventional wastewater treatment plants, which have a mechanical, biological and chemical purification stage at their disposal, are very often not able to eliminate antibiotic compounds from the wastewater circulation, when the compounds prove resistant to the plant bacteria (Baquero et al., 2008; Bouki et al., 2013; Michael et al., 2013; Wright, 2005). The intact, *i.e.* non-metabolized, antibiotics finally enter surface water from where they might re-circulate into drinking water treatment plants or travel further to the oceans. The presence of antibiotics is supposedly a worldwide environmental problem (Fatta-Kassinos et al., 2011; Lumaret et al., 2012; Sui et al., 2015; World Health Organization, 2014) Serving as an example, **Azi** was found in Serbia, Switzerland,

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Spain and China, **Ery** in Hongkong, China and **Tyl** in Australia (Fatta-Kassinos et al., 2011). Intense efforts are currently undertaken in the field of Advanced Oxidation Processes (AOPs) to enable and accelerate the complete elimination of pharmaceutically active compounds (Andreozzi et al., 1999; Oppenländer, 2003; Parsons, 2004). In the future, AOPs based fourth purification stages in wastewater treatment plants might be envisioned. As an example of AOPs, the formation of hydroxyl radicals is suitable to destroy and remove organic compounds from water samples. Such hydroxyl radicals may be formed by irradiation of water with UVC light containing wavelength well below 200 nm (Andreozzi et al., 1999; Kim and Tanaka, 2009; Oppenländer, 2003). The degradation of the macrolides **Azi**, **Ery**, **Tyl** using hydroxyl radical reactions has been investigated in earlier studies (Alatrache et al., 2010; Batchu et al., 2014; Laoufi et al., 2013; Tassalit et al., 2011; Tong et al., 2011). However, it is not fully understood, which degradation products thereby may arise and how the kinetic and the mechanism of the reaction proceeds. The exact kinetics of the photo transformation products have not been studied until now.

In this study, the effect of UV irradiation on the three macrolides, **Azi**, **Ery**, **Tyl**, will be analyzed qualitatively and quantitatively in aqueous environment at acid pH 3, neutral pH 7 and basic pH 9 to simulate conditions in wastewater treatment plants. To better evaluate the influence of UVC irradiation and hydrogen peroxide addition, molar extinction coefficients, quantum yields or efficiencies for the degradation processes of the parent macrolides will be determined, using ferri-oxalate actinometry for the photon fluence rate (Hatchard and Parker, 1956; Kuhn et al., 2005). Since macrolides have a 14- to 16-membered lactone ring with different kinds of glycosidically bound sugar moieties, such as desosamine or cladinose and the cleavage of theses sugars causes a loss of the antibacterial effect, the chemical structure and the life-time of the degradants in the water-environment are of prime interest (Delaforge et al., 1983; Hansen et al., 2002). The focus of this investigation will thus be laid on the identification of the degradation products and the chemical kinetic of their photoreactions. To this purpose, high-performance liquid chromatography (HPLC) coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-qTOF-MS) will be used. Based on the chemical structure of the degradants, negative potential environmental effects may be indicated. Structure-property or structure-activity relationships have been described (Delaforge et al., 1983; Hansen et al., 2002). In contrast, favorable conditions of an applied Advanced Oxidation Process may be forecasted from the kinetic data generated.

2. Materials and methods

2.1. Chemicals and reagents

Azithromycin-dihydrate (Alfa Aesar, purity 98%) consisted of form A (**Azi A**) and B (**Azi B**), erythromycin free base (BioChemica AppliChem, purity 95%), consisted of form A (**Ery A**), B (**Ery B**) and F (**Ery F**) and tylosin-tartrate (Alfa Aesar, purity 95%) consisted of form A (**Tyl A**) and B (**Tyl B**), see Fig. 1. Note that macrolide antibiotics are usually produced by genetically modified organisms such that a mixture of different forms, *e.g.* different sugar constituents, is obtained. The mixture is then chemically modified to yield the drug substance which in turn reflects the biologically produced mixture. All macrolide solutions were prepared using MilliQ water (Millipore System Simplicity 185). Methanol (LiChrosolv Merck for liquid chromatography), MilliQ water and formic acid (Fluka, LC-MS-Grade) were used as eluent. Hydrochloric acid and ammonia (approximately 25% Riede-de Haen; *pro analysis*, Reag ISO, Reag Ph. Eur.) were used to adjust the pH-value of the solution. For experiments with addition of H_2O_2 a 30% stabilized H_2O_2 solution (Roth) was used.

All compound structures and their abbreviations used are given in

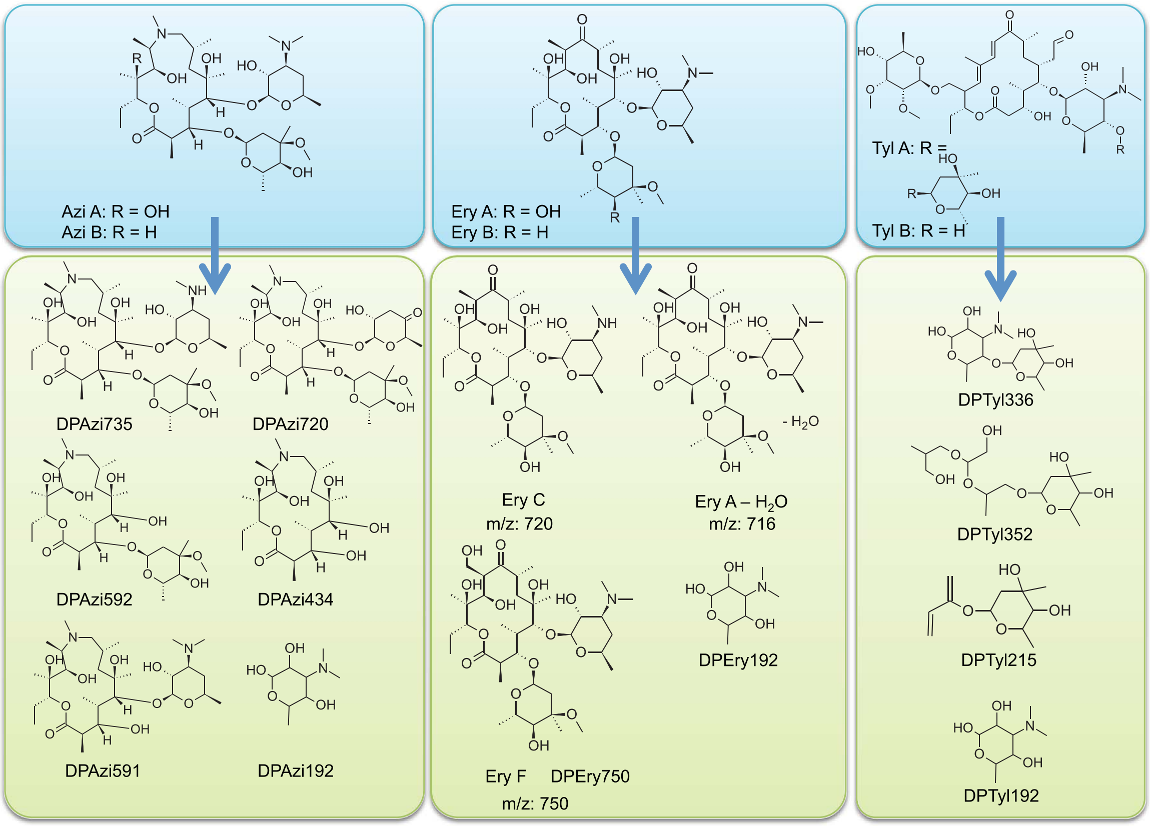

Fig. 1. Chemical structures of forms A and B of azithromycin, erythromycin, tylosin, and their photodegradation products. The *m/z* ratios observed in HPLC-MS experiments are given as nominal mass for simplicity reasons. The structures of degradation products were confirmed by MS/MS experiments where indicated.