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Degradation of macrolide antibiotics by ozone: A mechanistic case study with clarithromycin

Franziska Lange [a], Sjef Cornelissen [b], David Kubac [b], Myint M. Sein [c], Justus von Sonntag [d], Christoph B. Hannich [e], Alfred Golloch [c], Hermann J. Heipieper [b], Monika Möder [a], Clemens von Sonntag [d,e,f,*]

[a] *Department of Analytical Chemistry, Center for Environmental Research (UFZ) Leipzig-Halle, Permoserstr. 15, 04318 Leipzig, Germany*
[b] *Department Bioremediation, Center for Environmental Research (UFZ) Leipzig-Halle, Permoserstr. 15, 04318 Leipzig, Germany*
[c] *Universität Essen-Duisburg, Fachgebiet Instrumentelle Analytik, Lotharstr. 1, 47048 Duisburg, Germany*
[d] *Leibniz-Institut für Oberflächenmodifizierung (IOM), Permoserstr. 15, 04303 Leipzig, Germany*
[e] *Deutsche Projekt Union (DPU), Graeffstr. 5, 50823 Köln, Germany*
[f] *Max-Planck-Institut für Bioanorganische Chemie, Stiftstr. 34–36, 45413 Mülheim an der Ruhr, Germany*

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Abstract

Macrolide antibiotics are widely used (in the order of 1 g per person per year). They pass the body largely unchanged and are also not degraded in wastewater treatment plants. With not too much effort, they may be eliminated from their effluents by ozonation. The macrolide antibiotics have all a dimethylamino group at one of the carbohydrate residues in common. This functional group is the target of the ozone reaction, and clarithromycin has been selected here for a more detailed study. Since only the free amine reacts with ozone, the rate of reaction is pH dependent (at pH 7: $k = 4 \times 10^4\ M^{-1}\ s^{-1}$). In analogy to the ozonolysis of trimethylamine, the main reaction is a transfer of an O-atom yielding the *N*-oxide (identified by HPLC/MS–MS). A minor product (10%, based on formaldehyde yields) is demethylated clarithromycin (identified by HPLC/MS–MS). The dimethylamino group is thought to be essential for the binding of the macrolide antibiotics to their target. As a consequence, chemical changes of this functional group, notably the formation of the *N*-oxide that is no longer a proton acceptor, inactivates these drugs as assayed by the suppression of the growth of *Pseudomonas putida*. This is most important for wastewater treatment, as mineralization of clarithromycin by ozone would require 100 times as much ozone.

Keywords: Pharmaceutical; Macrolides; Wastewater treatment; Ozone; Reaction mechanism

1. Introduction

Macrolide antibiotics such as erythromycin, clarithromycin and roxithromycin (Fig. 1) are widely used to fight many serious infections induced by pneumococci, staphylococci and streptococci, the causative organisms of diseases such as diphtheria, scarlatina, pertussis and anthrax. They also hamper the growth of *Legionella* species. Macrolide binding to the 50S unit of the ribosomes suppresses protein synthesis (Goldman et al., 1990; Schlünzen et al., 2001).

Erythromycin has originally been isolated from *Streptomyces* species, but macrolide antibiotics are now also synthesized chemically. Clarithromycin, for example, has been developed from erythromycin to achieve a higher acid stability, and this is of advantage in the treatment of *Helicobacter pylori*. Interestingly, the use of the various macrolide antibiotics strongly depends on the country. For example, the consumption of erythromycin is high in the UK (near 1200 mg per person per year) and low in Switzerland (24 mg per person per year) (McArdell et al., 2003). On the other hand, the use of clarithromycin is low in Germany (near 20 mg per

* Corresponding author. Address: Max-Planck-Institut für Bioanorganische Chemie, Stiftstr. 34–36, 45413 Mülheim an der Ruhr, Germany. Tel.: +49 208 31806.
E-mail address: clemens@vonsonntag.de (C. von Sonntag).

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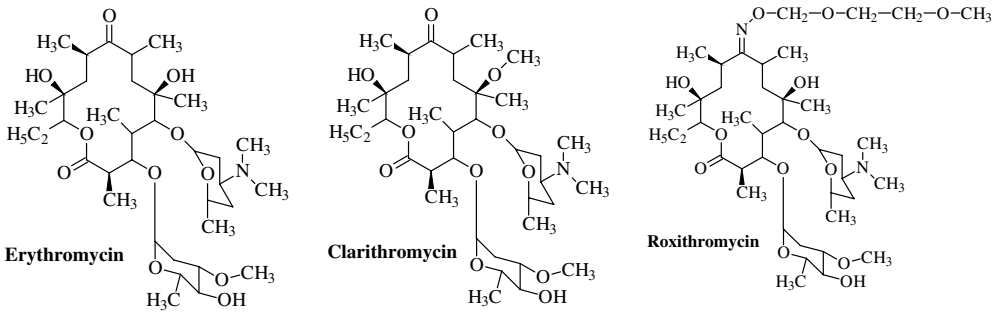
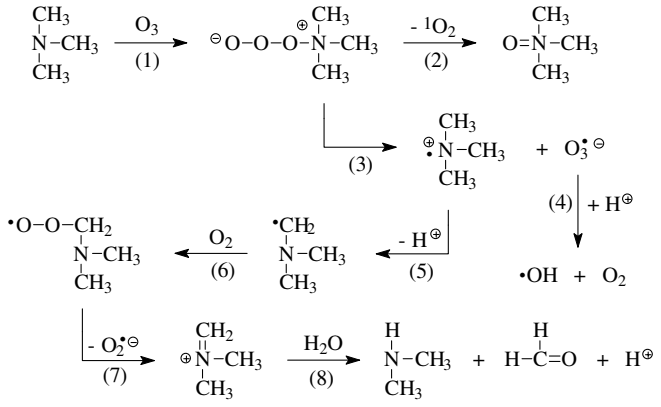


Fig. 1. Chemical structure of erythromycin, clarithromycin and roxithromycin.

person per year) and more than ten times higher in Switzerland, Austria and France (for the UK, no data seem to be available). The total amount of macrolide antibiotics prescribed in 1995 in Germany has been estimated at 8.3–28.6 tons per year (Hirsch et al., 1999). Excretion rates of unchanged macrolide antibiotics are high (>60%) (Hirsch et al., 1999). The common treatment of the wastewater in municipal wastewater treatment plants (WWTPs) does not fully eliminate these drugs, and they are found in their effluents (McArdell et al., 2003). For example, clarithromycin, the most abundant macrolide drug in Switzerland, was detected in three WWTPs at concentrations varying between 57 and 330 ng l^{-1}. It is desirable to eliminate these biorefractory antibiotics further, before the purified wastewater is discharged. It has been shown that this may be achieved upon treating the effluents with ozone (Huber et al., 2003, 2004, 2005; Ternes et al., 2003).

In the present paper, we have studied in details the ozonolysis of clarithromycin in aqueous solution as a typical representative of the macrolide antibiotics. They all have the tertiary dimethylamino group in common, and this is also their site of ozone attack. In previous work, detailed kinetic and product study on tertiary amines, including trimethylamine and triethylamine have been carried out; the latter are much smaller and more soluble tertiary amines that allowed us to study mechanistic details in depth (Muñoz and von Sonntag, 2000; Muñoz et al., 2001). In the first step, ozone adds to the lone electron pair at nitrogen thereby forming an ozonide ammonium zwitterion [reaction (1)].



The ozonide zwitterion decays via two routes. It looses dioxygen yielding the *N*-oxide [reaction (2)] and dissociates into the ozonide radical anion and the amine radical cation [reaction (3)]. Because of spin conservation rules the dioxygen is released as singlet dioxygen ($^1O_2\Delta_g$) (Muñoz et al., 2001). In this model system, reaction (2) dominates (90%) over reaction (3) (10%). The ozonide radical anion is stable at high pH only, but near pH 7 it is rapidly protonated by water and decomposes into O_2 and $^{\bullet}OH$ [reaction (4)]. The amine radical cation deprotonates at the α-carbon [reaction (5)]. The ensuing carbon-centered radical adds O_2 [reaction (6)] and the peroxyl radical thus formed eliminates superoxide, $O_2^{\bullet-}$ [reaction (7)]. The iminium ion hydrolyzes giving rise to the secondary amine and the aldehyde [reaction (8)]. Starting from the carbon-centered radical, the kinetics of the whole sequence of events have been studied in detail (Das et al., 1987). The free-radical reactions occur at the μs time scale. The hydrolysis into dimethylamine and formaldehyde is considerably slower.

In this paper, it will be shown that these reactions also dominate in the ozonolysis of clarithromycin. In order to assess whether these small changes result in the desired loss of the antibiotic activity, the corresponding bio-assays have been carried out as well.

2. Experimental

2.1. Chemicals and ozonation

Clarithromycin (Wako) was used as received. Concentrated stock solutions were made up in Milli-Q-filtered (Millipore) water. Ozone was generated with a dioxygen-fed ozonator (Philoz 04, Philaqua, Gladbeck). The ozone concentration of its stock solutions was determined spectrophotometrically taking $\varepsilon(260\ \text{nm}) = 3300\ \text{M}^{-1}\ \text{cm}^{-1}$ (Forni et al., 1982; Hart et al., 1983). Stock solutions, of clarithromycin and ozone (typically 3×10^{-4} M) were mixed in appropriate ratios to achieve the desired turnover in the product studies. For the kinetic studies, the clarithromycin concentration was 1×10^{-4} M and the ozone concentration near 10^{-5} M, that is, conditions prevailed that guaranteed the kinetics of O_3 decay to follow a first-order law. The pH was adjusted with phosphate buffer. The temperature was room temperature (20 °C).