Identification, Mechanisms and Kinetics of Macrolide Degradation Product Formation under Controlled Environmental Conditions

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Abstract

Erythromycin, azithromycin, clarithromycin and roxithromycin are antibiotics belonging to the widely used macrolide group. Their presence in the environment has been much investigated, despite the rapid degradation of Erythromycin to its spiroketal degradation product. In this study, the formation of macrolide degradation products was investigated in various aqueous solutions, each containing 100 µg/mL of the respective macrolide, under controlled artificial conditions: three phosphate buffer solutions (pH 5, pH 7 and pH 8.5), and a buffer solution at pH 7 with the addition of humic acids. Two solutions from natural sources were also examined: secondary effluent and tap water. The obtained degradation products were identified by their HRMS and NMR spectra (for Erythromycin-spiroketal, obtained from pure compounds isolated by preparative HPLC) as: N-oxide, N-desmethyl and N-didesmethyl forms of all examined macrolides. These degradation products were obtained only under irradiation by sunlight, while the Erythromycin-H2O degradation products were also obtained in the shade. The secondary effluent was the most significant medium for achieving macrolide degradation products. According the degradation product’s 1/2 values obtained in the secondary effluent, the azithromycin was most rapidly degraded (23 hours). Furthermore, results suggested that the degradation process was activated by sunlight irradiation energy, and that the degradation mechanism started with the transfer of an electron from the amine group to O2 to produce the radical ions RMe· and O2· as intermediates and production of the N-oxide and N-desmethyl macrolide degradation products. The kinetics of macrolide degradation was calculated as a first-order reaction.

Keywords: Macrolide; Degradation product; Secondary effluent; Humic acid; Photodegradation; LC-HRMS

Introduction

Degradation products (DPs) obtained from antibiotic residues are a recognized but mostly under-studied group of contaminants [1,2]. They may find their way into the aquatic environment where they are widely dispersed and persist for much longer than previously thought [3,4]. Moreover, drug DPs may be the result of natural biodegradation and/or chemical degradation (including advanced oxidation processes) during wastewater treatment. These DPs are suspected of being more resistant to degradation, and potentially more toxic, than their parent compounds [5-8]. For instance, the macrolide DPs N-desmethyl and N-didesmethyl macrolides have been reported to be biologically active [9,10].

The macrolides are an important group of antibacterial compounds that are commonly used for the treatment of upper and lower respiratory-tract infections. Erythromycin (ERY) is the first and most widely prescribed, orally administered member of this group. Due to its severe side effects, pharmaceutical manufacturers modified this compound to produce three other macrolide drugs: clarithromycin (CLA), roxithromycin (ROX) and azithromycin (AZI), which are widely used in livestock and human medicine. This group of molecules consists of a 14-membered (ERY, CLA and ROX) or 15-membered (AZI) lactone ring, with 10 asymmetric centers and 2 groups of sugar residues: L-cladinose and D-desosamine (Figure 1). ROX and AZI are distinguished from ERY by modifications in ERY’s 14 lactone residues: L-cladinose and D-desosamine (Figure 1). Additionally, CLA is distinguished from ERY by replacing the R2 position from OH to O-CH3 (Figure 1).

Macrolide DPs are frequently found in aquatic environments and their presence has been widely investigated, with a focus on their parent compounds ERY, CLA, ROX and AZI [12-18]. Moreover, several studies have reported detection of the ERY DP ERY-H2O, which is probably the spiroketal product [14,19-22]. In fact, ERY-spiroketal is the only macrolide DP ever detected in the environment. In addition, a non-environmental study designed to obtain macrolide DPs recently revealed a potential effect of ERY on its tissue distribution and bioaccumulation in fish, and its metabolism via demethylation to its N-desmethyl and N-didesmethyl DPs [23]. Studies analyzing related manufactured drug substances in macrolides found N-desmethylerythromycin E, erythromycin E N-oxide, anhydroerythromycin C, N-desmethylerythromycin B, anhydro-N-desmethylerythromycin A and pseudoeerythromycin E enol ether [24]. Others, examining benzamycin which is a combination of benzoyl peroxide and ERY by liquid chromatography–mass spectrometry (LC-MS) showed the underlying oxidation process that produces DPs such as ERY-desmethyl and ERY-N-oxide [25].

In all of the studies in which macrolide DPs have been obtained, these compounds were synthesized. Freiberg [9] synthesized macrolides in which the two 3-dimethyl amino substituents of the desosamine and mycaminose moieties were N-demethylated and N-didemethylated by reaction with a halogen (preferably iodine) in the presence of a base to control pH. Napoleton [26] synthesized ERY, CLA and AZI DPs using UV irradiation, describing the synthesis of N-desmethylerythromycin and its tissue distribution and bioaccumulation in fish, and its metabolism via demethylation to its N-desmethyl and N-didesmethyl DPs [23]. Studies analyzing related manufactured drug substances in macrolides found N-desmethylerythromycin E, erythromycin E N-oxide, anhydroerythromycin C, N-desmethylerythromycin B, anhydro-N-desmethylerythromycin A and pseudoeerythromycin E enol ether [24]. Others, examining benzamycin which is a combination of benzoyl peroxide and ERY by liquid chromatography–mass spectrometry (LC-MS) showed the underlying oxidation process that produces DPs such as ERY-desmethyl and ERY-N-oxide [25].

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Obtaining DPs under sunlight irradiation and shade

Four solutions containing, respectively, ERY, CLA, AZI and ROX (each at a concentration of 100 µg/mL) were prepared. Three different phosphate buffer solutions were examined under controlled artificial conditions pH 5, pH 7 and pH 8.5 and a fourth phosphate buffer solution at pH 7 contained 5 mg/L humic acid. The three different pHs were selected to examine the behavior of macrolides under natural environmental conditions, which typically present a pH range of 5 to 8.5. In addition, two solutions from natural sources were examined: SE and TW to simulate environmental conditions. The initial, non-environmental macrolide concentration of 100 µg/mL was chosen because it was high enough to enable monitoring DPs at the various obtained concentrations, but low enough to avoid intermolecular reactions. The sample solutions were prepared in sealed Pyrex glass bottles under natural sunlight (winter at 18°C, latitude: 32°, altitude: sea level), and in the shade (as a control). They were kept for 14 days (336 h), with sampling at 0, 2, 6, 32, 120 and 336 h. These times were chosen after preliminary tests to determine the optimal period for the degradation process. Each experiment was run in replicate and relative standard deviation (RSTD) was calculated.

N-oxide, N-desmethyl and N-didesmethyl ERY, CLA, AZI and ROX DPs were obtained only under solar irradiation. These DPs were also obtained for ERY-H₂O (Table 1).

Analytical measurements

LC-MS analysis of macrolides and their DPs (after exposure to sunlight irradiation) was performed by high-performance liquid chromatography (HPLC, Agilent 1100) coupled to MS (Q-Tof, Waters, model Premier) via an ESI interface in positive mode, using a C18 ACE column (250 × 2.1 mm, 5 µm particle size). The column temperature was set to 28°C, the flow rate to 0.5 mL/min, and the injection volume was 10 µL. The HPLC mobile phase consisted of water with ammonium formate (0.05 M) adjusted to pH 8 with ammonia (A) and acetonitrile (B). The elution gradient was initiated with 20% B, increased to 80% over 14 min, and then held at 80% for 5 min. ∆H and ∆13C nuclear magnetic resonance (NMR) analyses were carried out for ERY and ERY-spiroketal using a Bruker 500 MHz model ADVANCE II. The samples were dissolved in CDCl₃.

Sample preparation for N-oxide DPs

The four N-oxide DPs for ERY, CLA, AZI and ROX, respectively, were prepared and used as markers. In this procedure, 1 g of macrolide (ERY, CLA, AZI or ROX) was dissolved in 8 mL of methanol and 2 mL of H₂O₂ (30% in water), then heated to 60°C for 4 h. The four products were diluted to the appropriate concentration and then injected into the LC-MS. According to this analysis, the four N-oxide DPs were obtained at high conversion (more than 90% according to area percent).

Sample preparation of ERY-H₂O for NMR analysis

ERY·H₂O [peak at retention time (RT)=10.7 min] was isolated from the ERY working solution after its degradation (at pH 5), using semi-preparative HPLC (Agilent 1100) with a Vydac C18 column (250 mm length, 10 mm ID. and 10 µm particle size). The mobile-phase composition and the elution-gradient program were the same as for the HPLC analytical method (section 2.3), except that the flow rate was set to 5 mL/min. The obtained fractions were lyophilized; they were chromatographically similar before and after the lyophilization procedure. The isolated ERY·H₂O was identified by MS and NMR spectra and used as a marker for the ERY-spiroketal compound (Tables 3-5).
### Quality Control

All data were obtained in triplicate and the deviations (RSTD) were always less than 20%, and usually less than 10%. The correlations (R²) were higher than 0.98 (except for experimental conditions with a very low degradation process) for the results obtained in the experiments run under sunlight.

### Kinetics calculations

Kinetics calculations were based on the following equations:

\[
\ln [C] = -k_1 t + \ln [C_0], \text{first order},
\]

\[
[C] = \frac{[C_0]_{\text{Initial}}}{1 + k_2 t}, \text{second order}.
\]

where:

- \([C]\) (mM) = macrolide concentration at a given time
- \([C_0]\) (mM) = macrolide concentration at starting time
- \(k_1\) (hr⁻¹) = macrolide degradation-rate constant
- \(t\) = time (hr)

### Results and Discussion

The macrolide DPs were obtained in controlled laboratory experiments under sunlight irradiation.

### Macrolide DPs laboratory experiments

A variety of macrolide DPs have been produced under controlled environmental conditions. The main DPs of ERY, CLA, AZI and ROX (obtained only under sunlight irradiation) were N-oxide, N-desmethyl and N-didemethyl (Figure 1). All results were obtained in triplicate and calculated as area percentage obtained from the MS detector. Tables 1 and 2 present the results after 336 h. In addition, DP’s identity was confirmed by their MS spectra (Tables 4 and 5).

Three different phosphate buffer solutions (pH 5, 7 and 8.5), containing 100 µg/mL of each macrolide drug (prepared separately), were examined under controlled artificial conditions. In addition, the pH 7 macrolide-containing solution was also examined with the addition of humic acid. Solutions from natural sources SE and TW were also examined (Tables 1 and 2).

According to our observations, the photodegradation mechanism responsible for these DPs has never been presented. Chen et al. [28] investigated the autoxidation of tertiary amines using trimethylamine as a model, at a temperature of 100°C and pressure of 153 atm of O₂ to obtain the N-oxide products (Figure 2a). The study presented a mechanism that starts with electron transfer from the amine group to O₂ to obtain the radical ions Me₂N⁺ and O₂⁻ (a second-order reaction). In the present study, sunlight irradiation at room temperature was used to obtain the N-oxide and N-desmethyl products.
products (Figure 2b) in different solutions (Tables 1 and 2), and the O· and O₂·-

Structural elucidation of the DPs

Structural elucidation of the macrolide DPs was carried out using the LC-HRMS and NMR techniques. The NMR analysis was carried out only for the ERY-H₂O (spiroketal product), following preparative separation and purification.

**NMR analysis of ERY-H₂O**

Definitive proof for the proposed ERY-H₂O structure was obtained by comparing the ¹H and ¹³C NMR spectra of ERY and the isolated ERY-H₂O (RT=10.89 min; Table 3). Full assignment was performed for ¹H and ¹³C spectra based on El-Bondkly et al. [31] for ERY and on Alam et al. [32] for ERY-spiroketal.

The most significant indication of the formation of ERY-spiroketal was disappearance of the ERY ketone carbon C(9) appearing at δ 221.90 ppm in the ERY-spiroketal spectrum a difference of 10.43 ppm. According to the above suggested mechanism, the macrolide DPs N-oxide, N-desmethyl and N-ndesmethyl cannot, for the most part, be produced at low pH (5 and 7), due to unavailability of the amine’s two non-bonding electrons as a result of its protonation according to its high pKa level (~9) (Table 1) [15]. In contrast, in a solution with high pH (8.5), the DPs were obtained, but at relatively low levels. The N-oxide DP was obtained at 0.2%, 0.8%, 0.4% and 0.6% for ERY, AZI, CLA and ROX respectively; the N-desmethyl DP was obtained at 0.3%, 8.4%, 0.7% and 4.2% for ERY, AZI, CLA and ROX respectively (Tables 1 and 2).

The addition of humic acid to the solution was expected to enhance production of the photodegradation products due to its ability to act as a sensitizer [30]. A comparison was made between solutions at pH 7 with and without humic acid to demonstrate the latter’s effect. The humic acid certainly enhanced AZI degradation, based on the level of AZI N-desmethyl: 10.3% with humic acid and 0% without. As for the other macrolides, no significant effect of humic acid was observed (Table 1). The N-desmethyl DPs of all examined macrolides were obtained quite rapidly in SE: 70% for ERY, 76% for AZI, 79% for CLA and 86% for ROX after 2 weeks of sunlight irradiation. The N-ndesmethyl DPs of all macrolides were also produced in SE, for a level of 1.0% for ERY, 23% for AZI, 0.5% for CLA and 3.2% for ROX, after 2 weeks under sunlight irradiation. The relatively high AZI-N-desmethyl production can be explained by the possible elimination of methyl from the N-methyl amine, which is part of the lactone ring (Figure 1). Nevertheless, no N-tridesmethyl DP was detected for AZI.

The N-oxide DP also appeared mainly in the SE, for ERY (5.5%), AZI (1.4%), CLA (4.6%) and ROX (3.7%). This can be explained by the fact that SE contains sensitizer molecules such as humic acids and others, which encourage the photodegradation process.

In the shade and in all experimental solutions, the macrolides AZI, CLA and ROX showed high stability with minor degradation, whereas ERY only showed high stability in the TW and SE solutions due to their relatively high pH (8.2 and 7.7 respectively). In contrast to the other solutions [phosphate buffer at pH 5 (90%), pH 7 (87%), pH 7 with humic acids (89%) and pH 8.5 (18%)], ERY degraded rapidly in the first 2 h, producing ERY-spiroketal. The second H₂O elimination product [ERY-H₂O(2)] degraded less to 2.0% (pH 5), 3.5% (pH 7), 4.0% (pH 7 with humic acids) and 26% (pH 8.5) (Table 2). According to these results, it can be concluded that production of the two ERY-H₂O elimination products is pH-dependent: the ERY-spiroketal is obtained mainly at low pH and ERY-H₂O (2) mainly at high pH.
Table 4: Main MS fragments of macrolides erythromycin (ERY), azithromycin (AZI), clarithromycin (CLA) and roxithromycin (ROX) and their degradation products.

Further examination of the $^{13}$C spectra indicated additional significant differences for the C(9)-adjacent carbon peaks C(10), C(11) and C(12), which were shifted downfield from δ 37.96, 68.93 and 74.73 ppm (for ERY) to 50.97, 87.86 and 83.86 ppm (for ERY-spiroketal), respectively. The two methyl carbon peaks, C(17) and C(21), also showed significant differences in chemical shifts: δ 9.18 and 16.23 ppm in ERY shifted downfield to δ 18.45 and 25.37 ppm in ERY-spiroketal, and C(12), which were shifted downfield from δ 37.96, 68.93 and 74.73 ppm (for ERY), to 50.97, 87.86 and 83.86 ppm (for ERY-spiroketal), showed significant differences for the C(9)-adjacent carbon peaks C(10), C(11) and C(12). The differences in chemical shifts of $^{13}$C NMR spectra in ERY shifted downfield to δ 18.45 and 25.37 ppm in ERY-spiroketal, respectively (Table 3). The differences in chemical shifts of $^1$H NMR spectral peaks of ERY and the spiroketal product were not as indicative as in the $^{13}$C NMR spectra (Table 3).

LC-MS analysis

LC–MS analysis was carried out for macrolides and their DPs using HRMS to examine their structures. The main DPs of the four examined macrolides were identified according to their molecular masses, mass fragmentation, empirical formulas and relative retention times in the chromatographic column (Tables 4 and 5). The MS spectra of the detected parent macrolides consisted of their molecular masses [MH]$^+$, which were 734.4713 (ERY), 749.5183 (AZI), 748.4838 (CLA) and 837.5335 (ROX). Their spectra were also characterized by elimination of the cladinose residue to obtain the main fragment [MH]$^+$ without...
### Table 5: Measured m/z, calculated m/z, accuracy (mDa and ppm), double-bond equivalent (DBE), formulae of protonated ion and retention time (RT) of macrolides

| Macrolide | Name    | Measured m/z       | Calculated m/z   | Accuracy (mDa) | Accuracy (ppm) | DBE | [MH]⁺ formula | RT (min) |
|-----------|---------|--------------------|------------------|----------------|----------------|-----|----------------|----------|
| ERY       | Parent  | 734.4713           | 734.4691         | 2.2            | 3.0            | 4.5 | C₁₆H₂₄NO₁₃   | 9.84     |
|           | N-Oxide | 750.4677           | 750.4640         | 3.7            | 4.9            | 4.5 | C₁₆H₂₂NO₁₄   | 9.30     |
|           | Desmethyl | 720.4538         | 720.4534         | 0.4            | 0.6            | 4.5 | C₁₆H₂₀NO₁₂   | 9.10     |
|           | Didesmethyl | 706.4390     | 706.4378         | 1.2            | 1.7            | 4.5 | C₁₆H₁₈NO₁₀   | 8.86     |
|           | M-H₂O   | 716.4612           | 716.4585         | 2.7            | 3.8            | 5.5 | C₁₆H₂₁NO₁₁   | 10.89    |
|           | N-Oxide -H₂O | 732.4569    | 732.4534         | 3.5            | 4.8            | 5.5 | C₁₆H₂₀NO₁₂   | 10.20    |
|           | Desmethyl -H₂O      | 702.4420     | 702.4429         | -0.9           | -1.3           | 5.5 | C₁₆H₁₈NO₁₀   | 9.10     |
| AZI       | Parent  | 749.5183           | 749.5164         | 1.9            | 2.5            | 3.5 | C₁₆H₂₄NO₁₃   | 9.95     |
|           | N-Oxide | 765.5155           | 765.5113         | 4.2            | 5.5            | 3.5 | C₁₆H₂₂NO₁₄   | 9.23     |
|           | Desmethyl | 735.5018        | 735.5007         | 1.1            | 1.5            | 3.5 | C₁₆H₂₀NO₁₂   | 9.26     |
|           | Didesmethyl | 721.4846     | 721.4851         | -0.5           | -0.7           | 3.5 | C₁₆H₂₀NO₁₂   | 8.93     |
| CLA       | Parent  | 749.5183           | 749.5164         | 1.9            | 2.5            | 3.5 | C₁₆H₂₄NO₁₃   | 9.95     |
|           | N-Oxide | 764.4803           | 764.4796         | 0.7            | 0.9            | 4.5 | C₁₆H₂₂NO₁₄   | 11.50    |
|           | Desmethyl | 734.4701        | 734.4691         | 1.0            | 1.4            | 4.5 | C₁₆H₂₀NO₁₂   | 11.02    |
|           | Didesmethyl | 720.4559     | 720.4534         | 2.5            | 3.5            | 4.5 | C₁₆H₂₀NO₁₂   | 10.65    |
| ROX       | Parent  | 837.5335           | 837.5324         | 1.1            | 1.3            | 4.5 | C₁₆H₂₄NO₁₃   | 12.31    |
|           | N-Oxide | 853.5284           | 853.5273         | 1.1            | 1.3            | 4.5 | C₁₆H₂₂NO₁₄   | 12.07    |
|           | Desmethyl | 823.5194        | 823.5167         | 2.7            | 3.3            | 4.5 | C₁₆H₂₀NO₁₂   | 11.33    |
|           | Didesmethyl | 809.5019     | 809.5011         | 1.8            | 2.2            | 4.5 | C₁₆H₂₀NO₁₂   | 11.02    |

* Kinetic data not available

HA – humic acids

### Table 6: Kinetics rate parameters of macrolides erythromycin (ERY), azithromycin (AZI), clarithromycin (CLA) and roxithromycin (ROX) in various solutions under sunlight irradiation. SE, secondary effluent; TW, tap water; HA, humic acid.

| Solution | ERY | AZI | CLA | ROX |
|----------|-----|-----|-----|-----|
| SE       | t₅₀ (hr) | t₁₅ (hours) | t₉₀ (hours) | k₅₀ (hr⁻¹) | tₙ₀ (hours) | k₉₀ (hr⁻¹) |
| 4.88E-03 | 142 | 3.04E-02 | 23 | 5.56E-03 | 125 | 7.60E-03 |
| TW       | 6.60E-05 | 10500 | 3.20E-04 | 2310 | 2176E-05 | 25109 | 9.00E-05 |
| pH 7 (HA) | * | * | 3.90E-04 | 1733 | 6.01E-05 | 11531 | 5.30E-05 |
| pH 5     | * | * | 0 | - | 3.62E-06 | 191436 | 8.80E-05 |
| pH 7     | * | * | 0 | - | 2.74E-05 | 25292 | 2.00E-05 |
| pH 8.5   | * | * | 3.20E-04 | 2310 | 5.02E-05 | 13805 | 1.58E-04 |

* Kinetic data not available

HA – humic acids

### Figure 2: Mechanism underlying autoxidation of tertiary amines, demonstrated by trimethylamine (a), and by exposing tertiary amines at room temperature to sunlight irradiation (b, c and d).

The three main DPs, which were obtained only under sunlight irradiation, were N-oxide, N-desmethyl and N-didesmethyl. The spectra of the macrolide N-oxide contained mainly the molecular masses [MH]⁺, which were 750.4634 (ERY), 765.5107 (AZI), 764.4791 (CLA) and 853.5268 (ROX). These DPs’ empirical formulas were confirmed by their HRMS spectra (Table 5). The N-desmethyl and N-didesmethyl DP spectra consisted of their molecular mass as well as their main fragment [MH]⁺ without cladinose related to the elimination of cladinose (Tables 4 and 5). For ERY, due to its rapid formation to a spiroketal product, two additional photodegradation products were also obtained: ERY-N-oxide-H₂O ([MH⁺] 732.4529) and ERY-N-desmethyl-H₂O ([MH⁺] 702.4423) [35].

### Kinetics

During the experiments, the macrolides degraded with time mainly under sunlight irradiation, following first-order kinetics (Table 6, Figure 3). No kinetic data could be obtained for the experiments in the shade, due to the relatively high stability of the macrolides under these experimental conditions, except for ERY, which was rapidly degraded in the artificial solutions (different pHs) (section 3.1). Regarding the...
Production of the main macrolide DPs only under sunlight irradiation can be explained by the decomposition of the parent molecules due to photoactivation, with degradation that is much faster than in the shade; this is in contrast to ERY in artificial solutions, which rapidly decomposes to its main product ERY-spiroketal [11] (Table 2). In the present study, the kinetics results are demonstrated in various solutions under solar irradiation, producing $k_1$ values ranging from 0.0304 hr$^{-1}$ for AZI in SE (Figure 3b) to 0 for AZI at pH 5 and 7 (Figure 3b1; Table 6). It was anticipated that humic acids would act as a sensitizer [30] for the induction of photodegradation products (N-oxide, desmethyl and didesmethyl) in comparison to the other solutions. However, no additional effect was observed in CLA or ROX in the presence of humic acids (Figure 3c1 and d1, Table 6), while the $k_1$ of AZI at pH 7 with the addition of humic acids was 0.000390 hr$^{-1}$, and without humic acids it was 0 (Figure 3b1, Table 6). As discussed in section 3.1, the degradation process is not favored at low pH due to the unavailability of the non-
The characterization, presented herein, is expected to enable the detection of such DPs in various aquatic environments. Moreover, N-desmethyl and N-didesmethyl DPs are still biologically active, potentially increasing their toxicity to humans. Thus, further research is warranted to examine the environmental toxicity and stability of these compounds, which might, through exchange, form other DPs.

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7. Fatta-Kassinos D, Kalievrosziotis IK, Koukoulakis PH, Vasquez MI (2011) The formation of a variety of macrolide DPs under controlled environmental conditions, followed by a characterization and kinetics behavior analysis of macrolide degradation under solar irradiation. Three macrolide DPs (N-oxide, N-desmethyl and N-didesmethyl) were produced following exposure of macrolides to solar irradiation. Investigating the N-oxide and N-desmethyl DPs enabled us to understand the reaction mechanism governing DP formation through the intermediate radical ions RMeO and R-O. The macrolides degraded rapidly under solar irradiation in the investigated aqueous solutions, mainly SE, whereas in the same solutions in the shade, macrolide degradation was negligible, except for ERY in the artificial solutions. The results of this study should direct further research into identified and suggested DPs, not only from macrolides but also from other drugs, which could potentially be found in aquatic environments.

To the best of our knowledge, this is the first study to examine the formation of a variety of macrolide DPs under controlled environmental conditions, followed by a characterization and kinetics behavior analysis of macrolide degradation under solar irradiation. Three macrolide DPs (N-oxide, N-desmethyl and N-didesmethyl) were produced following exposure of macrolides to solar irradiation. Investigating the N-oxide and N-desmethyl DPs enabled us to understand the reaction mechanism governing DP formation through the intermediate radical ions RMeO and R-O. The macrolides degraded rapidly under solar irradiation in the investigated aqueous solutions, mainly SE, whereas in the same solutions in the shade, macrolide degradation was negligible, except for ERY in the artificial solutions. The results of this study should direct further research into identified and suggested DPs, not only from macrolides but also from other drugs, which could potentially be found in aquatic environments.

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