

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



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Introduction: Degradation products (DPs) obtained from antibiotic residues may find their way into the aquatic environment where they are widely dispersed, persist and might be toxic.

Macrolides are an important group of antibacterial compounds including Erythromycin (ERY), Clarithromycin (CLA), Roxithromycin (ROX) and Azithromycin (AZI). Those 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).

The focus of this study was to use controlled environmental conditions to obtain selected macrolide DPs, to verify their chemical structure, and to understand their degradation mechanisms.

Experimental:

Various aqueous solutions, each containing 100 µg/mL of the respective macrolide, were examine under controlled artificial conditions:

Buffer solution at pH 5

Buffer solution at pH 7

Buffer solution at pH 8.5

Buffer solution at pH 7 & humic acids

Natural source solution of secondary effluent SE

Natural source solution of tap water

Those 4 solutions selected to examine the behavior of macrolides under natural environmental conditions, typically present a pH range 5-8.5

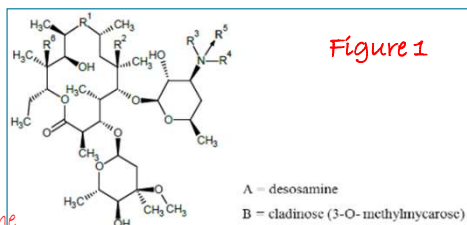


Figure 1

- ✓ The sample solutions were prepared under natural sunlight and in the shade (as a control).
- ✓ They were kept for 336 h with sampling at 0, 2, 6, 32, 120 and 336 h.
- ✓ Each experiment was run in triplicate and relative standard deviation (RSTD) was calculated.

To simulate environmental conditions

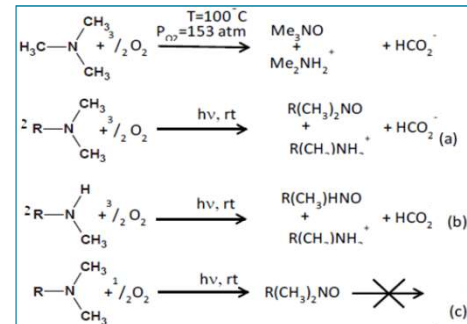


Figure 2: Mechanism underlying autoxidation of tertiary amines, demonstrated by trimethylamine (a), and by exposing tertiary amines at room temperature to sunlight irradiation (b, c and d)

Kinetics calculations:

Kinetics calculations were based on the following equations:

K_1 calculation

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

$[C_0]_{ERY, CLA, AZI, ROX} = 0.136, 0.133, 0.134, 0.120$ mM, respectively 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:

The main DPs of ERY, CLA, AZI and ROX were: N-oxide, N-desmethyl and N-didesmethyl, and obtained only under sunlight irradiation.

Sunlight irradiation at room temperature caused the formation of the DPs in different solutions and the O₂ dissolved naturally (first-order reaction) to formed N-oxide and N-desmethyl (Figure 2b).

In the shade and in all expeimental 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).

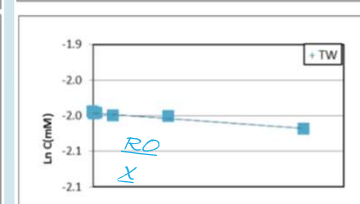
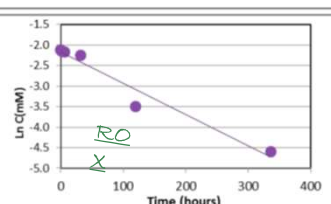
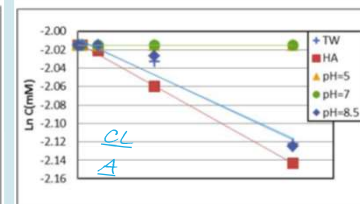
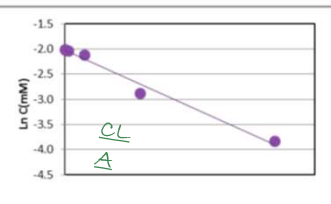
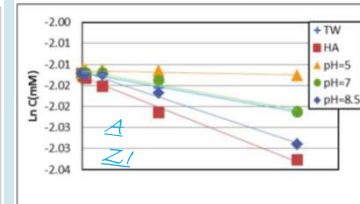
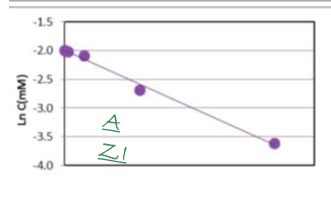
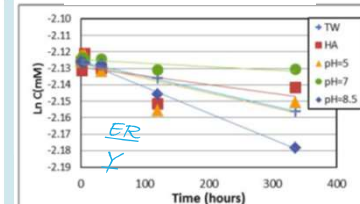
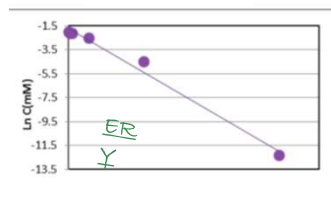
Summary and conclusions:

- The formation of a variety of macrolide DPs under controlled environmental conditions was demonstrated.
- The macrolide DPs and their kinetics behavior were

Figure 3: Degradation rates of macrolides spiked in various solutions and exposed to solar irradiation:

Secondary effluent

Phosphate buffer solutions



characterized.

- Three macrolide DPs were produced following exposure to solar irradiation.
- The reaction mechanism governing DP formation through the intermediate radical ions RME_2N^+ and O_2^- was recognized.
- The macrolide degradation was very fast under solar irradiation whereas it was negligible in the shade.