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Voltammetric investigation of macrolides by an HPLC-coulometric assay

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Abstract

Voltammograms of macrolides, including anhydroerythromycin A, azithromycin, erythromycin A, erythromycin A enol ether, pseudoerythromycin A enol ether, oleandomycin and tylosin have been investigated using a dual electrode cell in combination with a high-throughput LC method. The half-wave potentials ($E_{1/2}$) of the seven macrolides investigated ranged from 0.734 to 0.866 V, and the current responses reached the maxima at over 1.0 V. The current response of the downstream electrode displayed a non-linear behavior at high potentials over +0.75 V, probably because of polarization of solvent components, e.g., water. The HPLC-coulometric assay was optimized with the potentials of the upstream and downstream electrodes at +0.65 and +0.85 V, respectively. This method is suitable for detection of 14- and 15-membered macrolides (sensitivity < 0.05 μg ml^{-1}), but not for a 16-membered macrolide, tylosin (sensitivity > 0.1 μg ml^{-1}). The assay shows interferences from biomatrices in rat's blood plasma and serum, and human urine, but they were effectively removed by a cold acetonitrile extraction method.

Keywords: Coulometric assay; HPLC; Macrolides; Voltammogram

1. Introduction

Macrolides consist of a 12- to 16-membered ketolide ring, to which one or more sugar moieties are substituted. This class of antibiotics has been widely used in humans and food-producing animals. Because of extensive worldwide use, human clinical and veterinary macrolide antibiotics can be widely distributed in biological and environmental samples. Antibiotics released into the environment are of concern because they have potential to accelerate development of antibiotic resistance in bacteria from food-producing animals and resistance gene transfer to humans [1]. Foods and environments contaminated with antibiotics could function as reservoirs of antibiotic resistance genes. Particularly, erythromycin strongly inhibits the microalga, *Selenastrum capricornutum* (EC_{50} = 0.037 mg l^{-1}; ref. [2]). Therefore, it is necessary to develop an accurate and robust detection method for monitoring biological and environmental exposure to macrolide antibiotics.

Biological assays have been introduced to determine the concentration of a single type of macrolide using erythromycin-sensitive strains as indicators [3,4]. This method is routinely used for the determination of the efficacy and the susceptibility of antibiotics toward test microorganisms. The biological activities of antibiotics are semi-quantitatively measured, typically using twofold serial dilutions of drug concentrations in microtiter plates. Although it is convenient for the estimation of effective drug concentrations by logit analysis, it is difficult to distinguish activities of individual drugs in mixtures showing synergistic effects [2]. Most drawbacks arise from test accuracy and precision, because the bioassay is largely dependent on culture techniques (e.g., liquid culture and plate culture) and the physiological states of indicator microorganisms.

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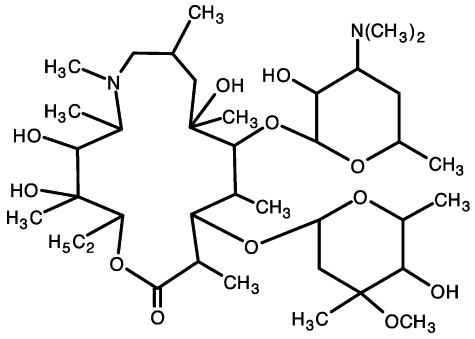
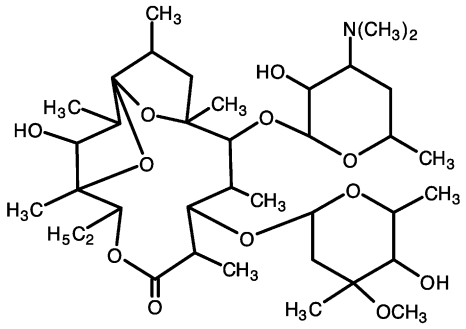
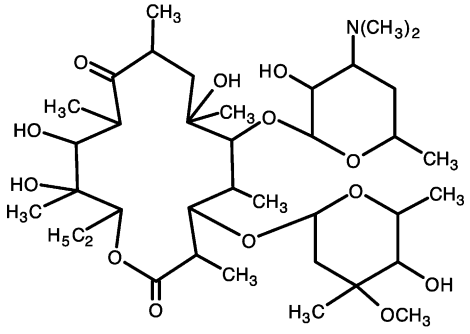


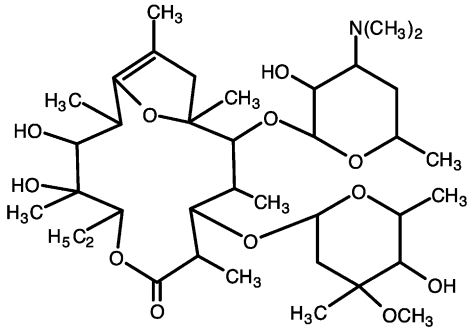
AZT: azithromycin



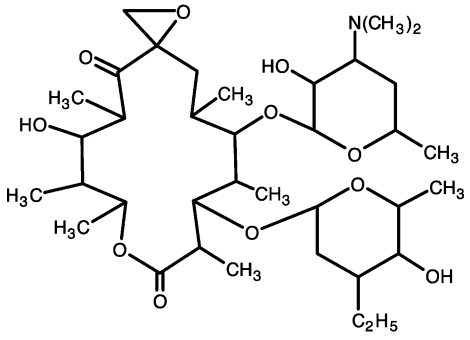
AEA: anhydroerythromycin A



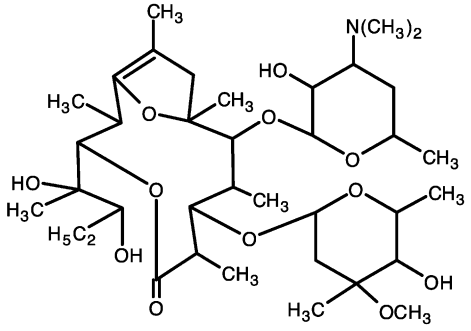
EA: erythromycin A



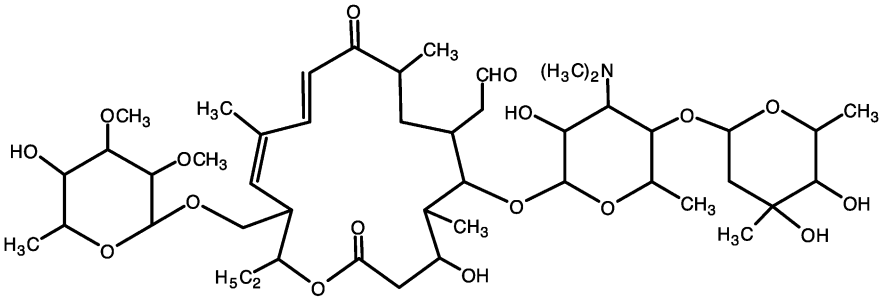
EMEN: erythromycin A enol ether



OM: oleandomycin



psEMEN: pseudoerythromycin A enol ether



TYL: tylosin

Fig. 1. Structures of macrolides.