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Transfer of tylosin across the H_2O/1,2-dichloroethane interface. Analysis of degraded product in acid solutions

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ABSTRACT

Cyclic voltammetry applied to liquid/liquid interfaces and nuclear magnetic resonance spectroscopy were used to analyze the stability of tylosin A in aqueous solutions in acid conditions. Cyclic voltammetry was employed to characterize the charge transfer processes for tylosin A and tylosin B across the H_2O/1,2-dichloroethane interface. Electrochemical study includes the transfer potential dependence of pH value, the elucidation of the mechanism of transfer using mechanical control of convective flux, and the determination of partition coefficient values and diffusion coefficient values employing the change of voltammetric current with external parameters. Degradation process of tylosin A was monitored and tylosin B or desmycosin was isolated and then identified and characterized by nuclear magnetic resonance spectroscopy and electrochemical techniques.

1. Introduction

Macrolide antibiotics constitute a group of antimicrobial drugs that have been used for several decades to treat clinical infections [1]. Tylosin A is an important veterinary antibiotic of the macrolide family, which has been shown to be an alternative to oxytetracycline for the control of some diseases of bacterial origin affecting honeybees [2]. It is produced by *Streptomyces fradiae* and is composed of 16-membered macrocyclic lactone substituted with a disaccharide chain in position 5, composed of mycaminose and mycarose, an aminosugar, and a neutral sugar, respectively. In addition, it is substituted with another neutral sugar, mycinose, in position 23 [3,4].

Fig. 1 shows the structure of tylosin A and tylosin B, and also the schematic representation of the hydrolysis reaction of tylosin A in acidic media. The stability of tylosin A in different acid–base media was previously studied and some degradation products were identified [5]. Therefore, in the last decades, the structures, conformations and carbon signal assignment of several macrolide antibiotics, e.g. tylosin, have been studied by NMR and computational techniques [6–9].

In previous works, we studied the stability of tetracycline (TC) and derivatives tetracyclines, oxytetracycline and chlortetracycline, in strong acid and alkaline solutions. The analysis of the degraded solutions was carried out with cyclic voltammetry applied to the H_2O/1,2-dichloroethane interface and the results obtained were complemented with measures of UV–Visible spectroscopy in a homogeneous phase [10]. For the TC acid degradation it was possible to electrochemically recognize anhydrotetracycline (AHTC) as the main degradation product. Our previous electrochemical studies at liquid/liquid interfaces have shown that AHTC is more hydrophobic than TC, and these differences in the hydrophobicity of TC and AHTC have been effectively used for quantifying each compound in a mixture [11].

Yudi et al. [12,13] have studied the transfer of erythromycin and that of their degradation products under different conditions of hydrolysis using cyclic voltammetry applied to the H_2O/1,2-dichloroethane interface. These authors conclude that the method proposed is suitable for the quantification of one antibiotic and for distinguishing it from its hydrolysis products [12,13].

In the present work, we offer a detailed characterization of tylosin A and tylosin B transfer processes, including the transfer potential dependence on the pH value (Section 3.1.1), the elucidation of the mechanism of transfer using mechanical control of convective flux (Section 3.1.2) [14] and the determination of diffusion coefficient and partition coefficient values using the variation in

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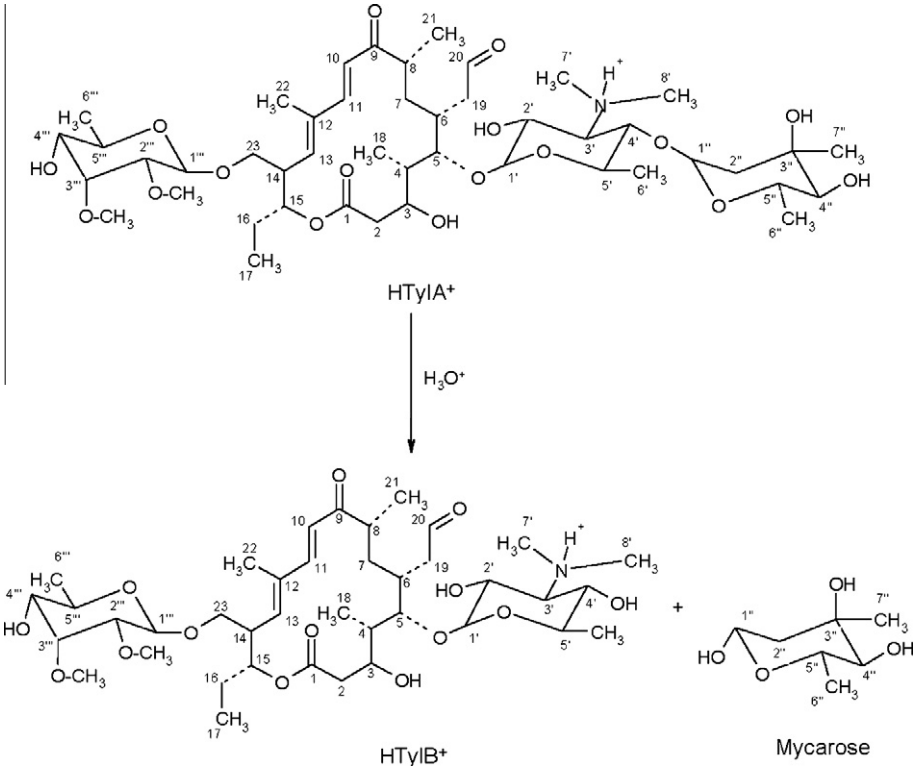

Fig. 1. Structure of tylosin A and degradation pathway in acid solutions. It indicates the formation of tylosin B for the release of neutral sugar mycarose.

the voltammetric current due to the control of external parameters (Section 3.1.3) [15].

Finally, we analyze the stability of tylosin A in aqueous solutions in extreme acid conditions. The degradation process was monitored and tylosin B or desmycosin was isolated and then identified and characterized by nuclear magnetic resonance spectroscopy and electrochemical techniques.

2. Experimental

Tylosin A tartrate (Sigma) was used without further purification. Tylosin B was synthesized with the following procedure: 500 mg of tylosin A tartrate were dissolved in 12 mL of water in a round-bottom flask, and the pH was adjusted to 2.8 by dropwise addition of commercial hydrochloric acid. The pale yellow solution was magnetically stirred at room temperature for 24 h, and the course of the reaction was followed by thin-layer chromatography (TLC) using ethanol as mobile phase. When the reaction completed, the solution pH was raised to 8.5 with sodium carbonate [16], after which the alkaline mixture was extracted three times with 15 mL of ethyl acetate. The organic phase was dried with anhydrous magnesium sulphate, filtered and evaporated under reduce pressure until constant weight. Desmycosin was recovered with 99% of yield as a white yellowish powder, which was characterized by nuclear magnetic resonance (NMR). Assignation of nuclear magnetic resonance signals in D_2O (ppm): 9.59 (H2O), 7.25 (H11), 6.55 (H10), 5.87 (H13), 5.10 (H15), 4.35 (H1′), 3.94 and 3.65 (H23), 3.88 (H5), 3.73 (H3), 3.57 (H2′), 3.47 and 3.57 (HOMe), 3.26 (H4′), 3.08 (H2‴), 2.57 (HNMe). Assignation of mycarose NMR signals in D_2O (ppm): 4.98 (H1″), 1.53 and 1.50 (H2″), 2.99 (H4″), 3.61 (H5″), 1.18 (H6″), 1.16 (H7″).

To obtain neutral tylosin A from tylosin A tartrate in a quantitative yield, 100 mg were dissolved in water and the pH was adjusted to 8.5 with sodium carbonate, and extracted three times with 15 mL of ethyl acetate. The organic phase was dried with anhydrous magnesium sulphate, filtered and evaporated under reduced pressure. The NMR of the product is in agreement with the assignation provided in the literature [17].

Tylosin A or tylosin B was always dissolved in the aqueous phase at different initial concentrations and the pH values were adjusted using sulphate, phosphate or acetate as a buffer.

The electrochemical experiments were performed in a four-electrode system using a conventional glass cell of 0.18 cm^2 interfacial area. Two platinum wires were used as counter-electrodes; the reference electrodes were $Ag/AgCl/Cl^-$. The reference electrode in contact with the organic solution was immersed in an aqueous solution of 1.0×10^{-2} M tetraphenylarsonium chloride (TPACl) (Merck p.a.). The potential values reported (E) are the potentials applied including $\Delta_o^w \phi'_{tr,TPA^+} = -0.364$ V for the transfer of the reference ion TPA^+. The supporting electrolytes are 1.0×10^{-2} M KCl (J.T. Baker p.a.), in ultrapure water, and 1.0×10^{-2} M tetraphenylarsonium dicarbollylcobaltate (TPADCC) in 1,2-dichloroethane, 1,2-DCE, (Dorwil p.a.). TPADCC was prepared as described in Ref. [18].

All the voltammograms shown in this work correspond to solutions in acid–base and partition equilibria. Aqueous and organic phases were equilibrated stirring different amounts of each phase in contact (initial system). This was performed in a stoppered flask for 1 h. The volume ratio r is defined as the ratio between the organic phase volume (V_o) and the aqueous phase volume (V_w). After equilibration, the electrochemical cell was filled with an aliquot of 5 mL of the aqueous phase and 1 mL of the organic phase. The aliquots were taken from the bulk of each phase in contact, to assure that their intensive properties (i.e., species concentrations) are the equilibrium properties. Thus, the electrochemical cell has been composed by two equilibrated solutions with the same properties as the initial system.

Cyclic voltammetry was carried out using a potentiostat which automatically eliminated the *iR* drop by means of a periodic current-interruption technique [19]. A Hi-Tek Instruments waveform generator and a data acquisition system were also employed.