Study of metal complexation of cardenolides with divalent metal ions by Electrospray Ionization Mass Spectrometry

Gastón E. Siless[a,b], Matias Butler[a,b], Gabriela M. Cabrera[a,b,*]

[a] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Orgánica, Ciudad Universitaria, Pabellón II, 3° piso, C1428EHA, Buenos Aires, Argentina
[b] CONICET-Universidad de Buenos Aires, Unidad de Microanálisis y Métodos Físicos aplicados a la Química Orgánica (UMYMFOR), Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, Pabellón II, 3° piso, C1428EHA, Buenos Aires, Argentina

ARTICLE INFO

ABSTRACT

Cardenolides are natural products with positive inotropic and cytotoxic activity that are able to interact with metals, although the possible role that these interactions may play in their biological activity is not known. Mixtures of the following cardenolides: digoxigenin (DgG), gitoxigenin (GxG), digitoxigenin (DxG), uzarigenin (UzG) and a butenolide, 2(5H)-furanone (Fur), with different metal cations, namely Ca^{2+}, Mg^{2+}, Cu^{2+}, Co^{2+} and Zn^{2+}, were studied by Electrospray Ionization Mass Spectrometry in a Quadrupole-Time of Flight. The relative stability of the most important adducts was studied by threshold collision induced dissociation, $E_{1/2}$. Computational modeling of the observed complexes with calcium was performed using DFT B3LYP/6-31G+(d,p) level of theory.

Complexes of stoichiometry $[nM+Me]^{2+}$, with n = 4 to 6 ligands and Me a metal cation, were observed for all studied compounds. The adducts $[4M+Me]^{2+}$ corresponded to the most intense peaks in most of the mass spectra and showed the highest $E_{1/2}$. GxG showed a higher tendency to form complexes with low coordination numbers. Calculations showed that the carbonyl oxygen of the butenolide moiety is the most important site of coordination and allowed the proposal of different binding modes to explain the differences observed in the GxG MS spectra.

A direct relationship was observed between experimental and computational data, which allowed to predict the MS behavior of these or similar compounds. The analysis can be extrapolated to other compounds with a furanone ring, and used as an analytical tool to characterize furanone compounds, or for the differentiation of DgG and GxG.

1. Introduction

Cardenolides are a family of natural compounds which share a steroidal framework with positive inotropic activity. Cardenolide glycosides, like digoxin, have been used for more than 200 years for the treatment of cardiac failures and have not been replaced by synthetic compounds. The steroid structure of the bioactive compounds must have a *cis* configuration between rings A–B and C–D and a *trans* configuration between B and C. It must also have an unsaturated γ-lactone ring attached to the 17β position, and two hydroxy groups at 3β and 14β positions. Sugar components are not related to the activity, but influence the pharmacokinetics [1].

The mechanism of action of cardenolides is attributed to the inhibition of Na^+/K^+-ATPase, raising the level of sodium ions in cardiac myocytes, which leads to an increase in the level of calcium ions via the Na^+/Ca^{2+} exchanger (NCX), and consequently an increase in cardiac contractile force. Recently, it was demonstrated that these compounds may activate multiple downstream signal transduction pathways that may be involved in the regulation of physiological and pathological conditions [2]. Remarkably, these compounds can also induce apoptosis and inhibit the growth of cancer cell lines at similar concentrations to those found in the plasma of patients with cardiac conditions. Several mechanisms seem to take part in these antitumor effects, and this is actually a topic of intense debate. Three compounds of this family are at this time in clinical trials [3].

The interaction between magnesium and digoxin has been studied, although from a pharmacological point of view [4]. NMR spectroscopy was used to demonstrate the formation of digoxin and digitoxin

complexes with calcium ions and this complex formation was related to pharmacological aspects. These experiments showed that digoxin in a high dose increases the calcium content in the heart muscle [5].

Metal complexation in solution, followed by effective transfer of charged complexes to the gas phase by Electrospray ionization (ESI) and subsequent analysis by Mass Spectrometry (MS) has proved to be an efficient way to recognize and identify these complexes [6,7]. Metal complexes of hydroxypyridine and pyrazine N-oxides [8,9], halogenated phenylmethylidenehydrazinecarbodithioates [10], cyclosporine [11], among others, have been identified previously by this technique. The characterization and the analysis of the relative stability of the complexes may be accomplished by Density Functional Theory (DFT) calculations, and these results may be used to explain and predict the behavior of these complexes in mass spectra [8].

Nowadays it is known that metal ions may be involved in the development of several diseases [12] and metal complex formation may be associated with ionophoric properties [13].

As these cardenolides exhibit biological activities in which the transport of ions is involved, a study was conducted of the interaction of four cardenolide genins: digitoxigenin (DxG), its 5-epimer uzarigenin (UzG), digoxigenin (DgG) and its isomer gitoxigenin (GxG),with different endogenous metal (II) ions such as Mg^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+}. The goal of this study was to determine binding selectivity and affinities for each metal and the different genins, to gather insight into the possible roles of these compounds in the biological activities. The observed differences between isomers were also taken into account (Fig. 1).

2. Experimental

2.1. Chemicals and reagents

The cardenolides digitoxigenin (DxG), uzarigenin (UzG), digoxigenin (DgG) and gitoxigenin (GxG), together with 2(5H)-furanone (Fur) were purchased from Sigma-Aldrich. LCMS grade methanol and HPLC grade water were purchased from Carlo Erba (Milan, Italy). The analyte solutions were prepared using methanol, each at a concentration of 10 mM. The metal ion stock solutions (10 mM) were prepared from $MgCl_2$, $CaCl_2$, $CoCl_2$, $CuSO_4$ and $ZnCl_2$ by diluting the appropriate amounts in water, as was determined in previous metal complexation studies by ESI-MS [8,9].

2.2. Instrumentation

Mass spectrometric analyses were performed using a BrukermicrOTOF-QII mass spectrometer (BrukerDaltonics, Billerica, MA, USA), equipped with an electrospray ion source. The instrument was operated at a capillary voltage of 4.5 kV with an end plate offset of −500 V, a drying gas temperature of 200 °C using N_2 as dry gas at 4.0 L min^{-1} and a nebulizer pressure of 0.4 bar.

Multi-point mass calibration was carried out using a sodium formate solution from m/z 50 to 1400 in positive ion mode. Data acquisition and processing were carried out using the Bruker Compass Data Analysis version 4.0 software supplied with the instrument.

The metal solutions were added in a 2:1 ratio to the sample solutions and then infused into the mass spectrometer. Sample solutions were infused into the source using a KDS100 syringe pump (KD Scientific, Holliston, MA, USA) at a flow rate of 180 μL min^{-1}. Each experiment was repeated at least three times in different days in order to ensure reproducibility.

For CID experiments, the quadrupole mass filter was set with a 1.0 Da window for transmission (isolation) of precursor ions. Fragmentation of the mass-selected ions (CID) was performed in a collision cell with UHP Argon as collision gas. The dissociation curves were built with data from sequential CID experiments, in which the potential was incremented in 1 eV steps, starting from 6 eV up to the potential in which the precursor ion signal fell to zero, plus 5 eV.

The Bruker msigma algorithm, which considers the isotopic profile together with the accurate mass, was taken into account to ascertain the identity of the ions.

2.3. Computational methods

Conformational search of $[nFur + Ca]^{2+}$ complexes (n = 3–6), where Fur is 2(5H)-furanone, was performed using molecular mechanics MM+ on Hyperchem 8.0, varying the dihedral angles involving calcium and oxygen atoms. Lowest energy conformers were selected for further optimization by DFT method using Gaussian 09 [14]. Frequency calculations were performed to characterize stationary points. Geometries of neutral, singly charged and doubly charged molecules were optimized at the B3LYP hybrid density functional level of theory (DFT) [15,16] using the 6–31 + G(d,p) basis set. This basis set featuring polarized orbitals proved to be well suited for the investigation of similar systems in previous studies [17,18]. The most probable coordination sites for the molecules were determined through the Gas Phase Calcium Affinity, which was calculated analogously to the Gas Phase Basicity GB [19] (Gibbs energy for the reaction $B + Ca^{2+} \rightarrow [B + Ca]^{2+}$). All the energies for protonation/complexation to Ca^{2+} were obtained by B3LYP/6–31 + G(d,p). The optimized structures were characterized by harmonic frequency analysis as local minima (all frequencies real). Corrections for zero-point vibrational energy were included using the same level of theory.

All energies expressed as $\Delta H°_0$ are relative enthalpies at 0 K; those expressed as $\Delta G°_{298}$ are relative free energies at 298 K, while those expressed as ΔE (in kcal/mol) are thermal energies. Calculations were performed at the Centro de Cómputos de Alto Rendimiento (CeCAR),
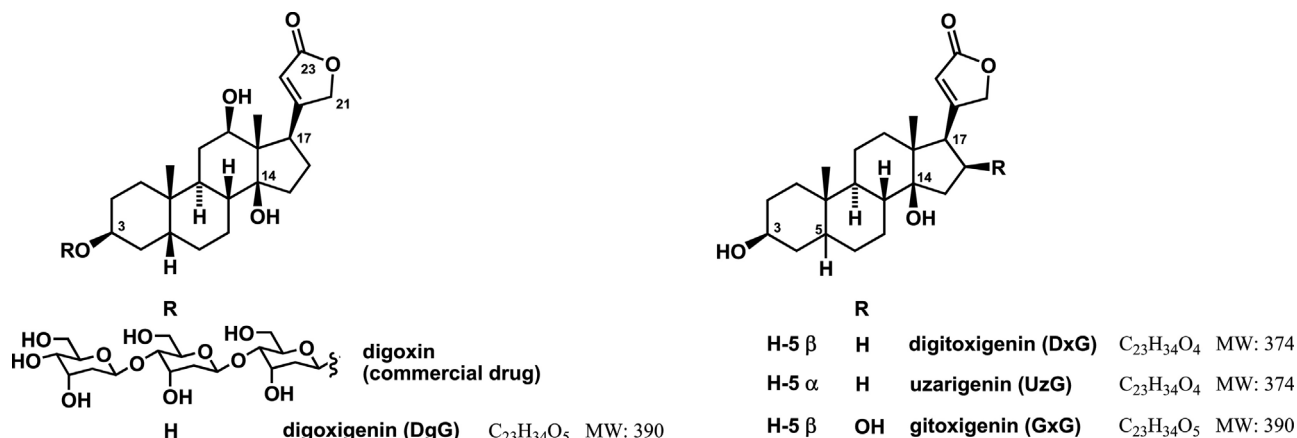

H-5 β	H	digitoxigenin (DxG)	$C_{23}H_{34}O_4$ MW: 374
H-5 α	H	uzarigenin (UzG)	$C_{23}H_{34}O_4$ MW: 374
H-5 β	OH	gitoxigenin (GxG)	$C_{23}H_{34}O_5$ MW: 390

digoxin (commercial drug)

H   digoxigenin (DgG) $C_{23}H_{34}O_5$ MW: 390

Fig. 1. Structures of the studied cardenolide genins and a commercial related drug.