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## Theoretical Characterization of The Protonation Sites of Mycolactones A/B and C by The Oniom Method

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### ABSTRACT

Mycolactones are infectious factors of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer. This work focuses on mycolactones A/B and C, the most virulent forms found in Africa and Australia. Each of these molecules is formed from a lactone ring and two side chains. The study focuses on the protonation reactions on each of the heteroatoms of the two mycolactones, in order to determine their preferential site. By implementing the ONIOM method, at the level (B3LYP/6-311+G(d,p): AM1) we determined, for each of the molecules and for each of the heteroatoms, the proton affinity (PA) and the gas phase basicity (GB). The choice of the Density Functional (DFT/B3LYP) allowed us to obtain very encouraging results. As for the choice of split-valence and triple-dzeta bases, they are sufficiently extended and the consideration of diffuse and polarization functions is important when it comes to intermolecular interactions. In total, the computational results identified the O5sp<sup>2</sup> heteroatom as the likely site of gas-phase protonation of the two mycolactones. The identification of this site constitutes a significant advance in the implementation of a methodology for the annihilation of the infection and destruction effects of these two toxins.

**Keywords:** *Mycobacterium ulcerans*, Mycolactone, ONIOM, protonation, Quantum Chemistry, Buruli ulcer

## 1. INTRODUCTION

Buruli ulcer is an infectious disease caused by *Mycobacterium ulcerans*, a microorganism belonging to the family of bacteria responsible for tuberculosis and leprosy [1]. Long neglected, this disease, which occurs in humid tropical and subtropical countries, is present on four (04) continents and has been reported in thirty-three (33) countries worldwide, twenty-seven (27) of which are in Africa. It is currently the third most common mycobacterial disease in the world after tuberculosis and leprosy [2]. Its global influence has led the World Health Organization (WHO) to classify it as an emerging disease and to recognize it as a public health and development problem [3].

*Mycobacterium ulcerans* enters the human body through the bite of an aquatic insect (*Naucoris cimicoides*) or through a skin break on a previously contaminated area. In the infected body, *Mycobacterium ulcerans* secretes a cytotoxic and immunosuppressive polyketide toxin called mycolactone that necroses the skin and soft tissue. To date, six (06) different molecular structures from several naturally occurring mycobacterial strains have been isolated [4]. Mycolactones A/B and C, which are produced by *Mycobacterium ulcerans* from Africa and Australia are the most virulent and destructive [5], are the subject of our study. Since 2005, the WHO has recommended a drug treatment consisting of a combination of antibiotics such as rifampicin and streptomycin for eight (08) weeks, regardless of the stage of the disease [6]. Evaluation after a few years showed that the introduction of antibiotic treatment reduced the relapse rate from 17% for surgery to 2%. It is true that the introduction of antibiotic treatment has significantly reduced relapses but it has not permanently reduced the sequelae of the disease [7]. The mode of treatment of Buruli ulcer remains limited, despite the progress observed in medical management [8]. Indeed, antibiotic therapy and reconstructive surgery, with their high cost and numerous relapses (16 to 28%), remain the reference treatments [9].

Moreover, the lack of knowledge of the mode of action of mycolactone in the literature and in the medical world is an obstacle to better management of Buruli ulcer. Most of the research work in this field is focused on the ecology of *Mycobacterium ulcerans*, the cutaneous part of the disease manifestation, the synthesis and structural characterization of mycolactones. The relationship between mycolactones, produced by *Mycobacterium ulcerans*, and the proteins that would be responsible for the appearance of Buruli ulcer are related to the conformation and interactions that these two types of molecules can establish. The research of the biological activities of mycolactones is crucial for a good understanding of their mode of action and also to establish a new therapeutic protocol in the framework of a preventive or even curative treatment of Buruli ulcer.

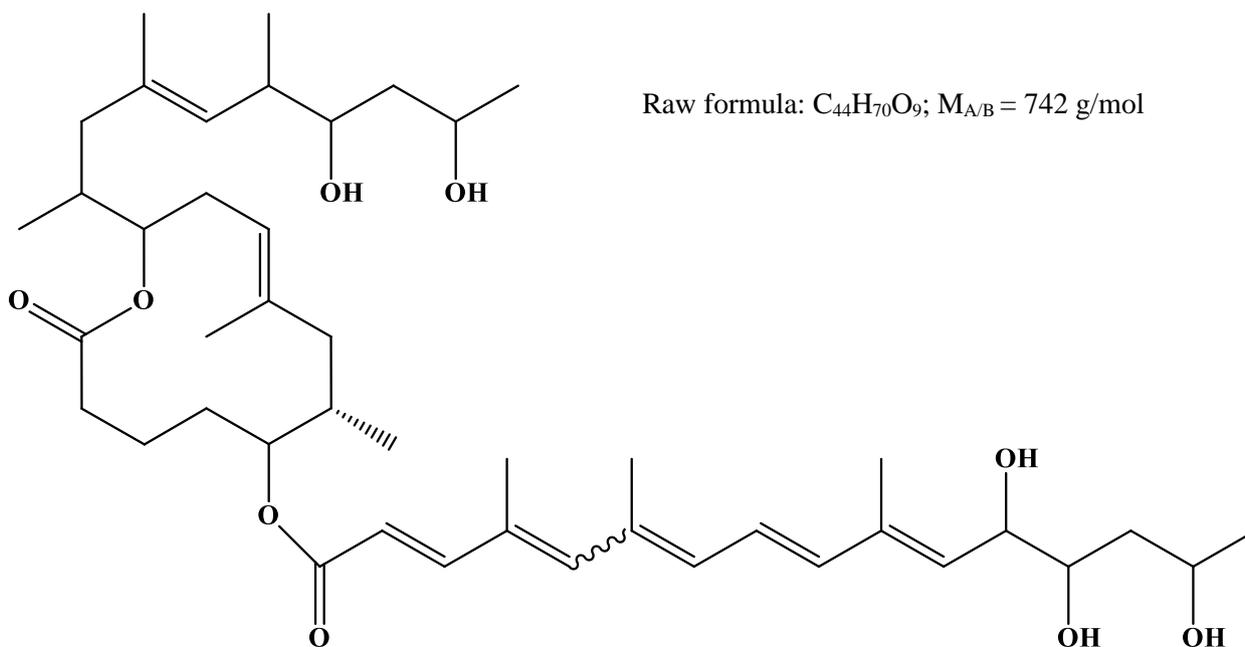
The knowledge of the molecular conformation and the interactions necessarily pass by the determination of the physicochemical parameters through the methods of Quantum Chemistry. Among the different properties of a biological molecule, its ability to protonate is likely to affect its fate in the environment, both in terms of its transport, its stay, its reactivity in the surrounding environment to the target molecule and in terms of its recognition by the receiver. Protonation is defined as a chemical reaction in which a proton ( $H^+$  ion) is added to

an atom, molecule or ion. Proton transfer reactions are important because they play a key role in the interactions of biological molecules or systems [10]. Biologically active molecules are usually derivatives of polyfunctional groups. The sites of protonation or deprotonation depend strongly on the acid-base properties of individual functional groups, interactions, and the intra- and intermolecular environment [11].

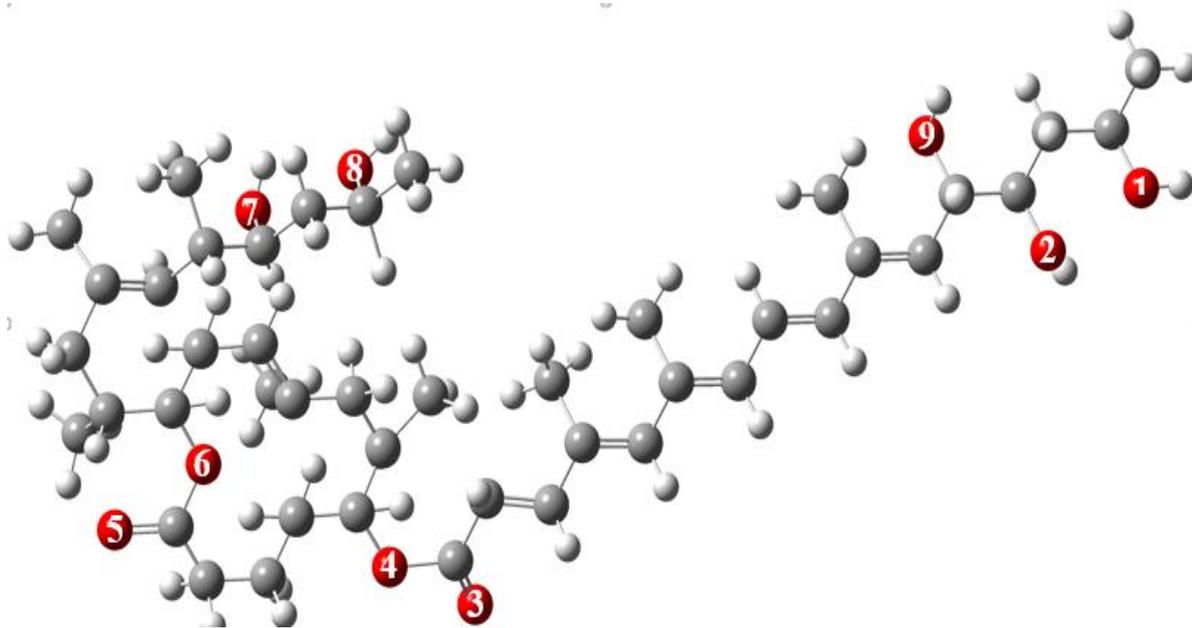
Protonation or deprotonation is the first step in many fundamental chemical rearrangements and in most enzymatic reactions. To characterize the ability of a molecule in the gas or condensed phase to accept a proton, two quantities are used. The gas phase basicity (GB) which is the opposite of the free energy change associated with the protonation reaction ( $GB = -\Delta G_o$ ) and the proton affinity ( $PA = -\Delta H_o$ ) [12]. Theoretical chemists have at their disposal a set of tools and approaches to evaluate the molecular properties of various systems, which can be easily calculated from the molecular structure. Thus, the development and improvement of increasingly powerful computer tools facilitate the study of complex systems as well as the study of very advanced techniques and levels of calculation. By implementing the methods of quantum chemistry, this work aims at determining, for each molecule, the probable sites of molecular interactions by protonation. The calculations are performed at the ONIOM level (B3LYP/6-311+G (d, p): AM1). Such a study will allow to propose a theoretical model of annihilation of the destructive effects of mycolactones A/B and C.

## 2. COMPUTATIONAL DETAILS

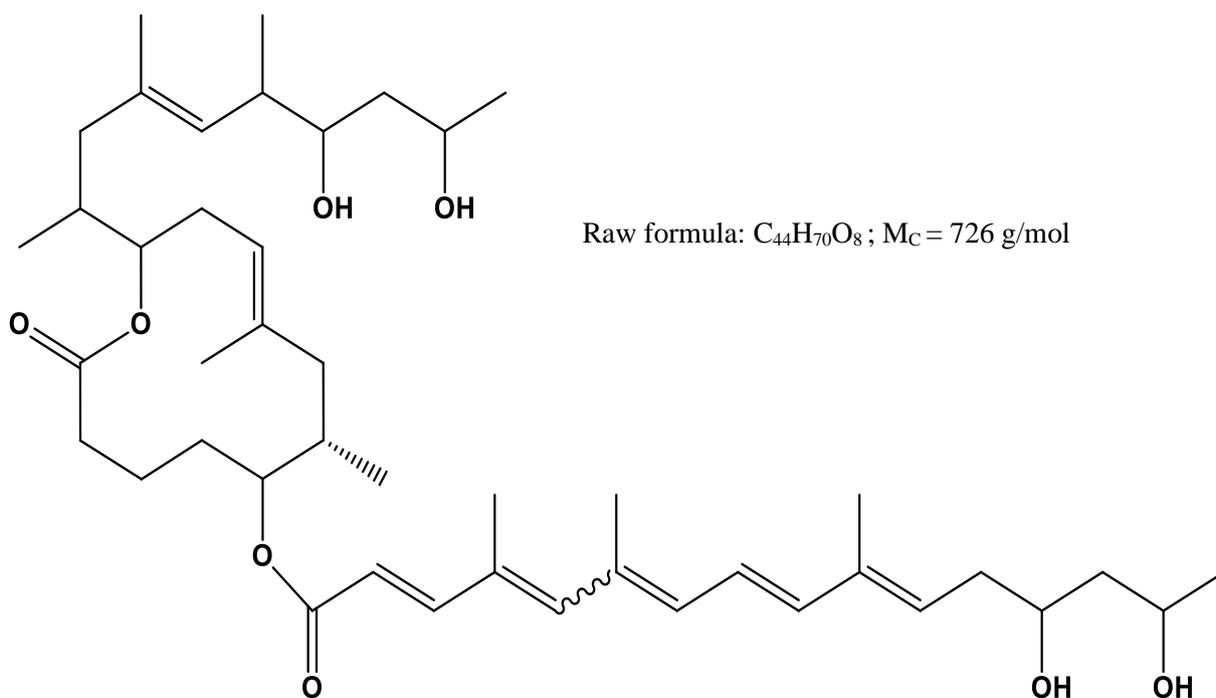
### 2.1. The Calculation Level



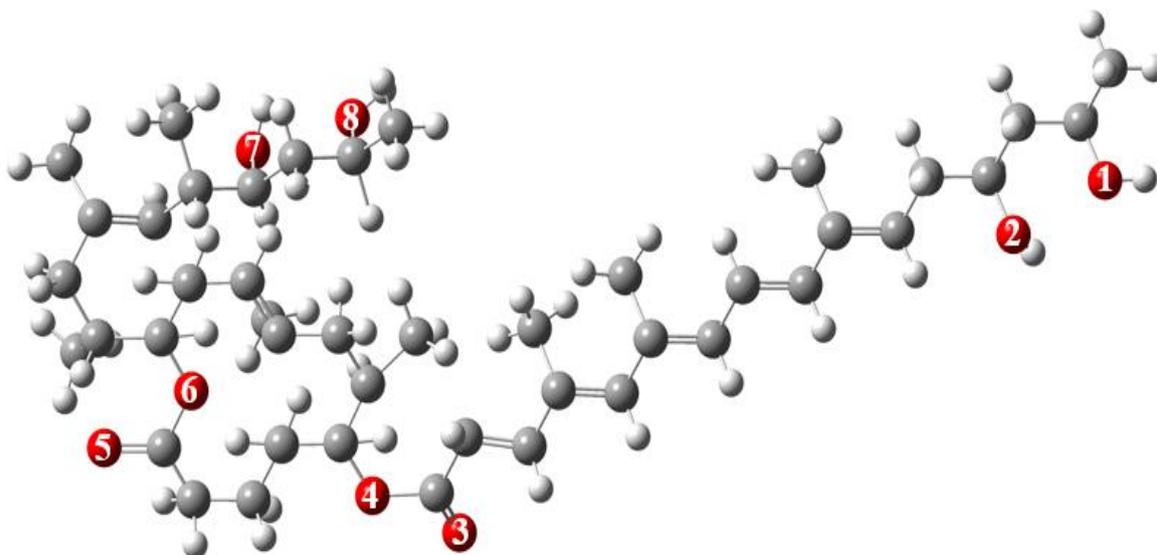
**Figure 1.** 2D schematic of mycolactone A/B (Figure obtained from the software ChemDraw software).



**Figure 2.** 3D schematic of mycolactone A/B. (Figure obtained from GaussView 03 software)



**Figure 3.** 2D schematic of mycolactone C (Figure obtained from the software ChemDraw software).



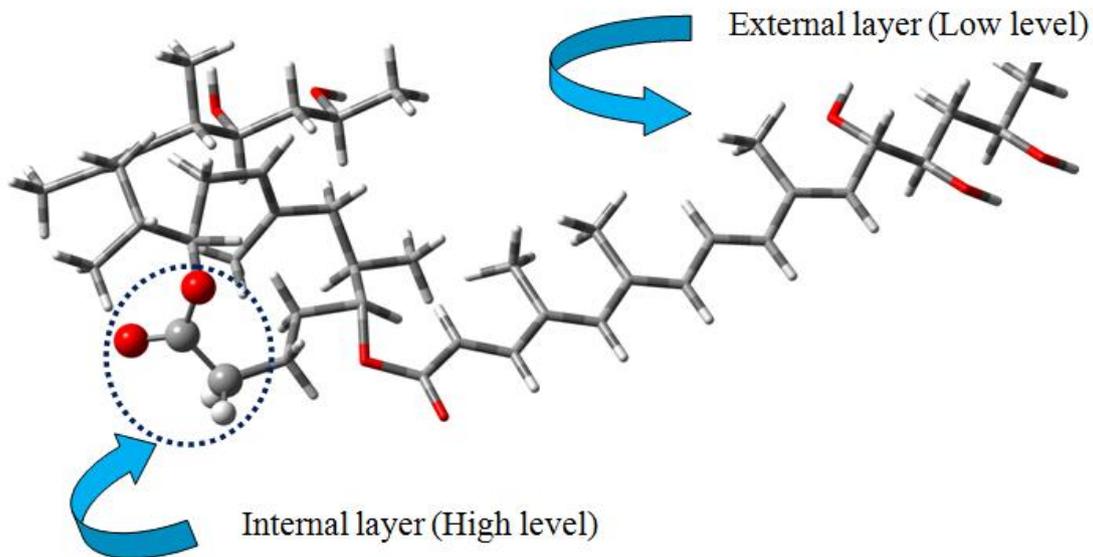
**Figure 4.** 3D schematic of mycolactone C. (Figure obtained from GaussView 03 software)

The non-optimized molecular structures of mycolactones A/B and C (2D and 3D) are presented by Figures 1, 2, 3 and 4. The gross formula and molecular weight of the different structures of the two mycolactones are specified. The heteroatoms of the mycolactone A/B or C, which consist only of oxygen atoms, are numbered from the main chain, going from the right to the left via the lactone ring. Thus, the numbers 1, 2, 3, 4, 5, 6, 7, 8 and 9 designate not only the heteroatoms but also the names of the different complexes formed. This numbering is done in such a way that the heteroatoms of the two identical mycolactones bear the same number.

In this work, the ONIOM method, developed by Morokuma *et al* [13-15], is used to determine some physicochemical properties of mycolactones A/B and C and their different protonated complexes. [16-19]. The ONIOM method also allows the study of large systems at a high computational level and at a reduced time. It also consists in splitting the studied system into several layers, each layer being treated at a different computational level. It thus allows to describe in a precise way the part of the system that is of particular interest for the study, called the internal layer or model system, and to describe in a less precise way the rest of the system, called the external layer or environment [20]. The addition of the inner layer and the outer layer constitutes the overall system. We have selected this method both for practical reasons, as it is integrated with its most recent developments under the Gaussian 03 software, used in this work; but also because of its successful use on large molecules. Thus, the reliability of the ONIOM method has been used to determine the dissociation energies of organic molecules, or the chemical reactivity of carbon nanotubes [21, 22].

The validity of this method has also been demonstrated for purely organic compounds by comparing theoretical results with those obtained using more elaborate levels of theory and experimental data [23]. In the case of a two-layer system (ONIOM 2) such as ours, the outer layer or environment will be the real system while the inner layer, the part of particular interest for our study, will be the model system. The real system will be treated at a low level (lower part). The model system we are interested in will be treated first with a high level of theory

(high part) and then at a low level, the final goal being to extrapolate the energy of the real system at the high level. Figure 3 shows a model of mycolactone A/B splitting with the ONIOM 2 method.



**Figure 5.** Cutting model of a mycolactone complex with the ONIOM 2 method.

The ONIOM method allows to obtain the energy of the real system at a high level of calculation. The total energy of the real system determined by extrapolation, is obtained from three independent calculations. The theory behind this method is that the difference in energy between a high-level ( $E_{real}^{high}$ ) and a low-level ( $E_{real}^{low}$ ) calculation for the real system is equal to the difference in energy between a high-level ( $E_{model}^{high}$ ) and a low-level ( $E_{model}^{low}$ ) calculation for the model system [24, 25]. Since the high and low level calculation methods are the same in both cases, the following relationship can be established:

$$E_{real}^{high} - E_{real}^{low} = E_{model}^{high} - E_{model}^{low} \quad (1)$$

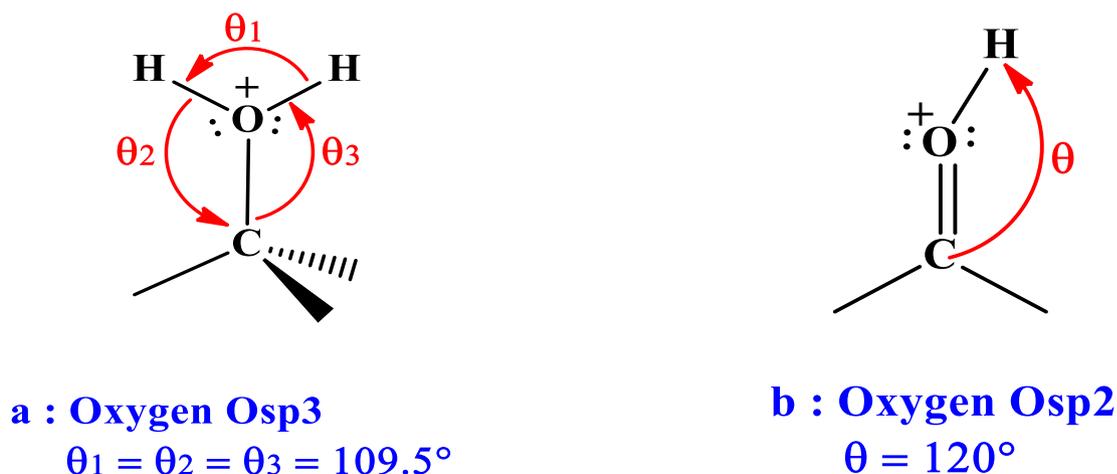
The knowledge of three of these terms gives access to the fourth without the need to calculate it. We can therefore have the energy of the real system at high computational level from three less expensive calculations. The total interaction energy obtained from the ONIOM 2 calculation [26], called the extrapolated energy, is defined as follows:

$$E^{ONIOM\ 2} \approx E_{real}^{high} = E_{model}^{high} + E_{real}^{low} - E_{model}^{low} \quad (2)$$

All the calculations were performed at the ONIOM level (B3LYP/6-311+G (d, p): AM1) with the Gaussian 03 software [27]. The choice of the Density Functional (DFT/B3LYP) allows us to obtain relatively less cumbersome results but is considered efficient [28]. The presence of the diffuse and polarization functions is important to take into account the free doublets of the heteroatoms. Also when it comes to intermolecular interactions.

## 2. 2. Geometry Optimization

The geometric parameter chosen for this study is the average valence angle  $\theta$  around the protonated sites of the A/B and C mycolactones. Prior to optimization, the protonated forms were initialized such that the valence angles between the hydrogen, the heteroatom of interest, and the adjacent atoms were equal. Thus, for the  $sp^3$  and  $sp^2$  oxygen atoms the average valence angles are  $109.5^\circ$  and  $120^\circ$  respectively. The most likely covalent bond that can be formed between the  $H^+$  proton and each basic site, is the one whose average valence angle  $\theta$  will tend at best towards the optimal angle which is  $120^\circ$  for  $sp^2$  oxygen and  $109.5^\circ$  for  $sp^3$  oxygen according to the Gillespie method or V.S.E.P.R method (Valence Shell Electronic Pair Repulsion) [29]. Figure 6 shows the initial geometries of the protonated forms on the  $sp^2$  and  $sp^3$  oxygens with the  $H^+$  proton.



**Figure 6.** Geometrical initialization schemes of the protonated forms on the oxygen atoms  $sp^3$  and  $sp^2$

## 2. 3. Energetic Parameters

Two thermodynamic protonation quantities are selected to characterize the ability that mycolactones A/B and C have in the gas phase to accept a proton ( $H^+$ ). These are gas-phase proton affinity (**PA**), which is the opposite of the enthalpy change associated with the protonation reaction ( $PA = -\Delta_r H^0$ ) and gas-phase basicity (**GB**), also defined as the opposite of the free enthalpy of protonation reaction ( $GB = -\Delta_r G^0$ ). The evaluation of the variations of the different electronic contributions to the internal energy at 0 K and 298.15 K between the product and the reactants contributes to the energetic characterization of the protonation reaction [30, 31]. For a given energy parameter X, its variation is determined through the relation :

$$\Delta X = \sum (\text{Products}) - \sum (\text{Reagents}) \quad (3)$$

By optimizing the geometries and calculating the frequencies of the free and interacting molecules, the variation of the internal energy at 0 K ( $\Delta E_{0K}^0$ ) and 298 K ( $\Delta E_{298K}^0$ ) relative to the studied relationship can be determined. All energy parameters are obtained from a frequency

calculation on each molecule and complex. The variation of the internal energy at 298 K, is a sum of the different electronic, translational, rotational, vibrational contributions and the internal energy at 0K through the relation :

$$\Delta E_{298K}^0 = \Delta E_{elec}^0 + \Delta E_{trans}^0 + \Delta E_{rot}^0 + \Delta E_{vib,thermal}^0 + \Delta E_{0K} \quad (4)$$

The calculations of the frequencies of the optimized molecules allow to access all the components of the internal energy. In addition,  $E_{0K}$  commonly called ZPVE (Zero Point Vibrational Energy) corresponds to the contribution of the vibration of molecules at 0 K. It reflects the vibrational energy at zero point induced by the normal modes of vibration of frequency  $\nu_i$  of the  $N$  nuclei at 0 K. It is defined by the relation (5) :

$$ZPVE = \frac{1}{2} R \sum_{i=1}^{3N-6} \frac{h \nu_i}{k} \quad (5)$$

with  $3N-6$  the number of normal modes of vibration of a nonlinear molecule ( $3N-5$  for a linear molecule);  $k$  Boltzmann's constant;  $h$  Planck's constant;  $R$  the perfect gas constant). This energy is the energy that corresponds to the internal energy at 0 K ( $\Delta E_{0K}$ ). During a temperature rise from 0 K to  $T$  (K), it is necessary to take into account the additional energy term corresponding to the population of vibrational energy levels during the temperature change. This term is defined by:

$$E_{vib,therm}^0 = R \sum_{i=1}^{3N-6} \frac{h \nu_i/k}{e^{(h\nu_i/T(K))} - 1} \quad (6)$$

As for the contributions of rotation and translation, they are derived from the perfect gas approximation by the relation :

$$\Delta E_{trans}^0 = \Delta E_{rot}^0 = - \frac{3}{2} RT \quad (7)$$

The change in internal energy  $\Delta E_{298K}$  at 298 K can then be written :

$$\Delta E_{298K}^0 = \Delta E_{elec}^0 + \Delta ZPVE + \Delta E_{vib,thermal}^0 - 3RT \quad (8)$$

In this relation, the enthalpy of reaction at 298 K is deduced. It corresponds to the change in internal energy corrected for the term  $\Delta(PV)$ , i.e.  $\Delta nRT$  ( $\Delta n$  being the change in the number of gas moles during the reaction).

$$\Delta H_{298K}^0 = \Delta E_{298K}^0 + \Delta nRT \quad (9)$$

The translational entropy contributions  $S_{trans}$ , rotational entropy  $S_{rot}$  and vibrational entropy  $S_{vib}$  of a given species at 298 K are combined into the total entropy term  $S$ . The reaction entropy is determined according to the relation.

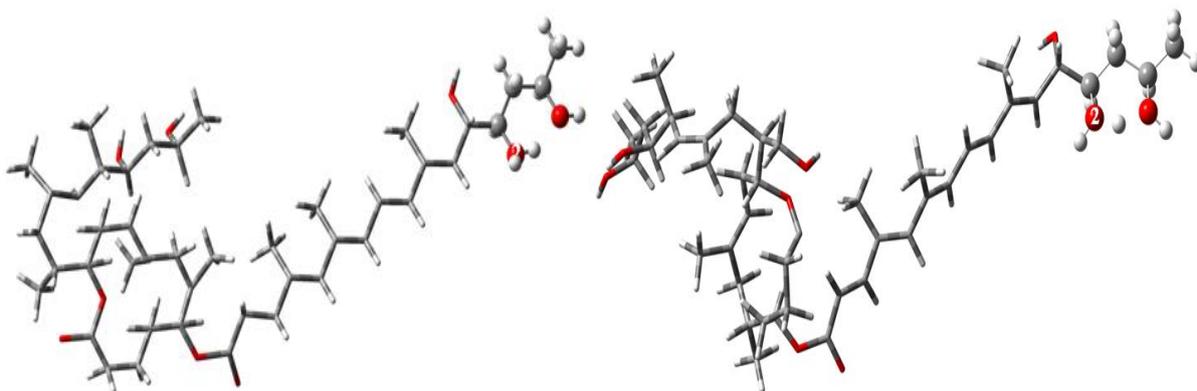
$$\Delta S_{298K}^0 = \sum S(\text{Products}) - \sum S(\text{Reagents}) \quad (10)$$

The Gibbs energy, at 298 K, related to the reaction is simply obtained by the relation :

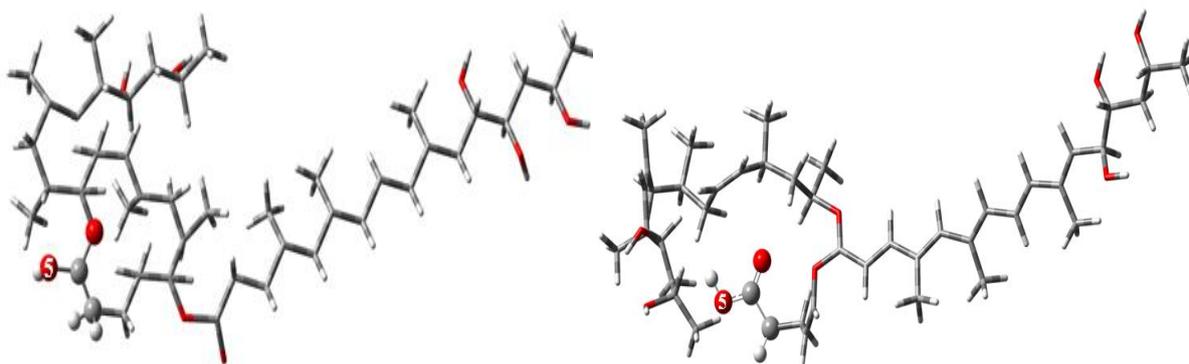
$$\Delta G_{298K}^0 = \Delta H_{298K}^0 - T\Delta S_{298K}^0 \quad (11)$$

### 3. RESULTS AND DISCUSSION

#### 3. 1. Analysis of Geometric Parameters



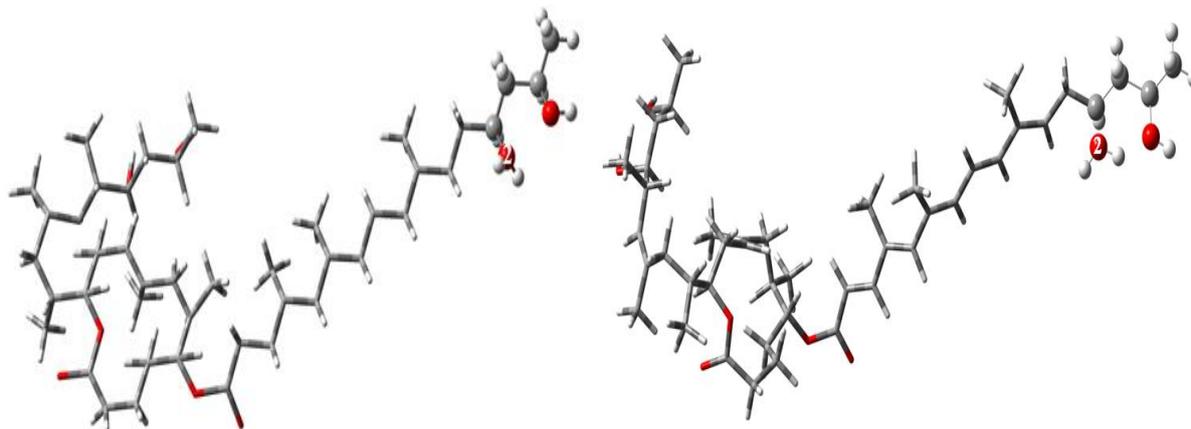
Initial (left) and optimized (right) structures of the protonation complex formed from mycolactone A/B on the  $O_{2sp^3}$  heteroatom



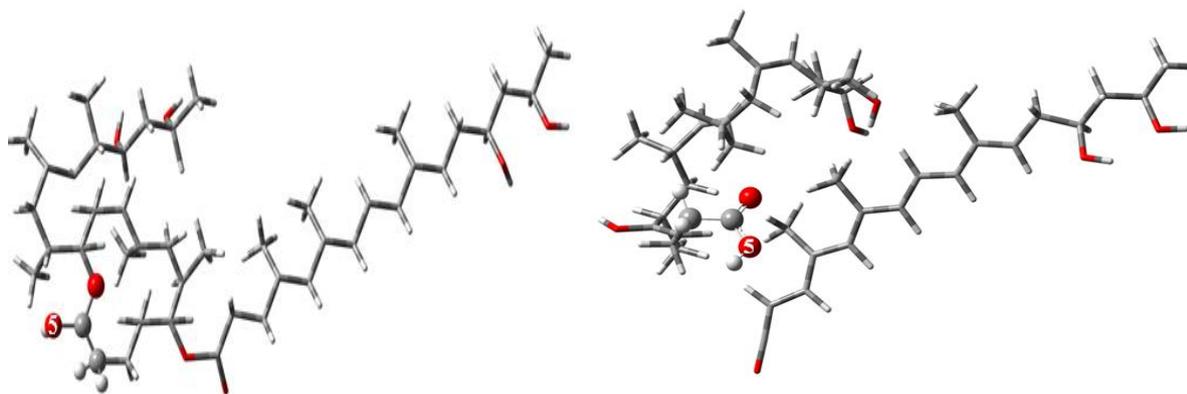
Initial (left) and optimized (right) structures of the protonation complex formed from mycolactone A/B on the  $O_{ssp^2}$  heteroatom

**Figure 7.** Non-optimized and optimized geometries of some protonated forms of mycolactone A/B

The geometric parameter chosen for this study is the average valence angle  $\theta$  around the protonated sites of mycolactones A/B and C. Figures 7 and 8 show the optimized geometries of the protonated forms of mycolactone A/B and C with the  $H^+$  proton at the ONIOM level (B3LYP/6-311+G (d,p): AM1).



Initial (left) and optimized (right) structures of the protonation complex formed from mycolactone C on the  $O_{2sp^3}$  heteroatom



Initial (left) and optimized (right) structures of the protonation complex formed from mycolactone C on the  $O_{5sp^2}$  heteroatom

**Figure 8.** Non-optimized and optimized geometries of some protonated forms of mycolactone C

Figures 7 and 8 were obtained from GaussView implemented in the Gaussian 03 software. The parts presented in tube are the external layers, optimized at low level precisely in AM1. The parts presented in "ball and stick", constitute the model system. They are optimized at the B3LYP/6-311+G (d, p) level.

Visualization of the different optimized geometries of the protonated complexes (Figures 7 and 8) showed that the optimizations of the complex geometries were successful for all heteroatoms of both mycolactones.

**Table 1.** Average valence angle values  $\theta$  (in degrees) of mycolactones A/B and C obtained at ONIOM (B3LYP/6-311+G (d,p) : AM1).

	Mycolactone A/B	Mycolactone C
<b>O<sub>1</sub>sp<sup>3</sup></b>	112.07	109.46
<b>O<sub>2</sub>sp<sup>3</sup></b>	113.65	105.56
<b>O<sub>3</sub>sp<sup>2</sup></b>	113.28	110.46
<b>O<sub>4</sub>sp<sup>3</sup></b>	110.33	119.84
<b>O<sub>5</sub>sp<sup>2</sup></b>	<b>120.45</b>	<b>121.85</b>
<b>O<sub>6</sub>sp<sup>3</sup></b>	112.15	111.38
<b>O<sub>7</sub>sp<sup>3</sup></b>	102.71	105.76
<b>O<sub>8</sub>sp<sup>3</sup></b>	107.20	109.29
<b>O<sub>9</sub>sp<sup>3</sup></b>	123.29	-

All values reported in Table 1 approach the optimal valence angle for all heteroatoms. However, the **O<sub>5</sub>sp<sup>2</sup>** heteroatoms of the two mycolactones show values of the valence angles closest to the optimal value. These values are respectively 120.45° for mycolactone A/B and 121.85° for mycolactone C. It is clear that the **O<sub>5</sub>sp<sup>2</sup>** heteroatom is the preferred site for the protonation of mycolactones A/B and C from the point of view of geometrical parameters.

### 3. 2. Analysis of Energy Parameters

**Table 2.** Energy descriptors of the protonation reactions of the different heteroatoms. Values (in (kJ.mol<sup>-1</sup>)) calculated at ONIOM (B3LYP/6-311+G (d,p) : AM1).

	Mycolactone A/B		Mycolactone C	
	PA	GB	PA	GB
<b>O<sub>1</sub>sp<sup>3</sup></b>	401.02	426.69	406.88	424.48
<b>O<sub>2</sub>sp<sup>3</sup></b>	405.33	443.05	436.29	459.11
<b>O<sub>3</sub>sp<sup>2</sup></b>	447.29	484.35	425.15	459.60
<b>O<sub>4</sub>sp<sup>3</sup></b>	407.93	430.34	418.24	443.16
<b>O<sub>5</sub>sp<sup>2</sup></b>	<b>516.60</b>	<b>522.48</b>	<b>616.24</b>	<b>675.42</b>

<b>O<sub>6sp</sub><sup>3</sup></b>	349.25	394.71	436.14	471.01
<b>O<sub>7sp</sub><sup>3</sup></b>	352.44	401.32	383.05	448.17
<b>O<sub>8sp</sub><sup>3</sup></b>	463.19	497.36	544.77	553.94
<b>O<sub>9sp</sub><sup>3</sup></b>	383.07	395.55	-	-

The spontaneity of each of the protonation reactions at each of the basic sites is indicated by the positive value of the gas phase basicity (GB). As for the positive value of the proton affinity (PA), it reveals the exothermicity of each of the protonation reactions. On the other hand, the highest values of proton affinity and gas-phase basicity of the two mycolactones, are observed with the O<sub>5sp</sub><sup>2</sup> heteroatom (**PA = 516.60 kJ/mol; GB = 522.48 kJ/mol** for mycolactone A/B and **et PA = 616.24 kJ/mol; GB = 675.42 kJ/mol** for mycolactone C). This shows that the O<sub>5sp</sub><sup>2</sup> heteroatom is the most basic site giving, the most stable protonated forms.

After examining the values of the geometric and energy parameters of protonation, it follows that the O<sub>5sp</sub><sup>2</sup> heteroatom gives the most stable protonated forms for both mycolactones. The positive values of proton affinities and gas phase basicity account for the spontaneity of these processes.

#### 4. CONCLUSION

The use of the ONIOM method, allowed to determine the geometrical and energetic parameters of protonation; and this for each atomic heteroatom of the mycolactones A/B and C. The calculation, followed by the analysis of the results related to the protonation descriptors on the different heteroatoms, such as the proton affinity (PA) and the gas phase basicity (GB), clearly showed that the **O<sub>5sp</sub><sup>2</sup>** heteroatom is the preferential protonation site.

The identification of such a site constitutes a significant advance in the project of annihilating the infection effects of both mycolactones. Indeed, the design of a molecule able to annihilate such a site would render ineffective the action of *Mycobacterium ulcerans*.

All the results obtained in this work offer to the researchers a new interesting axis of investigation in the project of annihilation of the destructive and necrotizing effects of mycolactones. Research efforts can be focused on further understanding the interaction of mycolactones A/B and C via the oxygen atom **O<sub>5sp</sub><sup>2</sup>** for a better consideration of Buruli ulcer. It is, moreover, possible to improve these results on the theoretical characterization of the physicochemical properties of mycolactones A/B and C by using other levels of calculation with more extended bases.

Thus, we envisage, in the immediate future:

- to extend the study of protonation interactions to other mycolactones in order to better understand their inhibitory effects and their biological activities;
- to characterize the hydrogen bonding interactions of the different mycolactone complexes in order to predict their residence time in subcutaneous fat.

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