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An Investigation of Chlorophenol Proton Affinities and Their Influence on the Biological Activity of Microorganisms

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The proton affinities of 15 chlorophenols are calculated by ab initio methods. Straight correlation between proton affinities and changes in the electronic structure is observed. The proton affinities decrease linearly with the electronic density gain on the chlorine atoms, as the liberation of the proton increases. To confirm the importance of the proton affinities on the toxicity of chlorophenols, calorimetric responses of these molecules and related ones where the acid proton is changed to a methyl group (anisol and its chlorinated derivatives) were used to verify their effects on *Chromobacterium violaceum*. The results confirmed that the chlorophenols are more toxic than the respective chloroanisols and suggest that high proton affinities are associated with low toxic activity. The toxicity of the chlorophenols can be associated with the respiratory mechanism in some microorganisms.

1. Introduction

Increasing exposures to toxic wastes have motivated scientists to study mechanisms to minimize the degeneracy of life quality on our planet. As an example, of a set of approximately 100 000 chemical compounds commercially available in 1994, 20 000 were considered dangerous and only 2500 had been effectively classified and analyzed in terms of their toxicity.[1] Among this large number of compounds, chlorophenols are an important class of organic molecules that play a major role in the chemical industry even though their impact on living organisms is alarming, when their toxic effects are considered. The enormous interest in their biological activity in vitro, in vivo, and in the possible alternatives to eliminate them from the environment is demonstrated by a variety of research in recent years as reported in the literature. A simple search in the literature shows that chlorophenols were studied or cited in approximately 110 papers in the year of 2003.[2−4] Despite all these efforts, present knowledge is still unsatisfactory and little is known about their action in living organisms or even more fundamental information like thermochemical or molecular properties of compounds of this family. Current studies, using a diversity of modern experimental and computational tools, have been concentrated on the kinetic and thermodynamic stabilities of the phenolic bond (O−H bond dissociation) and how these stabilities are affected by the number, nature, and position of the substituents in the aromatic ring.[5] One of the interesting perspectives on the biological activity of substances like chlorophenols suggests that their ability to cross membranes can be associated with the acid−base properties of the substances.[6] Weak organic acids and bases are usually more lipophilic and diffuse across the lipid regions of membranes more quickly than stronger ones. In organic chemistry, this dependence of proton affinity on structural features is often explained in terms of mesomeric or electrostatic substituent effects and is also associated with electronic reorganization when a molecule releases a proton.[5] Qualitative and quantitative correlations between proton affinities and properties associated with charge distribution can be found in the literature. For instance, Souza et al.[7] have observed an almost linear correlation between proton affinities and generalized atomic polar tensor (GAPT) charges for hydrides involving elements from groups 14 to 17 of the periodic table. Voets et al.[8] observed a linear correlation between proton affinities and Mulliken charges for some 2-subtituted pyridinium ions using AM1 calculations. Howard and Platts[9] studied a relationship between phosphine proton affinities and lone pair density properties using Bader's Atoms-In-Molecules theory.

The present work has three objectives: (a) to employ theoretical ab initio methods such as the Hartree−Fock (HF) and density functional theories (B3LYP) to estimate the proton affinities of 15 chlorophenols, most of which have not been determined experimentally, (b) to use these values to assess our understanding of the substituent effects and also to relate the proton affinity of chlorophenol compounds with the electronic distribution of their chemical structures ,and (c) to associate the influence of this property on toxic action of the chlorophenols on microorganisms.

2. Computational Methods

High-level ab initio methods using Gaussian basis functions and complete basis set schemes are remarkably accurate, typically providing acidities within 2 or 3 kcal·mol^{-1} of the experimental values.[7,10−15] For this reason, in this work ab initio calculations are performed to determine the molecular structure and proton affinity of a set of 15 chlorophenols.

The proton affinities reported are calculated from differences of absolute enthalpies (H) of products and reactants for the reaction:

$$A_{(g)}^{-} + H_{(g)}^{+} \rightarrow HA_{(g)}; \quad \Delta H_{298K} \tag{1}$$

where $\Delta H_{298K} = H(HA_{(g)}) - H(A_{(g)}^{-}) - H(H_{(g)}^{+})$.

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Thus, the proton affinities are calculated by the equation:

$$\Delta H_{298K} = \Delta E^{elec} + \Delta E^{vib}_{298K} + \Delta E^{rot}_{298K} + \Delta E^{trans}_{298K} + \Delta (pV)_{298K} \quad (2)$$

where $\Delta E^{elec} = E^{elec}(ClPhO^-) - E^{elec}(ClPhOH)$, which represents the difference between the total energy of the neutral chlorophenol (ClPhOH) and the energy of the unprotonated molecule ($ClPhO^-$) for each basis set at two levels of theory: Hartree−Fock and density functional theory (DFT) through the B3LYP hybrid functional. The structures of the neutral and anionic species are fully optimized at the two levels of theory for each different basis set. ΔE^{vib} is the vibration energy computed using harmonic approximation, standard statistical mechanical formulas and computed using the respective level of theory and basis set. ΔE^{rot} and ΔE^{trans} are rotation and translation contribution, respectively, and computed classically. $\Delta(pV)$ is replaced by ΔnRT, using the ideal gas law.

Compact effective core potentials (CEP) developed by Stevens, Basch, and Krauss,[16]are used along with three basis sets: CEP-31G, CEP-31++G**, and a basis set modeled by the generator coordinate method (GCM),[17,18] which provides a more general procedure based on the adjustment of basis sets with the CEP pseudopotential, reducing considerably computational demands when compared with calculations employing all electrons. The GCM basis set for C, O, and Cl, including polarization and diffuse functions, are presented in Table 1. Sets of 5d functions are used when polarization is considered either using CEP or GCM basis sets. The cc-pVDZ basis set is used for hydrogen. Only B3LYP calculations are carried out with the GCM basis set.

All the calculations are carried out with the Gaussian/2003 program.[19]

TABLE 1: GCM Basis Set for C, O, and Cl Including Diffuse and Polarization Functions to Be Used along with SBK Pseudopotential, Where the cc-pVDZ Basis Set Was Used for Hydrogen

s function		p function		d function	
α_i	C_I	α_i	C_I	α_i	C_I
GCM Basis Set for Chlorine Atom [4s2s1s|3p2p1p]					
0.067 82	1.000 00	0.046 60	1.000 00		
0.171 29	0.359 06	0.120 84	0.258 49		
0.432 62	0.771 53	0.313 33	0.517 03		
1.092 64	−0.00665	0.812 45	0.361 05	0.812 45	1.000 00
2.759 64	−0.29359	2.106 65	−0.02878		
6.969 89	0.046 55	5.462 46	−0.02262		
17.60354	−0.00950				
GCM Basis Set for Carbon Atom [4s2s1s|5p2p1p]					
0.036 75	1.000 00	0.028 59	1.000 00		
0.088 68	0.161 19	0.069 31	0.103 22		
0.214 02	0.507 86	0.168 05	0.333 34		
0.516 49	0.426 99	0.407 42	0.390 32		
1.246 45	0.042 71	0.987 78	0.251 07	0.987 78	1.000 00
3.008 07	−0.13580	2.394 80	0.116 10		
7.259 42	−0.03713	5.806 05	0.032 03		
		14.07641	0.015 98		
GCM Basis Set for Oxygen Atom [4s2s1s|6p2p1p]					
0.070 18	1.000 00	0.043 50	1.000 00		
0.168 96	0.161934	0.103 50	0.068 55		
0.406 77	0.489018	0.246 23	0.262 35		
0.979 32	0.437239	0.585 79	0.361 97		
2.357 73	0.056543	1.393 61	0.309 55	1.393 61	1.000 00
5.676 30	−0.138682	3.315 47	0.166 68		
13.66585	−0.040101	7.887 66	0.067 51		
		18.76512	0.017 55		
		44.64331	0.007 50		

3. Effective Charges

Two different methods are used to determine the atomic charges of the chlorophenols: (a) Mulliken population analysis and (b) the generalized atomic polar tensor (GAPT).[20] GAPT considers that the effective atomic charges are obtained from the isotropically averaged atomic polar tensor:

$$Q_A = \frac{1}{3}\left(\frac{\partial \mu_x}{\partial x_A} + \frac{\partial \mu_y}{\partial y_A} + \frac{\partial \mu_z}{\partial z_A}\right) \quad (3)$$

where Q_A is the effective charge of atom A, μ_x, μ_y and μ_z are components of the dipole moment and x_A, y_A and z_A are the Cartesian coordinates of nucleus A. Applications of GAPT have presented interesting correlations with experimental properties allowing the interpretation of tendencies through simple physical pictures.[7] Another important aspect is that GAPT have proved to be not very sensitive to the basis set or correlation effects.[21] Therefore, the correlation between atomic charges and proton affinities is analyzed for the set of 15 chlorophenols.

4. Results and Discussion

Table 2 summarizes the theoretical and available experimental proton affinities (PA) of 15 chlorophenols. The absence of experimental data for all the compounds significantly limits the analysis of the accuracy of the theoretical calculations. However, Table 2 shows that calculated proton affinities are systematically larger than the experimental. Hartree−Fock proton affinities calculated with CEP-31G or CEP-31++G** reveal differences as large as 10 kcal·mol^{-1} compared with the experimental results. The inclusion of electronic correlation at the B3LYP level improves significantly the results and usually provides deviations not larger than 3 kcal·mol^{-1}, regarding the experimental results. It is worth noting that the inclusion of diffuse and polarization functions either at the Hartree−Fock or B3LYP levels provide small improvements of the final proton affinities. The differences between results expanding and not the basis sets are proportional to the number of chlorine atoms. The deviation between calculations with and without diffuse and polarization functions at the same level of calculation are approximately 0.5 kcal·mol^{-1} for monochlorophenols, 2.0 kcal·mol^{-1} for dichlorophenols, and up to 4.0 kcal·mol^{-1} for trichlorophenols.

To carry out an appropriate analysis of the chlorophenol tendencies, the molecules are divided into three categories: monochlorophenols (ClPhOH); dichlorophenols (DClPhOH) and trichlorophenols (TClPhOH).

Monochlorophenols. The theoretical and experimental sequence of proton affinities follows the tendency:

PA: 3-ClPhOH < 4-ClPhOH < 2-ClPhOH

This sequence can be interpreted in terms of the capacity of the molecules to form hydrogen bonds and the accommodation of the electronic density after proton elimination. Therefore, the largest proton affinity of 2-ClPhOH is a consequence of an intramolecular hydrogen bond between the oxygen and the chlorine atoms in neighboring positions. The optimized geometry of the 2-ClPhOH at different levels of theory shows the presence of the hydroxyl proton in a position compatible with a hydrogen bond. The 4-ClPhOH and 3-ClPhOH proton affinities can be understood considering an electronic rearrangement before and after proton extraction. Table 3 illustrates the

TABLE 2: Experimental and Theoretical Proton Affinities (PA, in kcal·mol^{-1}) of Chlorophenols (ClPhOH) Obtained from Different Levels of Theory at the Temperature of 298.15 K

molecule	PA[1]	PA[2]	PA[3]	PA[4]	PA[5]	expt
2-ClPhOH	−348.1	−348.9	−341.5	−341.0	−340.3	−337.1 ± 2.0[b]
3-ClPhOH	−345.6	−346.8	−338.3	−338.3	−337.7	−335.0 ± 2.0[c]
4-ClPhOH	−346.8	−348.0	−340.1	−339.7	−339.1	−336.2 ± 2.0[c]
2,3-DClPhOH	−340.3	−342.8	−333.8	−335.2	−334.4	
2,4-DClPhOH	−339.4	−342.4	−333.2	−334.8	−334.0	
2,5-DClPhOH	−338.1	−341.0	−331.3	−333.3	−332.5	
2,6-DClPhOH	−337.8	−340.6	−331.5	−333.2	−332.3	
3,4-DClPhOH	−338.7	−341.4	−332.0	−333.4	−332.7	
3,5-DClPhOH	−336.0	−339.1	−328.8	−330.9	−330.2	−327.5 ± 2.0[c]
2,3,4-TClPhOH	−333.8	−337.7	−327.8	−330.8	−329.9	
2,3,5-TClPhOH	−331.2	−335.4	−324.8	−328.2	−327.3	
2,3,6-TClPhOH	−330.7	−334.8	−324.4	−328.0	−326.9	
2,4,5-TClPhOH	−331.8	−336.1	−325.6	−328.8	−328.0	
2,4,6-TClPhOH	−330.1	−334.6	−324.2	−327.6	−326.6	
3,4,5-TClPhOH	−331.1	−335.1	−324.6	−327.7	−326.9	−323.9 ± 2.0[c]

[a] Key: ClPhOH, chlorophenol; PA[1], HF/CEP-31G; PA[2], HF/CEP-31++G**; PA[3], B3LYP/CEP-31G; PA[4], B3LYP/CEP-31++G**; PA[5], B3LYP/GCM. Harmonic frequencies are calculated for neutral and ionic forms at the respective level of calculation. [b] Reference 22. [c] Reference 23.

TABLE 3: Mulliken (Q_M) and GAPT Charge Distributions of the Chlorine Atom in Monochlorophenols (ClPhOH) in the Neutral (n) and Ionic Form (ClPhO$^-$), and the Electronic Density Gains on the Chlorine Atom (EDGCl) with Respect to the Ionic Form, Obtained Using B3LYP/GCM

	net charges on Cl		EDGCl	
molecule	Q_M	GAPT	Q_M	GAPT
2-ClPhOH	−0.118	−0.333		
2-ClPhO$^-$	−0.249	−0.448	0.131	0.116
3-ClPhOH	−0.097	−0.346		
3-ClPhO$^-$	−0.245	−0.521	0.149	0.175
4-ClPhOH	−0.105	−0.341		
4-ClPhO$^-$	−0.248	−0.454	0.143	0.113

changes in the electronic density on the chlorine atom from Mulliken and GAPT charge analysis calculated at the B3LYP/GCM level of theory.

The results obtained from other levels of calculation present the same qualitative tendency. The electronic density gain on the chlorine atom (EDGCl) is correlated with its position on the benzene ring and also with the proton affinity; in other words, the largest electronic density gain on the chlorine atom is directly associated with the lowest proton affinity and vice versa. The order of proton affinity (PA) follows exactly the opposite tendency of the electronic density gain of the chlorine atom after proton abstraction. Changes in the electronic distribution associated with other atoms do not present as good correlation with the proton affinities when all mono, di and trichlorophenols are analyzed. In some sense the results suggest that large dispersions of the electronic density on the phenol ring favors stronger attractive forces acting on the hydroxyl proton. If the chlorine tendency to withdraw electrons is emphasized after the elimination of the hydroxyl proton, then it can be considered that the electronic distribution was being localized in a position where the proton was subjected to stronger attractive forces, preventing its elimination from the molecule.

In summary, there is a direct correlation between the acidity of ClPhOH and the capacity of the chlorine atom to attract electronic density after the elimination of the hydroxyl proton. The electronic density displacement depends on the position of the chlorine atom (*ortho*, *meta*, and *para*) on the aromatic ring.

Dichlorophenols. The analysis of the tendency of the proton affinities for DClPhOH is not as simple as for ClPhOH. For example, 3,4-DClPhOH presents a larger proton affinity than 2,6-DClPhOH and 2,5-DClPhOH (see Table 2). The last two

TABLE 4: Mulliken (Q_M) and GAPT Electronic Density Gains in the Chlorine Atom (EDGCl) with Respect to the Ionic Form in Dichlorophenols (DClPhOH ↔ DClPhO$^-$) and Trichlorophenols (TClPhOH ↔ TClPhO$^-$), Obtained Using B3LYP/GCM

	EDGCl			EDGCl	
dichlorophenols	Q_M	GAPT	trichlorophenols	Q_M	GAPT
2,3-DClPhOH	0.239	0.228	2,3,4-TClPhOH	0.347	0.268
3,4-DClPhOH	0.258	0.246	2,3,5-TClPhOH	0.365	0.347
2,4-DClPhOH	0.264	0.212	3,4,5-TClPhOH	0.365	0.347
2,5-DClPhOH	0.265	0.264	2,4,5-TClPhOH	0.368	0.323
2,6-DClPhOH	0.277	0.242	2,3,6-TClPhOH	0.384	0.335
3,5-DClPhOH	0.282	0.309	2,4,6-TClPhOH	0.403	0.321

molecules can confine the hydrogen from hydroxyl group due to hydrogen bonding, which would suggest stronger forces acting on this proton. 3,4-DClPhOH suggests that the net effect is not only a predominance of hydrogen bonds over the proton energy but the combination of more complex balances in these molecules. 2,6-DClPhOH presents the second lowest proton affinity with respect to other DClPhOH though it is able to form hydrogen bonds with both of the chlorine atoms. The presence of two chlorine atoms close to the hydroxyl group removes electrons not only from the benzene ring but also from the hydroxyl group, which minimizes the attractive effects over the proton, reducing the magnitude of the proton affinity.

An excellent correlation between proton affinity and the sum of the electronic density gain of the chlorine atoms is also obtained for this class of molecules. Table 4 shows the chlorine charges and the electronic density gain on the chlorine atom from Mulliken and GAPT methods calculated at the B3LYP/GCM level of theory.

While the individual charges do not present any specific tendency, a comparison between proton affinities and the total electronic density gain on the chlorine atoms shows an interesting correlation (see Tables 2 and 4):

PA:

3,5-DClPhOH < 2,6-DClPhOH < 2,5-DClPhOH < 3,4-DClPhOH < 2,4-DClPhOH < 2,3-DClPhOH

total EDGCl (Q_M):

3,5-DClPhOH > 2,6-DClPhOH > 2,5-DClPhOH > 2,4-DClPhOH > 3,4-DClPhOH > 2,3-DClPhOH

In general, the information obtained for monochlorophenols is also satisfied for dichlorophenols considering Mulliken's

charge: the proton affinity is directly correlated with the total electronic migration to the chlorine atoms after the elimination of the hydroxyl proton. Changes in the level of calculations and charge model modify the sequence presented above for a pair of molecules. This is consequence of the small differences in the calculated proton affinities, which can be smaller than 0.5 kcal/mol. However, even with such small differences between the proton affinities, the general trend between acidity and the total electronic density gain on the chlorine atoms is observed.

Trichlorophenols. The same analysis applied for trichlorophenols shows that the sum of the electronic density gain on the chlorine atoms presents a significant correlation with its proton affinity. In other words, the smallest sum of the electronic density gain on the three chlorine atoms confers the largest proton affinity to the molecule and vice versa (compare Tables 2 and 4).

The proton affinity sequence for the trichlorophenols molecules in general follows the tendency

PA:

2,4,6-TClPhOH < 2,3,6-TClPhOH < 3,4,5-TClPhOH < 2,3,5-TClPhOH < 2,4,5-TClPhOH < 2,3,4-TClPhOH

while the order of the sum of the Mulliken electronic density gain on the three chlorine atoms of trichlorophenols molecules after the proton liberation follows the same tendency:

EDGCl (Q_M):

2,4,6-TClPhOH > 2,3,6-TClPhOH > 2,4,5-TClPhOH > 3,4,5-TClPhOH > 2,3,5-TClPhOH > 2,3,4-TClPhOH

Table 4 also shows the results of the electronic density gain atomic in chlorine atoms obtained at the B3LYP/GCM level. These results are analyzed via Mulliken charge and GAPT for the chlorine atoms of chlorophenols in neutral and ionic species. These methods show the same good correlation between the proton affinity of the trichlorophenol molecules and the electronic density gain on the chlorine atoms after proton liberation.

Figure 1 presents a general picture of the proton affinities vs charge density gain for the 15 chlorophenols. The linear correlation is common to all the molecules and both charge models, suggesting a strong association between the electronic distribution and the energy necessary to remove a proton. GAPT presents a smaller standard deviation than Mulliken charges.

Table 5 shows the linear regression for chlorophenol proton affinities (PA) and the electronic density gains in the chlorine atoms calculated with B3LYP and the three basis sets used in this work. All three calculations present excellent correlation coefficients and standard deviations suggesting that the proton affinity of any other chlorophenol can be estimated from any of the three methods. Once again it is worth noting that the presence of diffuse and polarization functions at the present level of calculation are not very significant. The linear regression indicates B3LYP/CEP-31G as the best fitting between proton affinity and the electronic density gain.

5. Proton Affinity Influence on the Biological Activity of Chlorophenols

Several mechanisms describing the chemical action of substances on microorganisms, which may influence their mitochondrial respiratory functions, have been suggested in the literature.[24] Reverse electron transfer can be affected by inhibitors of the enzymatic complexes involved in this process

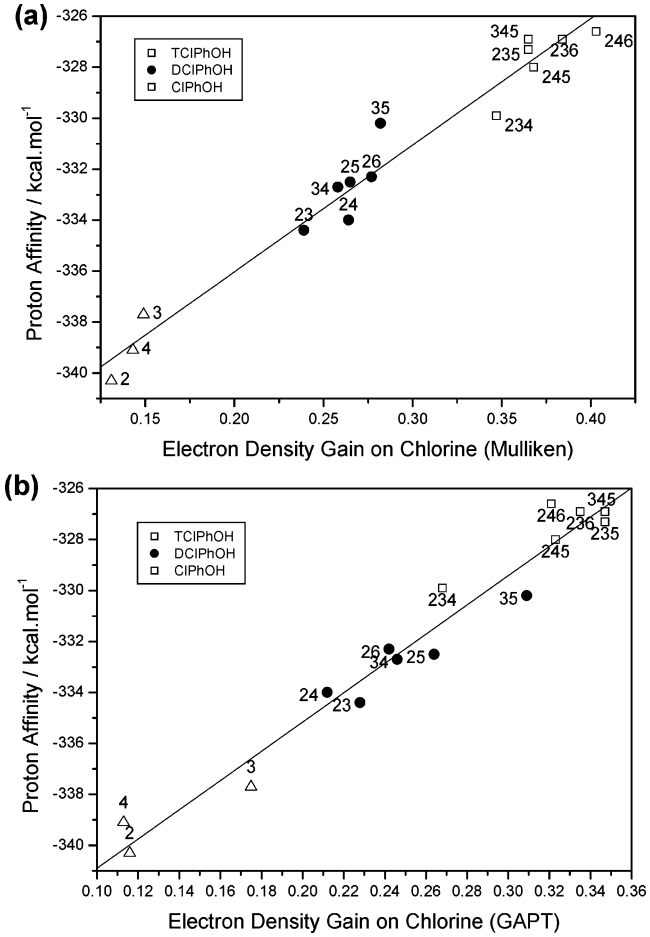

Figure 1. Proton affinities calculated at the B3LYP/GCM level of theory vs (a) Mullinken and (b) GAPT electronic density gains in the chlorine atom.

TABLE 5: Linear Regression for Chlorophenol Proton Affinities (PA) and the Electronic Density Gains on the Chlorine Atom (δ_{EDGCl}) with Respect to the Ionic Form, Obtained Using B3LYP/CEP-31G, B3LYP/CEP-31++G, and B3LYP/GCM**

method	a^a	b^a	r^b	SD^c
B3LYP/CEP-31G	−350.39	58.27	0.994	0.660
B3LYP/CEP-31++G**	−346.74	54.58	0.963	1.242
B3LYP/GCM	−346.65	57.45	0.980	0.936

[a] PA = $a + b\delta_{EDGCl}$. [b] Correlation coefficient. [c] Standard deviation.

(succinate dehydrogenase, NADH dehydrogenase, and ATP synthase), by uncouplers of oxidative phosphorylation, and also by the presence of drugs that cause damage to the membrane.[24]

Classical uncouplers are moderately weak acids, characterized by the presence of a bulky hydrophobic aromatic system, with electron withdrawing substituents.[25] These compounds, commonly called protonophoric uncouplers, act by translocating protons across the lipid bilayer of the mitochondria inner membrane. In the mitochondria, stored energy in foods is converted to a flow of electrons, which convert ADP to ATP. ATP is essential for all biological activities that require energy for all aerobic organisms, Figure 2a, and death will result if the process is stopped.

The chlorophenols are considered protonophoric uncouplers.[26] They are weak organic acids with pK_a values that range from 5.4 to 8.9; consequently, at the working pH of the medium for bacterial assay (pH = 7.5) the concentration of the neutral form is larger for the monochlorophenols than for di- or trichlorophe-

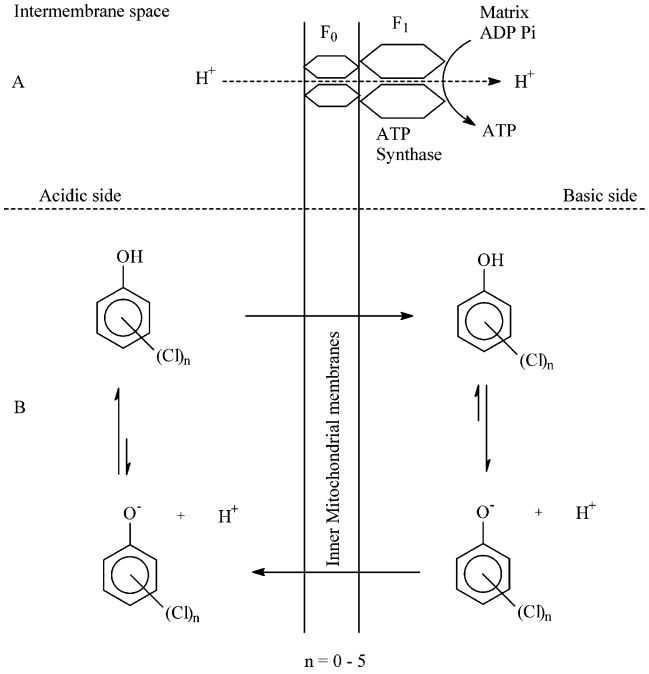

Figure 2. Schematic illustration comparing (A) ATP synthesis driven by proton motive force, (B) a possible mechanism to explain the uncoupled effect of chlorophenols inside living cells of *C. violaceum*.

nols. As a result, the presence of chlorophenols in the culture medium (Figure 2b) inhibits or decreases ATP synthesis.

One of the most important responses related to the inhibition mode of phenols, which profoundly affects the microorganisms, is their proton affinity.[27] For this reason, we focused our attention on the proton affinity of phenol, some of its chlorinated derivatives and the respective molecules, changing the acid proton to a methyl group (anisol and its chlorinated derivatives) in order to understand the biological activity of these compounds. The influences of these compounds were tested experimentally on *Chromobacterium violaceum* respiration metabolic rate, to verify if the calorimetric response can be partitioned into contributions of the acidic property of chlorophenols.

The inhibition mode and acidity characteristics were investigated using a flow microcalorimetric method.[6] Microcalorimetry has been proposed because it is a general, nondestructive, and highly sensitivity technique and, in principle, is capable of investigating any process or reaction, since all chemical and physical mechanisms give rise to changes in enthalpy.[28] An isothermal twin conduction flow microcalorimeter Thermometrics 2277 TAM (thermal activity monitor) was used for these studies. A continuous flow of a buffer solution between the calorimeter and an external reactor is maintained by a peristaltic pump to establish the experiment baseline. Then, a suspension of microorganism in buffer solution without chlorophenol is pumped through the calorimeter and this experiment is defined as control.

The variable calorimetric response, CR, is defined by comparing the maximum deflection of the baseline or the height of the thermal power vs time curve for each compound with the maximum height of the control curve.[29] The calorimeter was chemically calibrated according procedure described by O'Neill et al.[30] Each calorimetric experiment takes 45 min and was always repeated more than two times with a reproducibility of 4%.

A comparison between the calorimetric response of two groups of chlorophenols and chloroanisoles, based on the methoxyl and hydroxyl groups, and also on the number of chlorine substituents, reveals that, in general, chlorophenols are more toxic than chloroanisoles and that pentachlorophenol is more toxic than the others (see Table 6). The highly toxic behavior of this fully chlorinated phenol is presumably a result of its greater lipophilicity[31] and acidity, which facilitates proton transfer across the inner mitochondrial membrane.

TABLE 6: Calorimetric Responses (CR) of Phenol, Anisole, and Chlorinated Derivatives on the Respiration of *C. violaceum*, Compared with Controls in Which the Maximum Value of (CR) Represents 100% of the Microcalorimeter Baseline Deflection

molecule	CR (μW)	%	molecule	CR (μW)	%
control	92	100	control	92	100
phenol (PhOH)	73	79	anisole (An)	86	93
4-ClPhOH	68	74	4-ClAn	71	77
3,4-DClPhOH	29	31	3,4-DClAn	68	74
2,4,6-TClPhOH	16	18	2,4,6-TClAn	67	73
2,3,4,5-TClPhOH	7	8	2,3,4,5-TClAn	53	58
PClPhOH	6	7	PClAn	70	76

[a] Key: 4-ClPhOH, 4-chlorophenol; 3,4-DClPhOH, 3,4-dichlorophenol; 2,4,6-TClPhOH, 2,4,6-trichlorophenol; 2,3,4,5-Cl_4PhOH, 2,3,4,5-tetrachlorophenol; Cl_5PhOH, pentachlorophenol; 4-ClAn, 4-chloroanisole; 3,4-DClAn, 3,4-dichloroanisole; 2,4,6-TClAn, 2,4,6-trichloroanisole; 2,3,4,5-TClAn, 2,3,4,5-tetrachloroanisole; PClAn, pentachloroanisole.

An overview of the physicochemical properties of chlorophenols can be helpful for the interpretation of the results described above and for elucidation of the mechanism of action of these compounds. The results of our investigation on chlorophenols confirm that their high or low biological activity is associated with their proton affinity, considering the acidity difference between chlorophenols and chloroanisoles. However, it is clear that the toxicity of these compounds is not only associated exclusively with the proton affinity but also depends on a combination of other effects, such as chlorine content, hydrophobic interaction, partition coefficient, heat of formation, molecular volume, etc.

6. Conclusion

Ab initio calculations are used to estimate and interpret the proton affinity tendencies of 15 chlorophenols. Electronic correlation effects are significant to describe proton affinities with an error smaller than 3 kcal/mol with respect to the experimental results. At the Hartree−Fock level of theory, the error can be as large as 10 kcal·mol^{-1}.

Linear correlations between the mono-, di-, and, trichlorophenol proton affinities and the respective electronic density gains on their chlorine atoms after the liberation of the proton are observed. The proton affinity is directly associated with the capacity of the chlorine atoms to reduce the electronic density of the aromatic ring. Consequently the number and the geometry of chlorine atoms bonded to the aromatic ring determine the larger or smaller capacity of the phenol to release its proton.

Experimental tests comparing the behavior of chlorophenols and chloroanisols on *Chromobacterium violaceum* respiration metabolic rate confirm that their biological activity can be associated with the proton affinity. The toxic behavior of chlorophenols is probably a result of the number of chlorine atoms bonded to the aromatic ring, which change lipophilicity and acidity, facilitating proton transfer across the inner mitochondrial membrane. Of all possible factors, proton affinity seems to be one of the most important aspects responsible by the toxicity of these compounds.

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References and Notes

(1) Dearden, J. C.; Calow, P.; Watts, C. *Chem. Br.* **1994**, *30*, 823.
(2) Chen, J, Jiang, J, Zhang, F.; Yu, H, Zhang, J. *Cell Biol Toxicol.* **2004**, *20*, 183.
(3) Im, W. T.; Bae, H. S.; Yokota, A.; Lee S. T. *Int. J. Syst. Evol. Micr.* **2004**, *54*, 851.
(4) Blaha, L.; Klanova, J.; Klan, P.; Janosek, J.; Skarek, M.; Ruzicka, R.; *Environ. Sci. Technol.* **2004**, *38*, 2873.
(5) Santos, R. M. B.; Simões, J. A. M. *J. Phys. Chem. Ref. Data* **1998**, *27*, 707.
(6) Basheer, M. M.; Volpe, P. O. L.; Airoldi; C. *Int. J. Pharm.* **2004**, *282*, 163.
(7) Souza, H. F.; Guadagnini, P. H.; Custodio, R.; Goddard, J. D. *J. Comput. Chem.* **2000**, *21*, 1119.
(8) Voets, R.; François, J.-P.; Martin, J. M. L.; Mullens, J.; Yperman, J.; van Poucke, L. C. *J. Comput. Chem.* **1989**, *10*, 449.
(9) Howard, S. T.; Platts, J. A. *J. Phys. Chem.* **1995**, *99*, 9027.
(10) Smith, B. J.; Radom, L. *J. Phys. Chem.* **1995**, *99*, 6468;.
(11) Smith, B. J.; Radom, L. *Chem. Phys. Lett.* **1995**, *245*, 123;.
(12) Morgon, N. H.; Riveros, J. M. *J. Phys. Chem. A* **1998**, *102*, 10399;.
(13) Morgon, N. H. *J. Phys. Chem. A* **1998**, *102*, 2050;.
(14) Rassolov, V.; Pople, J. A. *J. Chem. Phys.* **1998**, *109*, 7764;.
(15) Curtiss, L. A.; Redfern, P. C.; Raghavachari, K.; Rassolov, V.; Pople, J. A. *J. Chem. Phys.* **1999**, *110*, 4703.
(16) Stevens, W. J.; Basch, H.; Krauss, M. *J.*; *Chem. Phys.* **1984**, *81*, 6026.
(17) Hill, D. L.; Wheeler, J. A. *Phys. Rev.* **1953**, *89*, 1102.
(18) Griffin, J. J.; Wheeler, J. A. *Phys. Rev.* **1957**, *108*, 311.
(19) Gaussian 03, Revision B.05. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian, Inc.: Wallingford CT, 2003.
(20) Cioslowski, J. *J. Am. Chem. Soc.* **1989**, *111*, 8333.
(21) Cioslowski, J.; Hamilton, T.; Scuseria, G.; Hess, B. A., Jr.; Hu, J.; Schaad, L. J.; Dupuis, M. *J. Am. Chem.* Soc. **1990**, *112*, 4183.
(22) Kebarle, P.; McMahon, T. B. *J. Am. Chem. Soc.* **1977**, *99*, 2222.
(23) Fujio, M.; McIver, R. T., Jr.; Taft, R. W. *J. Am. Chem. Soc.* **1981**, *103*, 4017.
(24) Stockdale, M.; Selwyn, M. J. *Eur. J. Biochem.* **1971**, *21*, 416.
(25) Terada, H. *Biochim. Biophys. Acta* **1981**, *639*, 225.
(26) Hudson, J.; Cass, A. E. G.; Prabhananda, B. S. *Phys. Chem. Chem. Phys.* **2002**, *4*, 2702.
(27) Rutgers, M.; Van Bommel, S.; Breurg, A.; Van Andel, J.; Buetz, W. *Environ. Toxicol. Chem.* **1998**, *17*, 792.
(28) Beezer, A. E.; Hunter, W. H.; Storey, D. E. *J. Pharm. Pharmacol.* **1980**, *32*, 815.
(29) Volpe. P. L. O. *J. Braz. Chem. Soc.* **1997**, *8*, 343.
(30) O'Neill, M. A. A.; Beezer, A. E.; Labetoulle, C.; Nicolaides, L.; Mitchell, J. C.; Connor, J.; Orchard, J.; Kemp, R. B.; Olomolaiye, D. *Thermochim. Acta* **2003**, *399*, 63.
(31) Chlorophenols Other Than Pentaphenol. *Environmental Health Criteria 93*; World Health Organization: Geneva, 1989.