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Theoretical approach of the complexation of Zn (II) with a toxic amino acid, l-canavanine and its analogue, l-arginine.

Habiba Eljazouli, Mohamed Laabd, Maria Elamine, Abdallah Albourine*

Team of Analytical and Environmental Chemistry, Laboratory of Materials and Environment, Faculty of Science, Ibn Zohr University, B.P: 8106, Dakhla City. Agadir, Morocco

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albourine@yahoo.fr

Abstract

The experimental study of binary complexation of l-canavanine and l-arginine amino acids with Zn (II) was completed by a quantum approach. This examination is performed using calculations based on a semi-empirical method Austin Model 1 (AM1). It concerns the electronic, structural and energetic aspects to the neutral, deprotonated and complexed states of these molecules. We found that for both molecules that the three functional groups: carboxyl, guanidyl and amino may participate in the complexation of Zn (II) given the high negative net charges of the active sites of each group: oxygen atom of the carboxyl functional group and nitrogen of the amine and guanidyl functions. However, despite that the two molecules have similar structures; they have two different behaviors with Zn (II). In fact, the l-canavanine gave zinc chelates tridentate ZnCan $(H_2O)_3$ and $ZnCan_2$ in which the molecule intervenes with the three functional groups. While the l-arginine gave two complexes: $ZnArg(H_2O)_4$ et $ZnArg_2(H_2O)_2$. From a structural point of view, the optimized geometries are octahedral structures for all complexes. In addition, the formed coordinate-covalent bond ligand-metal is more rigid with the guanidyl and carboxyl groups compared to that with the amine functional group. Nevertheless, the bond formed between Zn (II) and amino group of the l-canavanine is more rigid to that formed with the l-arginine.

1. Introduction

L-canavanine or 2-amino-guanidinoxy-butyric acid, a structural analog of 1-arginine [1,2], is included in the composition of certain plants and legumes such as soybean, alfalfa and clover [3, 4]. It was isolated in 1929 from "jack bean" flour by TOMIYAMA et al. [5]. Specifically, l-canavanine is an anti-metabolite of 1-arginine. They are found in plants of the genus Sutherlandia. It protects the plant from various predatory insects.

L-canavanine is generally presented as a toxic compound of plant proteins. This toxicity is due to its structural likeness to l-arginine was related to two mechanisms which are the inhibition of the enzymes of the metabolism of l-arginine and the denaturation of the functional properties of the proteins in which it is incorporated [2]. It also induces similar lesions to those caused by cadmium on ribonucleic proteins [2]. This justifies the need for quality control, which should make it possible to verify its absence in plant proteins intended for human or animal consumption.

If l-canavanine has been extensively studied experimentally [6-9] then for the theoretical study we do not have any bibliographic data concerning the complexation of these two acids with the metal ions. For this reason, we are interested in the theoretical study of the interactions of 1-canavanine and 1-arginine with the Zn (II) ion. This experimental results of the difference in the protonic equilibrium in the solution of l-canavanine and 1-arginine, as well as their complexation with Zn (II), in the experimental study [4,10]) [11] which seems equally important to us.

To complement the previous experimental data on complexation by a theoretical study, it is necessary to characterize the different ligands in the neutral, deprotonated and complexed states by quantum computation. For this, we used the semi-empirical AM1 method, which is very adapted to molecules and large-scale complexes such as ours [12-14]. This method developed by the group of Dewar et al. [15] has found a wide range of applications in both basic and applied research. This procedure, implemented in the AMPAC and MOPAC programs [16], has proved to be very effective. The introduction of pseudo-diagonalization at SCF iterations [17] and the accuracy of the results obtained makes this method widely used in several fields of chemistry [18,19]. Moreover, it is a very reliable method to calculate the enthalpies of formation [15, 20-23].

Our study will determine:
- The active sites of molecules via analysis of net atomic charges.
- The optimized equilibrium geometries of neutral and complexed molecules and their enthalpies of formation.
The theoretical results will be compared with experimental data [4,10,11].

2. Results and discussion

Determination of the energetic, structural and electronic parameters of each molecule is carried out using the Davidon-Fletcher-Powell algorithm [24] in AM1 implemented in MOPAC 6.0 program (RHF) version with the keyword << PRECISE >>.

2.1. Atomic charges

The analysis of the net charges of the X heteroatoms (O and Cl) makes it possible to identify the active sites with respect to the metal ion Zn (II). In AM1 the atomic net charge Q_X is calculated according to the equations (1):

$$Q_X = z_X - \sum_{p \in X} P_{pp} \quad \text{with} \quad P_{pp} = 2 \sum_i C_{ip}^2 \quad (1)$$

Z is the atomic number of the atom X, P_{pp} is the electronic population of the atomic orbital p and C_{ip} is the coefficient of the LCAO development. The obtained results after optimization of the geometry of each molecule in the neutral and deprotonated state make it possible to carry out an analysis of the net charges of l-canavanine and l-arginine (FIG. 1).

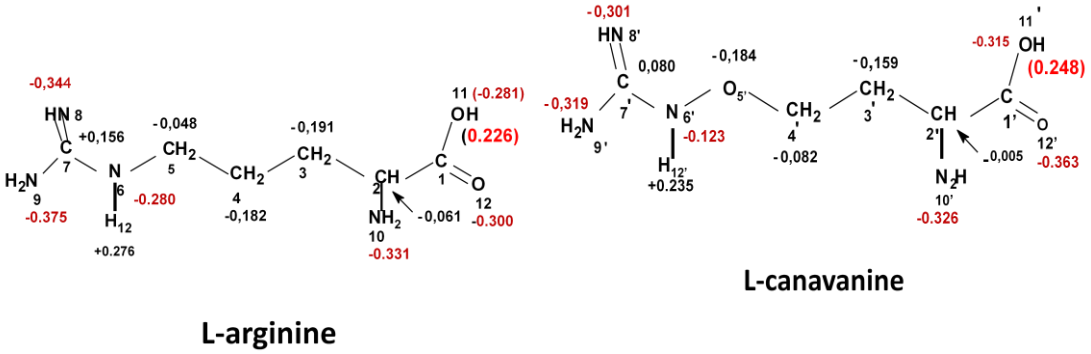


Figure 1: Net atomic charges of neutral forms of the l-arginine and l-canavanine.

The comparison of the atomic charges in the two molecules leads to the following results:
- The negative charge of the N6 atom of 1-arginine is much greater than that of the N6' atom of 1-canavanine, which is probably due to the attractive inductive effect of oxygen Oα. Also, the N8', N9' and N10' atoms of 1-canavanine exhibit lower negative charges than those of the N8, N9 and N10 atoms in the 1-arginine molecule.
- The oxygen atoms of the acid function carry lower net charges than those of the amine and guanidyl groups in l-arginine. On the other hand, in l-canavanine, this classification is reversed, which shows the influence of the oxygen atom O5' on the nucleophilic susceptibility of these functional groups.
- The influence of oxygen Oα is very sensitive and reaches, in particular, the hydrogen atoms of the acid function of the two molecules. In fact, the net charges of these atoms are different with values of 0.248e and 0.222e for l-canavanine and 1-arginine, respectively. This means that the l-canavanine deprotonates more easily than the 1-arginine. This behavior is in good agreement with the experimental results (Table 1) [11]. The AM1 method involving a polyelectronic wave function, therefore, makes it possible to evaluate the influence of oxygen Oα of the l-canavanine molecule on the electronic charges of its atoms.

Table 1: Proton stability constants of the l-canavanine and 1-arginine at T = 298K [11].

	Carboxylic group	Guanidyl group	Amine Group
L-canavanine Log K	2.15	6.98	9.16
L-arginine Log K	2.22	11.58	8.98

2.2. Complexation with Zn (II)

The experimental results obtained by Albourine [11] on the complexation of the zinc ion by these two types of amino acids show a notable difference between l-canavanine and 1-arginine in the deprotonation order

of the two guanidyl and amine functional groups. In the case of l-canavanine, the guanidyl group (logk = 6.98) deprotonates before the amine group (logK = 9.16). While the l-arginine, deprotonates well beyond (logk = 11.85) [11].

The direct consequence of this difference is manifested by different modes of complexation with respect to the Zn (II) metal. Effectively, for l-canavanine, the three groups (carboxyl, amine and guanidyl) deprotonate in the pH range of the complexation of Zn (II) and are therefore likely to participate in chelation. However, in the case of 1-arginine, only the carboxyl and amino groups can be involved in the chelation of this metal [11].

Theoretically, the obtained results for the deprotonated molecules (FIG. 2) using AM1 method indicate that the oxygen atoms of the carboxyl groups (O11 for 1-arginine and O11' for 1-canavanine) possess the highest negative charge of the same order (Q_{O11} = -0.571e and $Q_{011'}$ = -0.577e). For amino groups (Q_{N10} = -0.326e and $Q_{N10'}$ = -0.320e) and guanidyl (Q_{N9} = -0.320e and $Q_{N9'}$ = -0.318e), the charges are also of the same order except for N6 (-0.288e) and N6' (-0.133e). This means that the three functional groups are likely to participate in the complexation with Zn (II) ion. This is in perfect agreement with the experimental results [11].

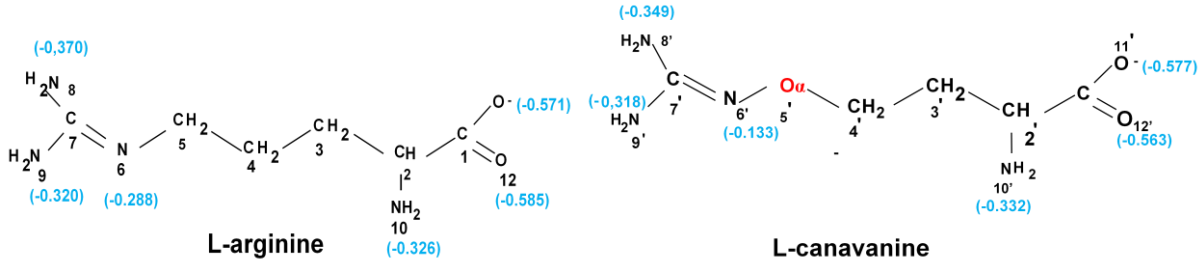


Figure 2: Net atomic charges of deprotonated forms of 1-arginine and 1-canavanine.

As far as we are concerned, we performed our calculations on complexes resulting from the complexation behavior obtained experimentally [11]. So, l-canavanine yielded stable tridentate zinc chelates $ZnCan(H_2O)_3$ and $ZnCan_2$ formed by the contribution of the three functional groups: amino groups, carboxyl and guanidyl (FIG 3).

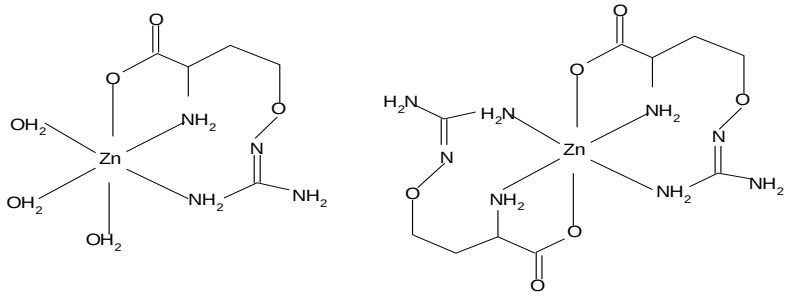


Figure 3: Complexes of the l-canavanine with Zn (II) ion.

As for the 1-arginine, it gave rise to carboxyl and amino groups being added to bidentate complexes: $ZnArg(H_2O)_4$ and $ZnArg_2(H_2O)_2$ (FIG. 4).

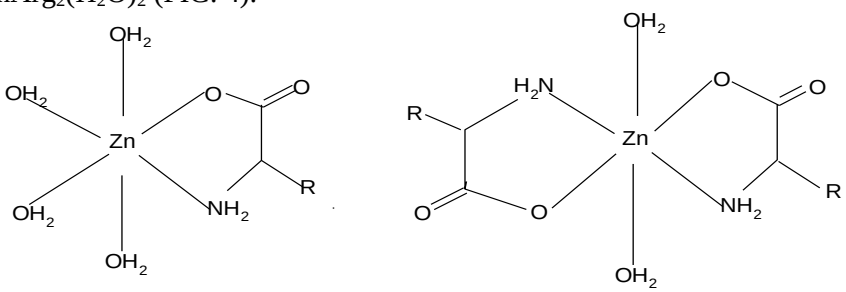


Figure 4 : Complexes of l-arginine (R = - $(CH_2)_3$-$NH(NH_2)NH$) with Zn (II) ion.

2.2.a. Energetic aspect

The stability of these complexes requires the calculation of their enthalpies of formation ΔH_f. Thus, the obtained values of ΔH_f for l-canavanine and 1-arginine using AM1 method were grouped in Table 2. Our objective is, on the one hand, to compare their relative stabilities to that obtained experimentally; on the other

hand, to examine the complexation of these two molecules by the same metal ion Zn (II). This constitutes a way of their distinction in view of their structural resemblance. At the same time, this will make it possible to examine the reliability of the AM1 method with regard to metal coordination.

Table 2: Enthalpies of formation of the zinc complexes with 1-arginine and l-canavanine.

l-arginine		**l-canavanine**	
Complexe	**ΔH_f (kcal/mol)**	**Complexe**	**ΔH_f(kcal/mol)**
$ZnArg(H_2O)_4$	-115.358	$ZnCan(H_2O)_3$	-56.47
$ZnArg_2(H_2O)_2$	-165.877	$ZnCan_2$	-63.36

The analysis of the table 2 makes it possible to demonstrate chelating behaviors of each molecule. In fact, the values of the ΔH_f relative to the 1-arginine complexes show that these chelates exhibit a much better thermodynamic stability than the complexes formed with l-canavanine with Zn (II) metal ion. As a result, 1-arginine has a better affinity for Zn (II) than l-canavanine. This is in good agreement with experimental results [11] (Table 3).

Table 3: Stability constants of the zinc complexes with l-canavanine and 1-arginine.

Complexes	**Log β (exp)**
ZnCan	5.00
$ZnCan_2$	9.20
ZnArg	4.00
$ZnArg_2$	7.95

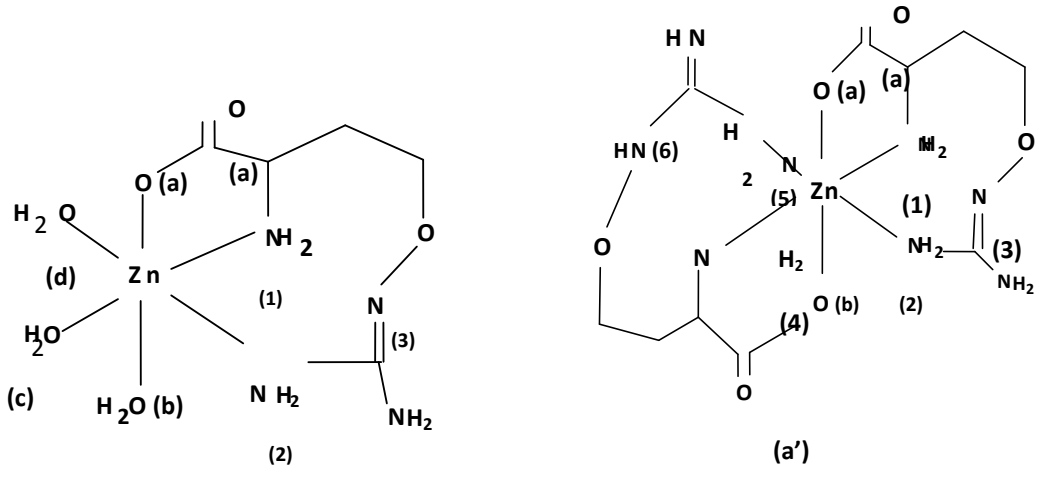
Thus, the complexation of these two acids with Zn (II) may be useful for the distinction between l-canavanine and 1-arginine.

2.2.b. Structural aspect

Structurally, it is interesting to evaluate the effect of complexation on the geometric parameters of the studied molecules, in particular, the immediate vicinity of the coordination site. The determination of the metal-ligand interatomic distance formed within the zinc complexes appears equally very important.

i) l-canavanine

The complexation of 1-canavanine with Zn (II) resulted in tridentate octahedral zinc complexes (FIG. 5): $ZnCan(H_2O)_3$ (I) and $ZnCan_2$ (II). In these complexes, the l-canavanine ligand is trident and the complexing sites are the nitrogen atoms N(1) (FIG. 5) of the amino group and N(2) (FIG.5) of the guanidyl group and the oxygen atom O (a) of the carboxyl functional group (FIG. 5).



(I) **(II)**

Figure 5: Structures of the zinc complexes with l-canavanine: (I) ZnCan(H2O)3 and (II) ZnCan2.

From the analysis of the geometrical parameters, the octahedral arrangement (FIG. 5) of the ligands is rigorously distorted, presumably because of the tridentate form. In fact, in ZnCan $(H_2O)_3$ by way of example, the Zn-Oa bond is shorter by 0.028 Å than the Zn-Ob bond, the Zn-N1 and Zn-N2 bonds differ by 0.008 Å and

Zn-Od bond exceeds Zn-Oc by 0.011 Å (Table 4). On the other hand, these differences are less important in $ZnCan_2$ and consequently, the distortion would be smaller because of the symmetry of the complex. Indeed, the Zn-Oa, Zn-Ob (Zn-N1, Zn-N2) and Zn-N4 and Zn-N5 bonds differ only by 0.014 Å, 0.005 Å and 0.003 Å, respectively. In addition, the dihedral angles of the atoms constituting the base of the octahedron (OcZnN2N1) and (OdZnOcN2) in $ZnCan(H_2O)_3$ are -166.5° and 170.8°, respectively. For $ZnCan_2$, these angles are closer and are of the order of -174.3° and 175.9°. For plane angles (N2CN3) and (N5CN6) have substantially equal values (respectively 119.8° and 120.6°). Also, the angles (N1CCa) and (N5CCa') have the same value of 119°.

Table 4: The Zn-L bond (Å) in the complexes of l-canavanine with Zn(II) optimized by AM1 method.

Complexe M-L	$ZnCan(H_2O)_3$	$ZnCan_2$
Zn-N1	2.343	2.348
Zn-Oa	2.214	2.208
Zn-N2	2.335	2.337
Zn-Ob	2.242	2.222
Zn-Oc	2.231	-
Zn-Od	2.242	-
Zn-N4	-	2.352
Zn- N5	-	2.349

ii) l-arginine

The compounds formed from the coordination of 1-arginine with the Zn (II) ion through the oxygen atom of the carboxyl functional group and the nitrogen atom of the amine function are octahedral complexes: ZnArg(H2O)4 (III), ZnArg2(H2O)2 (IV) (FIG 6)**.**

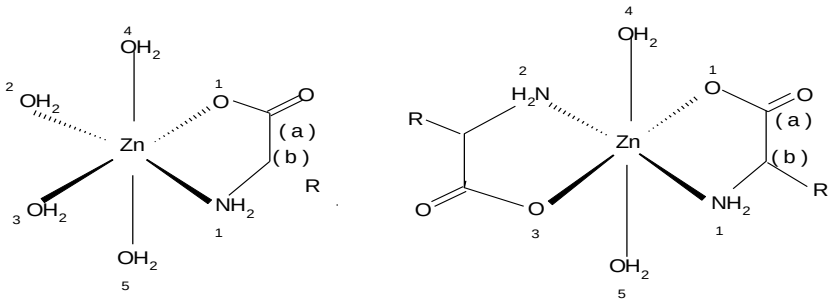


Figure 6: Complexes of the 1-arginine R = $-(CH_2)_3$-NH-C(NH_2)NH with Zn (II) ion.

In these complexes, the zinc ion is bi-cordoned with the ligand l-arginine via the atomic sites (O1 and N1). It should be noted that for the $ZnArg_2(H_2O)_2$ complex, we adopted the trans isomer having a higher stability (ΔH_f = -165.87 kcal/mol) than that of cis (-138.34 kcal/mol). This is due to the repulsion of the free doublets of the oxygen atoms which can destabilize the complex.

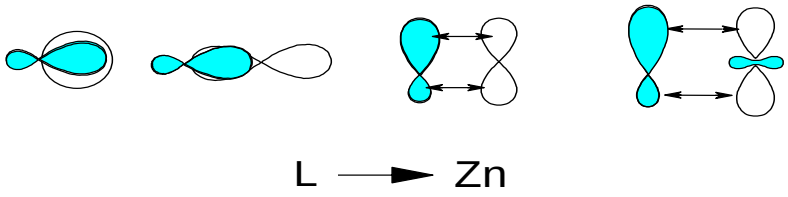
Optimization of the geometrical parameters of these complexes has shown that the octahedron is more deformed than the l-canavanine, presumably because of the homogeneity of the constituents of the octahedron base formed by four vertices of nitrogen atoms. On the other hand, the base of the octahedron of arginine is constituted by the nitrogen and oxygen atoms. Thus, the length difference of the Zn-O1 and Zn-N1 bonds reaches approximately 0.114 Å in the $ZnArg_2(H_2O)_2$ complex. The same order of length difference was obtained for the Zn-O3 and Zn-N2 bonds. Whereas the Zn-O4 and Zn-O5 bonds, as in the case of l-canavanine, differ only from 0.005 Å and 0.009 Å in $ZnArg_2(H_2O)_2$ and $ZnArg(H_2O)_4$, respectively.

Moreover, in the coordination of the 1-arginine ligand with Zn (II), the O1-C bond shortened by approximately 0.050 Å. Thus, a shorter shortening was observed in the 1-arginine-Zn(II) complex than that obtained in the case of l-canavanine (0.037 Å). While N1-C underwent an elongation of about 0.025 Å which is lower than that of zinc complexes with l-canavanine (0.039 Å).

In fact, the length increased from 1.443 Å (in the neutral state) to 1.468 Å (in the complexed state) in the $ZnArg_2(H_2O)_2$. Also, The O2-C and N2-C bonds varied in the same order as their O1-C and N1-C counterparts, which is probably due to the symmetry of the complex. The angles (O1CaCb) and (N1CbCa) have increased by about 4° and 3°, respectively. The first passes from 117.5° to 121.3° and the second from 112.2° to 115.1° in the ZnArg $(H_2O)_4$ complex. This variation is about the same for $ZnArg_2(H_2O)_2$ as (O1CaCb) increases from 117.5° to 121.9° and (N1CbCa) increases from 112.2° to 115.3°.

2.2.c. Zinc-ligand bonding

The complexation of a ligand (L) results in the formation of a bond between the ligand and metal ion (M). The nature of this bond continues to be an interesting point of research. In this perspective, we will examine the order of this binding and its effects on the geometrical structure of the various ligands. This leads to the formation of covalent coordination bonds by intervening with a doublet of free electrons of the nitrogen and oxygen atoms with Zn (II) having vacant atomic orbital's in its valence layer: $4s^0 4p^0 4d^0$ (FIG 7).



(O.A sp3 of the O or N atom) **(O.A 4s, 4p, 4d)**

Figure 7: Overlap of A.O forming the L-Zn coordination bonds.

As for the bond formed between l-canavanine, 1-arginine and zinc ion, several important conclusions can be drawn from the results recorded in Tables 5 and 6.

- ✓ In this bond, it appears that there is a considerable covalent character for the two molecules. They are rigid in the order of their lengths which is 2.208 Å as the minimum value of (Zn-O) in the case of $ZnCan_2$.
- ✓ In the case of 1-canavanine, the bonds formed through the guanidyl group (2.335 Å to 2.337 Å) are more rigid than those formed with the amino group (2.343 Å to 2.352 Å). This is probably due to the proximity of the carboxyl group to the amino group. This is absent in the case of the guanidyl group. Furthermore, the oxygen-Zn bond formed between the carboxyl group and Zn (II) in the $ZnCan(H_2O)_3$ and $ZnCan_2$ complexes, is shorter than that formed with the guanidyl group (2.335 Å and 2.349 Å) and that formed with the amino group (2.343 Å and 2.352 Å) and subsequently more rigid. This is logical since the oxygen atom is more electronegative than the nitrogen atom and therefore the Zn-O bond is shorter than Zn-N. These results in the following increasing ranking of the strength of the three bonds formed between the three groups of the l-canavanine molecule and Zn (II) ion:

amino-Zn(II) < guanidyl-Zn(II) < carboxyl-Zn(II).

Table 5: The Zn-L bond (Å) in the zinc complexes of l-canavanine optimized using AM1.

Complexe M-L	**$ZnCan(H_2O)_3$**	**$ZnCan_2$**
Zn-N1	2.343	2.348
Zn-Oa	2.214	2.208
Zn-N2	2.335	2.337
Zn-Ob	2.242	2.222
Zn-Oc	2.231	-
Zn-Od	2.242	-
Zn-N4	-	2.352
Zn- N5	-	2.349

For l-arginine, in the two zinc complexes, the bond established with the oxygen of the carboxyl group, ranging from 2.254 Å to 2.285 Å, is more rigid than that formed with the nitrogen of the amino group, ranging from 2.351 Å to 2.406 Å. Nevertheless, this binding is less rigid in l-arginine than in l-canavanine where these bonds are shorter. This means that the presence of the Oα oxygen atom in l-canavanine influences the amino-metal bond.

Table 6: Zn-L bond (Å) in the 1-arginine complexes optimized by the AM1 method.

Complexe M-L	**$ZnArg(H_2O)_4$**	**$ZnArg_2(H_2O)_2$**
O2(or N2)-Zn	2.275	2.372
O4-Zn	2.285	2.293
O5-Zn	2.294	2.288

The nitrogen-zinc bond of the amine function in the arginine complexes is ranging from 2.351 Å to 2.372 Å in the zinc complexes, so it is less rigid than that of the zinc complexes of l-canavanine, which is ranging from

2.343 Å to 2.352 Å. This can be explained by the tension of the five-center cycle formed during the complexation of 1-arginine with Zn (II) metal ion. This is also the case, and probably for the same reason that the oxygen-metal bond of the carboxyl group is more rigid in l-canavanine (from 2.208 Å to 2.242 Å) than in l-arginine (from 2.254 Å to 2.285 Å).

Conclusion

The complexation l-canavanin and l-arginine with semi-empirical Zn (II) metal ion were theoretically investigated using the AM1 method with satisfactory reliability. The analysis of the atomic net charges allows us to determine the active sites of each compound. This examination leads to the conclusion that the nucleophilicity of these amino acids varies as follows:

Guanidyl > Carboxyl > Amino

On the other hand, l-canavanine yielded stable tridentate zinc chelates $ZnCan(H_2O)_3$ and $ZnCan_2$ formed by the contribution of the three functional groups, while 1-arginine gave place to the carboxyl and amino groups to bidentate complexes $ZnArg(H_2O)_4$ et $ZnArg_2(H_2O)_2$.

As for the metal-ligand bonds, we found a difference between the 1-canavanin and 1-arginine complexes for both the nitrogen-zinc bond and the oxygen-zinc bond. In fact, these coordination bonds are more rigid in the case of the complexes of l-canavanine compared to those of the complexes of 1-arginine.

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