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Structure of the *t*-butyl *N*-(4-chlorophenyl)thiooxamate and related complexes with heavy metals

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Abstract

The structure of the title thiooxamate was studied by molecular modeling and i.r spectroscopy along with its $ZnBr_2$ complex and transformation products, thiooxamic acid and zinc thiooxamates. Similar results were obtained with heavy metal salts like Cd(II) and Hg(II) perchlorates with, moreover, formation constants determination of their complexes by ultra-violet spectrophotometry.

Keywords: Zinc; Cadmium; Mercury; Complexes; Molecular modeling; Formation constants

1. Introduction

t-Butyl thiooxamates are main products from aminolysis of sulfinamoyl acetates, suicide-type inhibitors of the cinnamoylalcohol dehydrogenase, a zinc-containing metalloenzyme [1].

On the assumption that thiooxamates should be generated at the active site of the enzyme, we focused our attention both on their complexing properties toward the zinc cation localized at the active site and on the transformation possibilities of thiooxamates by Lewis-acid type action of the ion.

We will successively study: (1) the structure of a selected ligand, the *t*-butyl *N*-(4-chlorophenyl) thiooxamate, along with its conformational analysis using molecular modeling, a computational aid to define intra and intermolecular interactions; (2) the action of $ZnBr_2$, a simplified model of metalloenzyme active site, on the studied ligand leading to either the corresponding acid by isobutene extrusion, or salts of the acid or complex(es) of the untransformed ligand; (3) the complexing abilities of the ligand toward other heavy metal ions, i.e. cadmium and mercury.

2. Experimental

Infra-red (i.r.) spectra were recorded on a Perkin-Elmer 883 spectrometer in KBr pellets for solids and in 0.05 M $CHCl_3$ solution by using NaCl 0.5 mm cells in the 4000–600 cm^{-1} frequency range or polyethylene cells in the 600–200 cm^{-1} range. ^{13}C magnetic resonance spectra (62.9 MHz) were recorded on Bruker AC-200 spectrometer. Mass spectra (MS) were performed on Perkin-Elmer SCIEX API 100 for electrospray (ES) or NERMAG R10-10C apparatus using fast atom bombardment (FAB) and a MNBA matrix. Elemental analysis were carried out by the ''Service Commun de Microanalyse Elementaire UPS-INP'' at Toulouse. Molecular modeling

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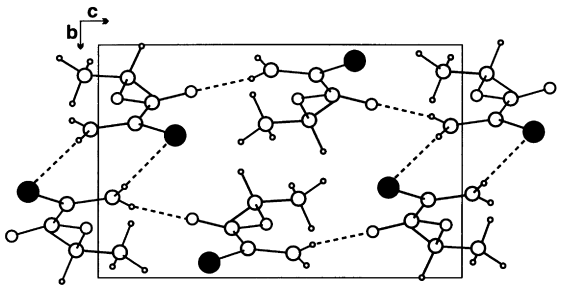


Fig. 1. X-ray structure of the ethyl thiooxamate.

was performed with the PCWIN program version 5.13 and the MMX force field from SERENA SOFTWARE, Bloomington, USA.

2.1. Synthesis of the t-butyl N-(4-chlorophenyl)thiooxamate (L)

The thiooxamate was prepared from the *t*-butyl *N*-(4-chlorophenyl)sulfinamoylacetate by aminolysis in acetonitrile with three-fold excess of imidazole, as previously described [1].

ES^-MS (CH_3CN): $m/z = 271$ $[M - H]^-$.

2.2. Action of $ZnBr_2$ on L

To a 0.05 M solution of thiooxamate in chloroform was added $ZnBr_2$, previously dried, following the selected stoichiometry ρ = [metal]/[ligand] = 0.5, 1 and 2. For $\rho = 2$ the excess of $ZnBr_2$ was decanted after centrifugation. In the orange solution (color of the thiooxamate) a white solid (X_1) slowly precipitated, and then was filtered off. The filtrate was concentrated under nitrogen stream and the resulting yellow solid dissolved into acetonitrile. A new solid (X_3) precipitated and was filtered off. After the solvent removed the filtrate gave a yellow solid (X_2).

X_1: Anal. calcd for $C_8H_6NO_2SCl$: C, 44.56; H, 2.80; N, 6.49. Found: C, 43.87; H, 2.83; N, 6.21.

X_3: Anal. calcd for $C_{16}H_{10}N_2O_4S_2Cl_2Br_2Zn_2$: C, 26.70; H,1.40; N, 3.89. Found: C, 26.87; H, 1.80; N, 3.88.

X_2: ES^-MS in $CH_3OH/CHCl_3$: m/z 358.0 $[L/ZnBr]^-$, 439.8 $[L/ZnBr_2]^-$, 492.9 $[L/_2Zn]^-$, 572.9 $[L/_2ZnBr]^-$ with $L/ = L - C_4H_9$.

2.3. Action of $Cd(ClO_4)_2$,6 H_2O on L

To 1 ml of a 0.3 M solution of thiooxamate in CD_3CN was added the hexahydrated cadmium perchlorate following the desired stoichiometry (ρ = 0.5 or 1). ^{13}C nuclear magnetic resonance (n.m.r.) spectra were immediately recorded after preparation and 2 h accumulation. After that time, i.r. spectra were performed on solutions four-times diluted.

2.4. Action of $Hg(ClO_4)_2$,3 H_2O on L

To 0.3 ml of a 0.05 M solution of L in acetonitrile was added 0.5 equivalent of trihydrated mercury perchlorate in acetonitrile. After dilution to around 5×10^{-4} M, the resulting solution was immediately analyzed by ES^+MS. The mother solution gave, after 1 h, a solid which was analyzed in KBr pellets.

ES^+MS (CH_3CN): $m/z = 743.0$ $[L_2Hg - H]^+$, 687.0 $[L_2Hg - C_4H_9]^+$, 630.9 $[L_2Hg - C_8H_{17}]^+$.

2.5. Formation constants determination of heavy metal complexes by ultra-violet (u.v.) spectrophotometry

To 2 ml of a 6.1×10^{-5} M thiooxamate solution in acetonitrile in the u.v. cell thermo-regulated at 25°C were added aliquots of 2.4×10^{-3} M (Hg or Zn) or 3.39×10^{-3} M (Cd) solution in acetonitrile of metallic perchlorates. The addition was performed in order to vary $\rho = [M^{2+}]/[L]$ from 0 to 1. The analysis of spectral variations and calculation of formation constants were realized as previously described [2].

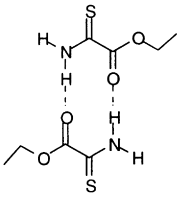
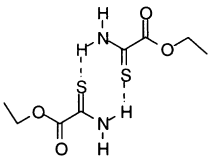
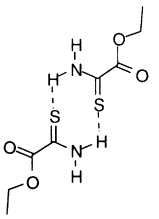
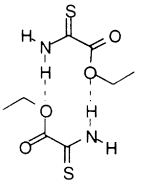
3. Results and discussion

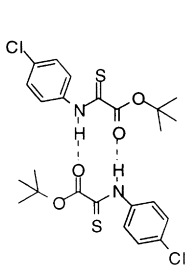
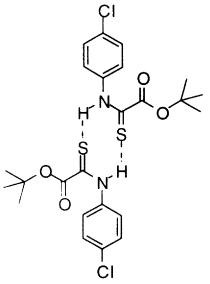
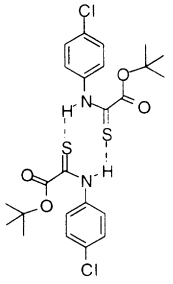
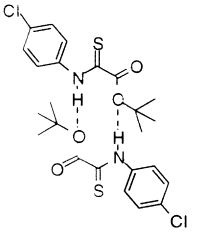
3.1. Structure of the t-butyl N-(4-chlorophenyl)thiooxamate

Due to lack of X-ray diffraction data with the studied thiooxamate we will analyze its structure by molecular modeling and i.r spectroscopy using the X-ray structure of the ethyl thiooxamate [3] as a model.

The ethyl thiooxamate presents a chelated structure of polymeric type, the six subunits of the elemental cell (Fig. 1) displaying a *syn* orientation of the C(O)C(S) group with H···S bonds (*syn*/H···S).

Table 1
Characteristics[a] of most stable dimeric associations of the ethyl thiooxamate and *t*-butyl *N*-(4-chlorophenyl)thiooxamate in the gas phase calculated with PCWIN. [a]Successively: conformational energies (Kcal mol^{-1}), i.e. total, stretching (Str), bending (Bnd), torsion (Tor), Van der Waals (VdW), electrostatic including H-bonding (QQ), H···X and N···X distances (Å), N···H···X angles (°) with X = O, S. Syn and anti refer to C(O)C(S) group.

	***anti* / H...O**	***anti* / H...S**	***syn* / H...S**	***syn* / H...O**
				
E total	55.5	59.4	52.7	> 80 Kcal. mol^{-1}
str	2.1	2.1	2.4	
Bnd	18.8	18.7	18.6	
SB	-1.3	-1.3	-1.4	
Tor	8.1	8.0	7.9	
VdW	6.8	8.2	2.5	
QQ	21.1	23.6	22.7	
d(N...X)	3.1	2.9	3.3	
N…H…X	147	3.9 114	170	

				
E total	67.8	76.1	74.1	> 100 Kcal. mol^{-1}
str	3.5	3.5	3.3	
Bnd	27.2	26.5	26.6	
SB	-1.1	-1.1	-1.1	
Tor	24.3	28.3	30.4	
VdW	10.3	12.4	8.4	
QQ	3.7	6.7	6.6	
d(N...X)	3.2	3.2	3.6	
N…H…X	130	158	137	

A molecular mechanics study (Table 1) was first performed on the ethyl thiooxamate in order to estimate the validity of the technique. It involves dimeric rather than monomeric forms as suggested by the X-ray structure.

The energy calculation of dimeric $H_2NC(S)C(O)OEt$ as *syn*/H···O or H···S and *anti*/H···O or H···S conformations gives results in agreement with the solid state. Thus, the *syn*/H···S conformer is 2.8 kcal mol^{-1} more stable than the *anti*/H···O and 6.7 kcal mol^{-1} than the *anti*/H···S conformer; the N···S distance is near 3.3 Å (3.45 Å

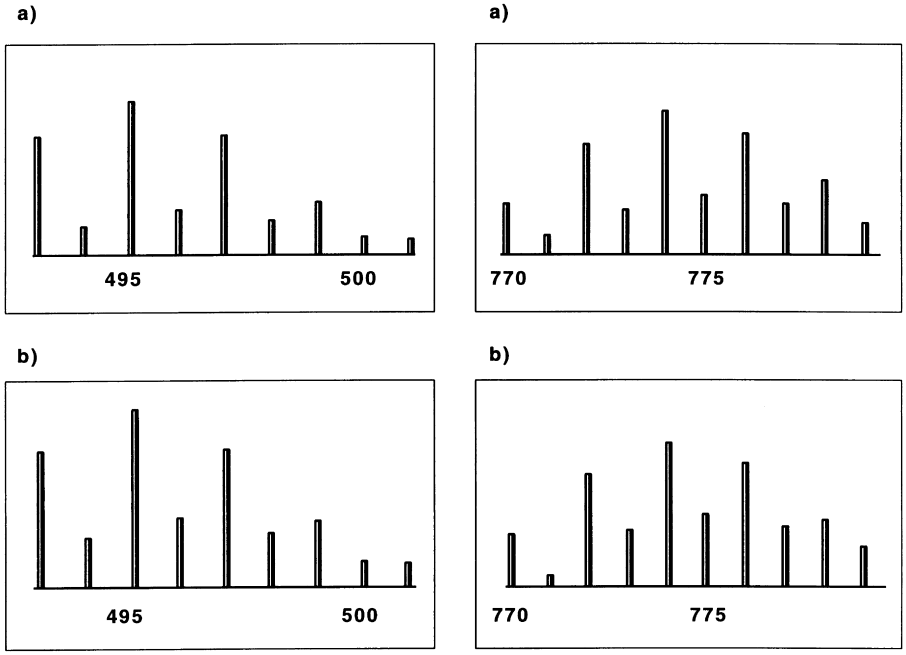


Fig. 2. Found (a) and calculated (b) isotopic patterns of zinc thiooxamates peaks.

from X-ray diffraction) and the N···H···S angle around 170° (160° from X-ray).

On the other hand, the *t*-butyl *N*-(4-chlorophenyl)thiooxamate shows a different structural feature related to the specificity of its substituents. In this case, the *anti*/H···O conformer (Table 1) is greatly favoured by 6.3 kcal mol^{-1} versus the *syn*/H···S and 8.3 kcal mol^{-1} versus the *anti*/H···S conformer. In the two thiooxamates the *syn*/H···O orientation is very unfavourable because the H-bond with the oxygen atom of the alkoxy group is destabilized by strong steric constraints giving energy higher than 30 kcal mol^{-1} related to the most stable conformer.

As an explanation of the inversion of stability for the two most stable conformers of the two thiooxamates, we found, as expected, a great dependence of the energy of the studied molecules with the H···X distance and the N···H···X angle. The best arrangement, i.e. shortest distance versus greatest angle for lowest energy gives a N···X distance of ~3.3 (in the most stable conformation of the two thiooxamates, the ethyl thiooxamate exercising a H-bond angle of 170° versus 130° for the *t*-butyl *N*-(4-chlorophenyl)thiooxamate. Furthermore, the electrostatic and torsional interactions appear more favourable in the *anti*/H···O form of the *t*-butyl derivative while Van der Waals interactions are the main stabilizing factor in the *syn*/H···S conformation of the ethyl derivative in relation to their substituent configuration.

The *anti* configuration of the carbonyl and thiocarbonyl groups in the *t*-butyl *N*-(4-chlorophenyl) thiooxamate is opposite to that (*syn*) of the ethyl thiooxamate. It is favourable to an electronic delocalization and, thus, to a more important conjugation of the π-orbitals. This phenomenon could account, in part, for the important decrease observed in the C = O stretching vibration frequency: ν(C = O) ester bands were observed at as low a frequency as 1701 (KBr pellet) or 1707 ($CHCl_3$) cm^{-1} versus 1739 (liquid) or 1722 ($CHCl_3$) cm^{-1} for *t*-butyl acetate. The withdrawing effect of the thioamide group should induce an opposite effect and cannot participate to the decrease. Another explanation should come from C = O···H–N bonding in the dimeric association. Indeed, the ν(NH) bands at 3314 ($CHCl_3$) and 3286 (KBr) cm^{-1} are indicative of H-bonded molecules as for potassium *N*-methyl

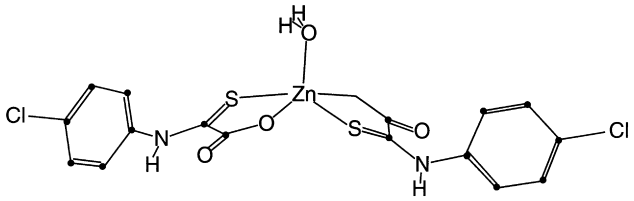


Fig. 3. Structure of $[L_{/2}Zn].H_2O$.

thiooxamate [4], where it is found at 3278 cm^{-1} in nujol or for arylsulfinamoylacetates [5].

Other bands will be of importance for the study of the complexation phenomenon, e.g. the four thioamide bands (TA) that we localize in KBr pellets at 1523 (TA I), 1386 (TA II), 1032 (TA III) and 735 (TA IV) cm^{-1} with respectively great character of ν(CN), δ(NH) and for the last two $\nu(C=S) + \nu(CC)$ vibrations, as well as NCS deformation bands in the 400–550 cm^{-1} range. These assignments were made according to works on dithiooxamides [6] or polythioamides [7].

3.2. Action of $ZnBr_2$ on the t-butyl N-(4-chlorophenyl)thiooxamate

$ZnBr_2$ has been used as rough model of zinc-containing metalloenzymes [8] and zinc complexes as chemical models of the active site of these metalloenzymes, especially of the enzyme–inhibitor interactions [9]. It was also our approach for determining the affinity of potential inhibitors of the cinnamoylalcohol dehydrogenase, i.e. *t*-butyl sulfinamoylacetates [1, 5].

The action of $ZnBr_2$ on *t*-butyl sulfinamoylacetates led to the formation of stable complexes [5], but also, in particular conditions, through isobutene extrusion, to the formation of the corresponding acid [10]. So, a similar behaviour can be expected with the thiooxamate.

The experimentation was conducted as follows. To a 0.05 M solution of thiooxamate in chloroform was added dried $ZnBr_2$ at different stoichiometries (ρ = 0.5, 1, 2). Into the resulting orange solution precipitated slowly a white solid (X_1) which was isolated by centrifugation. The liquid layer (yellow) contains essentially X_2 which was obtained by evaporation of the solvent under nitrogen stream. Then, when X_2 was dissolved into acetonitrile, X_3 precipitated.

X_1, X_3 and X_2 respectively identified to the *N*-(4-chlorophenyl)thiooxamic acid, zinc thiooxamate(s) and $ZnBr_2$-complexed thiooxamate will be successively studied.

3.2.1. N-(4-chlorophenyl)thiooxamic acid (X_1 or $L_{/}H$)

X_1 is a white solid essentially obtained with ρ = 0.5. The elemental analysis (see experimental section) is compatible with the thiooxamic acid of formula $C_8H_6NO_2SCl$.

The i.r. spectrum goes into the same way. OH stretching band in the 2500–3500 cm^{-1} range (dimeric associations), strong ν(C = O) band at 1656 cm^{-1} implying intermolecular H-bond like for dimeric forms of salicylic acid (1663 cm^{-1}) [11]a. Another possibility is the formation, in the KBr pellet, of potassium thiooxamate with $\nu_{as}(CO_2) = 1656\ cm^{-1}$ and $\nu_s(CO_2) = 1348\ cm^{-1}$; such exchanges sometimes occur, as previously noted [12], and as further observed for X_3. The bands observed at 1258 and 1153 cm^{-1} for the *t*-butyl *N*-(4-chlorophenyl)thiooxamate, respectively assigned to in-plane wagging of the tBu group [13] and ν(COC) of the OtBu group [11]b are missing in X_1.

The formation of thiooxamic acid is also supported by the known action of $ZnBr_2$ as a Lewis acid [14], as already shown with *t*-butyl acetates which easily give the corresponding carboxylic acids. With *t*-butyl sulfinamoyl- or sulfamoyl-acetates [10, 15], particularly, in aprotic 0.05 M CCl_4 or THF media, the formation of corresponding acids was observed and the isobutene extrusion was explained by an intramolecular process already described by Read [16] for *t*-butyl β-hydroxyesters. The mechanism that we propose proceeds via the $ZnBr_2$ complex and will be detailed in Section 3.2.3.

3.2.2. Zinc thiooxamates (X_3)

The yellow precipitate (X_3) was analyzed by

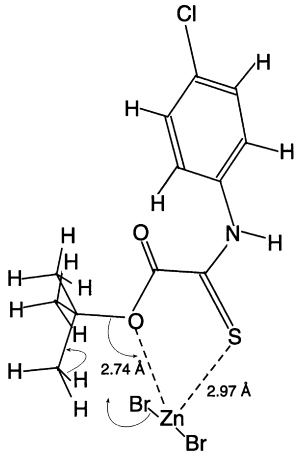


Fig. 4. Most stable conformation of the [$LZnBr_2$] complex.

MS–FAB$^+$. This spectrum exhibits four entities of respective mass 495 $[L/_2ZnH]^+$, 517 $[L/_2ZnNa]^+$, 774 $[L/_3Zn_2]^+$, 796 $[L/_3HZn_2Na]^+$ with compatible isotopic patterns given in Fig. 2. These entities indicate the presence, in the sample, of zinc salts of the thiooxamic acid L/H, especially $L/_2Zn$ and $L/_3Zn_2$. The elemental analysis (see Section 2) is compatible with a compound of global formula $C_{16}H_{10}N_2O_4S_2Cl_2Br_2Zn_2$ e.g. $L/_2Zn$, $ZnBr_2$.

The i.r. spectra in KBr pellets for different values of ρ show a diversity of ν(NH) and $\nu(CO_2)$ bands. In the ν(OH) and ν(NH) domains, bands near 3430 cm^{-1} can be indicative of coordinated water like with nickel thiooxamate ($\sim$ 3400 cm^{-1}) [17]. Bands at 3170 and 3210 cm^{-1} were also observed: the first one undergoes a 116 cm^{-1} decrease relative to the NH stretching vibration of the original ligand (L); it can indicate H···Br associations as early disclosed in the case of *t*-butyl sulfinamoylacetates with $ZnBr_2$ [5] where a variation of 110 cm^{-1} was noticed. The second band belongs to the chelated NH···O type.

In the $\nu_{as}(CO_2)$ range, several main frequencies were observed: 1630 and 1656 ($\rho = 0.5$), 1630 ($\rho =$ 1), 1630 and 1670–1680 ($\rho = 2$) cm^{-1}, some being bending vibrations of coordinated water which generally occur in the 1600–1650 cm^{-1} range [18]. However, the $\nu_{as}(CO_2)$ band is well localized in the 1660–1675 cm^{-1} domain for nickel and cadmium thiooxamates with two coordinated water molecules and ML_2 stoichiometry [17]. The nickel and cadmium complexes were found octahedral by X-ray diffraction [17], while the zinc thiooxamate with only one coordinated water molecule is a trigonal bipyramid [19]. The $\nu_s(CO_2)$ band is at 1385 cm^{-1} for the nickel salt. With the potassium thiooxamate [4] these bands occur respectively at 1633 and 1368 cm^{-1}. In the X_3 product ($\rho = 2$) the strong band at 1380 cm^{-1} can thus be assigned to $\nu_s(CO_2)$.

All these findings can be gathered in the molecule calculated by molecular mechanics and are represented in Fig. 3.

Remark. The analysis of i.r. spectra in KBr pellets shows some peculiar behaviour, previously noticed by Desseyn between KBr and metallic salts. For instance, the KBr matrix reacts with the dimethylacetamide/zinc or cadmium perchlorate to recover the ligand [12]b or with MCl_2 complexes to exchange halides [12]a. In our case, with $\rho = 1$, several modifications occurred in the spectrum of the X_3 solid after 30 days of life of the KBr pellet. The following shifts were observed: 1630 → 1657, 1374 → 1347, 1072 → 1053, 729 → 763 cm^{-1}. These variations could be explained by the slow hydrolysis of the complex in the pellet and consequent breaking of the metal–ligand bond, causing the shifting of the bands belonging to CO_2 (the first two) or CS (the last two) vibrators.

3.2.3. $ZnBr_2$-complexed thiooxamate (X_2)

In chloroform solution the solid X_2 is stable during at least 5 days. Its ES^-MS does not show the expected $L/ZnBr_2$ complex but peaks with isotopic patterns compatible with the carboxylate ion (L/): $L/ZnBr_2$, L/ZnBr, $L/_2Zn$, $L/_2ZnBr$ (see Section 2). This result may be explained by: (i) the facility for the ligand to lose the *t*-butyl group as isobutene by Lewis acid type action of $ZnBr_2$; and (ii) the insufficiently soft ES^-MS method to prevent fragmentations, e.g a $m/z = 170.1$ $[ClPhNHCS]^-$ fragmentation was detected for the ligand.

The i.r. spectrum of X_2 in chloroform presents, as main characteristics, very strong bands ν(C=O) at 1757 cm^{-1} and thioamide I at 1526 cm^{-1} with respective shifts of + 50 and + 14 cm^{-1} relative to the ligand. The same effects are observed in KBr pellet or in acetonitrile solution. Bands at 1403 and 1363 cm^{-1} correspond to the splitting of the symmetrical deformation band of the methyl group [11]c and state the presence of the *t*-butyl group in the thiooxamate and, thus, hold up a complexed thiooxamate. The ν(NH)

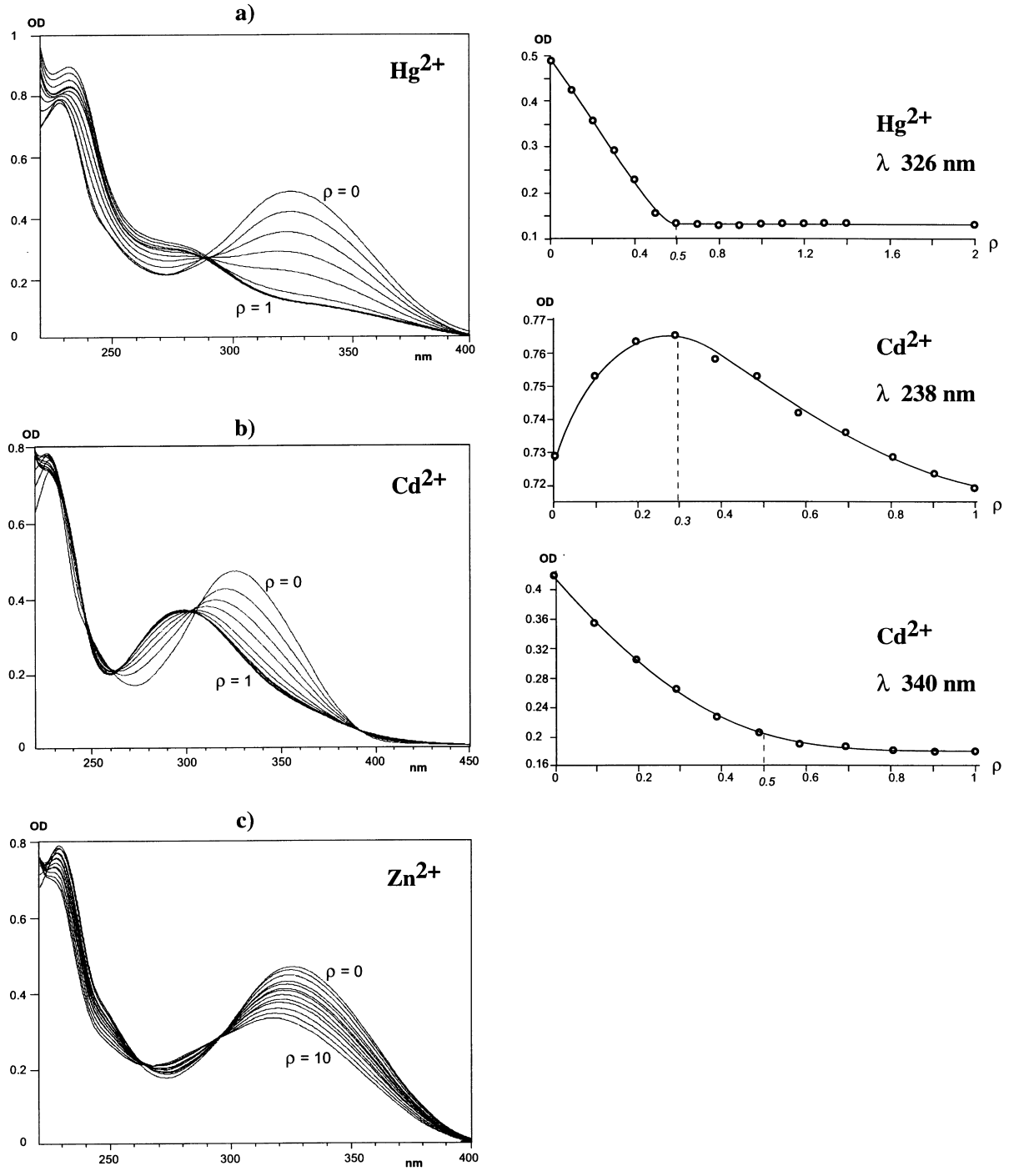


Fig. 5. Changes in u.v. absorption spectrum of *t*-butyl *N*-(4-chlorophenyl)thiooxamate (L) upon addition of metallic perchlorate: Hg^{2+} (a), Cd^{2+} (b), Zn^{2+} (c), and OD variations as a function of $\rho = [M(ClO_4)_2]/[L]$.

Table 2
^{13}C n.m.r. data (δ/TMS) for the *t*-butyl *N*-(4-chlorophenyl)thiooxamate (L) in CD_3CN and its Cd(II) adducts ($\rho = 0.5$ and 1)[a]

Assignment	Thioxamate	X_2' $\rho = 0.5$	$\Delta\delta$	X_3' $\rho = 1$	$\Delta\delta$
CH_3	27.9	27.9	0	–	–
$\underline{C}(CH_3)_3$	85.6	85.6	0	–	–
$\overline{C}2$, C6 (Ph)	125.9	126.5	+ 0.6	126.4	+ 0.5
C3, C5 (Ph)	129.8	130.1	+ 0.3	130.1	+ 0.3
C4 (Ph)	132.6	132.1	− 0.5	134.1	+ 1.5
C1 (Ph)	137.9	137.4	− 0.5	137.0	− 0.9
		137.2	− 0.7		
C=O	160.4	160.8	+ 0.4	161.4	+ 1.1
		160.1	− 0.3		
C=S	186.1	185.6	− 0.5	185.4	− 0.7

[a] $\Delta\delta$ is the chemical shift difference relative to the thiooxamate.

band is also informative: in KBr pellet the 3286 cm^{-1} band of the ligand decreases to 3249 cm^{-1} in X_2; that low frequency likely indicates a NH *cis*/CS conformer with no H···Br interaction, otherwise the $\Delta\nu$(NH), which is even lower in chloroform, will be at least twice [16]. The molecular mechanics study of the complex gives a significative insight on its structure and, consequently, a possible explanation of the observed spectral variations, of the isobutene extrusion and of the zinc carboxylate formation.

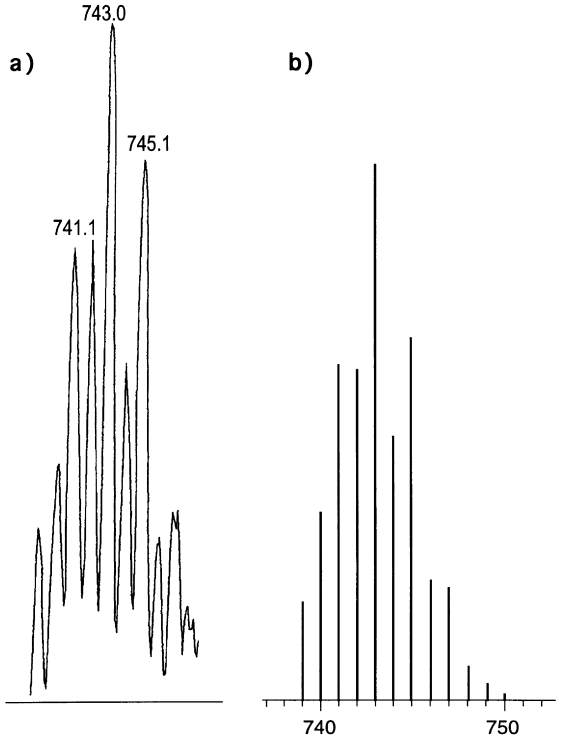


Fig. 6. Found (a) and calculated (b) isotopic patterns for the $[L_2Hg - H]^+$ peak.

The most stable conformation is shown in Fig. 4 with NH*cis*/CS and *anti*C(O)C(S) groups. The conformational difference between the complex and the ligand comes from the NH*cis*/CS conformation in the monomeric complex instead of a NH*trans*/CS conformation in the dimeric ligand. It is due to the zinc chelation by the sulfur and oxygen (OtBu) atoms, with several consequences: (i) the increase, as expected and observed, of the ν(CN) frequency (TA I band) by spill-over effect [12]a and of the ν(C=O) frequency by removal of the C=O···HN bond; (ii) the absence of H···Br interaction; (iii) the pre-organization of the complex to allow a direct extrusion of isobutene with HBr elimination and zinc salt formation; (iv) the thioamide IV band (ν(CS)) localized at 719 cm^{-1} showing, as expected [12]b, a decrease of 16 cm^{-1} relative to the ligand; (v) the observation of deformation NCS bands in the 400–600 cm^{-1} range near frequencies previously reported [12]b for zinc complexes of dimethylthioacetamide, i.e. 511(br), 454 (m), 425(sh); (vi) the ν(ZnS) vibration assigned to the 337 cm^{-1} frequency by comparison with similar bands at 321 or 330–341 cm^{-1} respectively in dimethylthioformamide/$ZnCl_2$ [20] or biuret/$ZnBr_2$ [21] complexes.

3.3. Action of metallic perchlorates on the thiooxamate

The progressive addition of $M(ClO_4)_2$, M = Zn, Cd, Hg, to a 10^{-4} to 10^{-5} M solution of *t*-butyl *N*-(4-chlorophenyl)thiooxamate in acetonitrile produces a significant variation of the u.v. spectrum of the thiooxamate (see Fig. 5). As a consequence the stoichiometry and the formation constants of possible complexes can be reached, but first the complexes must be well characterized.

As the occurrence of the $L/ZnBr_2$ complex was previously evidenced we also expected the formation of a $L/Zn(ClO_4)_2$ complex, the zinc perchlorate being a weaker Lewis acid than the zinc bromide.

3.4. Characterization of Cd(II) complexes

Two experiments were realized with 1:2 ($\rho = 0.5$) and 1:1 ($\rho = 1$) stoichiometries in 0.3 M CD_3CN (see Table 2).

3.4.1. 1:2 Stoichiometry

The ^{13}C n.m.r. spectrum was run 5 min after mixing thiooxamate and cadmium perchlorate, then accumulated during 2 h. Immediately after, the sample was diluted four times and its i.r. spectrum recorded in CD_3CN then, after solvent evaporation, in KBr pellet. By analogy with X_2 ($ZnBr_2$) the product was labelled X_2'.

The i.r. spectra in KBr pellets of X_2' and X_2 are very similar: ν(NH) bands at 3246 and 3249 cm^{-1} (3274 and 3275 cm^{-1} in CD_3CN), ν(C=O) at 1746(vs), TA I at 1539(ms) and 1535(m), TA IV at 721(ms) and 719(ms) cm^{-1}. Thus, we find again with Cd(II) the same great frequency shifts versus ligand as with Zn(II), i.e. $-$ 40 ($\Delta\nu$(NH)), + 45 ($\Delta\nu$(C=O)), + 16 ($\Delta\nu$(CN)), $-$ 14 ($\Delta\nu$(TA I)) cm^{-1}. The 600–200 cm^{-1} region is also informative with X_2': we found bands at 515, 485, 449 and 429 cm^{-1}, assigned to NCS deformations following Desseyn [12]b who studied the complex $CdL_4(ClO_4)_2$ with L = dimethylthioacetamide. The ν(CdS) band is at 311 cm^{-1}, very close to 308 cm^{-1} found with dimethylthioacetamide Cd(II) complex.

Similarities appear also between ^{13}C n.m.r spectra of X_2' and the ligand (Table 2). The signal of the methyl groups is weakly shifted while phenyl carbons undergo 0.2–0.7 ppm variations and the thiocarbonyl carbon a decrease of 0.5 ppm as expected for a complexation through the sulfur atom.

3.4.2. 1:1 Stoichiometry

The ^{13}C n.m.r spectrum in CD_3CN was also accumulated during 2 h. Then 2 days after a deep yellow solid was formed and filtered off. After removing the solvent the filtrate gave a yellow solid labelled X_3'. The i.r. spectra of the two solid compounds were recorded. The first one is a mixture of the thiooxamic acid and X_2'.

The X_3' compound shows again analogies of i.r. spectra with X_3 in KBr pellets. The same broad ν(OH) bands at 3468 (Cd) or 3460 (Zn) cm^{-1} of coordinated water like with zinc and cadmium thiooxamates as yet seen by X-ray diffraction [16], ν(NH) bands at 3180 (Cd) or 3166 (Zn) cm^{-1} of H-bonded amide groups, $\nu_{as}(CO_2)$ at 1670 and TA I at 1534 cm^{-1}. This latter band undergoes a variation of + 17 cm^{-1} versus ligand (+ 11 for X_3 with $ZnBr_2$), indicative of chelation by the sulfur atom.

Important variations of ^{13}C n.m.r. chemical shifts are observed versus thiooxamate (L) on the spectrum of X_3' (Table 2). The variation of the *t*-butyl group is not significant in that the reaction in the n.m.r. tube gives *t*-butyl unidentified products. For the other signals the variations are of 0.3–1.5 (phenyl carbons), + 1.1 (C=O) and $-$ 0.7 (C=S) ppm. The resonance of the thioamide ^{13}C at 185.6 (X_2') or 185.4 (X_3') ppm is in the 190 ppm region assigned to a N–C=S carbon type [22]. With the N=C–S carbon type of a deprotonated thioamide the chemical shift would be in the 170 ppm region [22]. The observed deshielded thiocarbonyl carbon correlates well with a complexation of the N–C=S···Cd type. The shielding of the carbonyl carbon could be related to a non H-bonded carbonyl oxygen atom.

3.5. Characterization of Hg(II) complexes

A unique experiment was performed with 1:2 (ρ = 0.5) stoichiometry in 0.05 M CH_3CN solution. After 10 times dilution, a positive electrospray mass spectrum (ES^+MS) was recorded. It shows peaks at *m/z* 743.0, 687.0 and 630.9, which correspond respectively to $[L_2Hg - H]^+$, and its derivatives after elimination of one and two isobutene molecules

respectively. The isotopic pattern of these peaks is consistent with these assignments (see Fig. 6). Thus, the obtaining of the L_2Hg complex and mercuric salts of the thiooxamic acid can be expected.

The i.r. spectrum of the grey solid obtained by precipitation from the acetonitrile solution shows bands at 3438 (coordinated water), 3263 (ν(NH)), 1742 (ν(C=O)), 1670 ($\nu_{as}(CO_2)$), 1543 (TA I) and 719 (ν(TA IV)) cm^{-1}. The carbonyl stretching vibration at 1742 cm^{-1} is very close to those found for zinc and cadmium complexes (1746 cm^{-1}), undergoing the same frequency decrease versus ligand; it can be assigned to the L_2Hg complex. Besides this product, the mercuric salt of the thiooxamic acid is characterized by the asymmetric carboxylate stretching vibration at 1670 cm^{-1}, here also in agreement with the same bands for zinc and cadmium salts. Other bands, such as TA I and TA IV, present respectively an increase of 20 cm^{-1} and a decrease of 16 cm^{-1}, characteristic of chelation by the sulfur atom of the thiooxamate.

The L_2Hg complex is less soluble in acetonitrile than the other two metallic complexes; so was obtained a solid which is composed of the two entities, complex and salt, without noticeable mercuric compound in the acetonitrile layer at the i.r. maximum concentration (0.05 M).

The variation of the absorption spectrum of the thiooxamate by progressive addition of mercuric perchlorate in acetonitrile shows (Fig. 5a) that, at the u.v. concentration ($\sim 10^{-4}$ M), the complexed thiooxamate is the unique entity in the medium. This is in accordance with its expected greater solubility than mercuric salts and also with the results of mass spectrometric analysis.

3.6. Spectrophotometric study of the complexes

Aliquots of metallic perchlorates (2.4×10^{-3} to 3.4×10^{-3} M) were added to a acetonitrile solution of *t*-butyl *N*-(4-chlorophenyl)thiooxamate (6.1×10^{-5} M) at 25°C. After dilution correction, spectra at different ρ = M/L values are gathered in Fig. 5. We note that, for $0 < \rho < 1$, the OD variation at $\lambda_{max} = 326$ nm is strong with Hg^{2+} (0.36), lesser with Cd^{2+} (0.23), and weak with Zn^{2+} (0.05).

The OD variation as a function of ρ is represented in Fig. 5 for all the complexes. Taking into account the weak variation with Zn^{2+}, only the two other complexes have been studied. With Hg^{2+} (Fig. 6a), a clear break in the graph is observed for $\rho = 0.5$ at $\lambda = 326$ nm indicating a ML_2 complex formation. However, whereas the formation of a new maximum was seen near 275 nm, an accurate formation constant for the ML_2 complex (β_{ML2}) cannot be calculated with the STAR program; only an approximate value of $\log\beta_{ML2} = 13.4 \pm 0.6$ is obtained. With Cd^{2+} (Fig. 5b), isosbestic points are observed at 298, 307 and 390 nm. On the other hand, slope changes of the OD versus ρ curves are found, for instance, at 340 nm for $\rho = 0.5$ and at 230 nm for $\rho = 0.3$, suggesting respectively ML_2 and ML_3 complexes. Taking into account these two complexes, two formation constants are found, i.e. $\log\beta_{ML2} = 10.0 \pm 0.1$ and $\log\beta_{ML3} = 14.0 \pm 0.3$. The species distribution along $0 < \rho < 1$ indicates that the ML_3 complex does not exceed 5%. Moreover, the absorption curves of the pure species show that the complexes absorb near 295 nm.

Finally, the ML_2 complex formation constant with Hg(II) is higher than that with Cd(II) by three logarithmic units, according to the affinity difference of these two metallic ions for the thioamide function of the ligand. This affinity difference can be also correlated to the λ_{max} shift of the complexes (51 and 31 nm respectively). In each case, it is an hypsochromic and hypochromic shift related to a lesser conjugation of the electronic system in the two ML_2 complexes.

4. Conclusion

In this study, we found that the potential enzyme inhibitor *t*-butyl *N*-(4-chlorophenyl)thiooxamate presents a different conformational pattern than the ethyl thiooxamate of known structure, i.e. *anti* carbonyl versus thiocarbonyl groups and H···O hydrogen bonds instead of *syn* configuration and H···S bonds in the ethyl thiooxamate due to favourable electrostatic and torsional interactions in that conformation of the *t*-butyl derivative. This behaviour explains, for instance, the great decrease of the ester carbonyl stretching band near 1700 cm^{-1}.

The action of a rough metalloenzyme model like $ZnBr_2$ on the *t*-butyl derivative led to the characterization of three entities: a Zn(II) complex of the thiooxamate; the thiooxamic acid obtained by isobutene

elimination; and its zinc salt(s). A mechanism is suggested displaying the great facility to produce zinc salts, and thus providing a possible process for the inactivation of zinc metalloenzymes.

The study was followed by the action of heavy metal perchlorates showing the formation of same complexes and salts as with zinc bromide. Finally, the stability constants of cadmium and mercuric complexes determined by u.v. spectrophotometry demonstrated the high affinity of the thiooxamate for mercuric versus cadmium and, to a greater extent, zinc ions.

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