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Equilibrium Studies of the Dimethyltin(IV) Complexes with Tyrosine, Tryptophan, and Phenylalanine

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ABSTRACT: The formation constants of the species formed in the systems H^+ + dimethyltin(IV) + tyrosine, tryptophan, and phenylalanine as well as H^+ + tyrosine, tryptophan, and phenylalanine have been determined in aqueous solution in the pH range (1.5 to 9.5) at a constant temperature (25 °C) and constant ionic strength (0.1 mol·dm^{-3} $NaClO_4$), using a combination of spectrophotometric and potentiometric techniques. The composition of the formed complexes were determined, and it was shown that dimethyltin(IV) forms three mononuclear 1:1 species with all the ligands, of the type MHL, ML, and $MH_{-1}L$, where M and L represent metal ion and each amino acid, respectively. A complex distribution curve depending on the pH and metal−ligand ratio is given, and influences of the ligands side chains on the stability constants of the formed species are discussed. ^{1}H NMR investigations in aqueous solution confirmed the species formation and led us to propose their probable structures.

INTRODUCTION

Organotin compounds have been shown to have high antitumor activity in vitro in a wide variety of human tumors.[1−15] The increasing interest in the chemistry and biochemistry of organotin complexes has led to extend studies on their interactions with different naturally occurring ligands, for example, carbohydrates, nucleic acid derivatives, amino acids, and peptides.[2,3,7−15] Several papers have revealed the coordination behavior of organotin cations toward biomolecules containing different types of donor atoms including both solid state and solution studies.[16−19]

Organotin compounds are generally very toxic, even at low concentrations. They are found both in fresh and marine waters, since they are among the most industrially used organometallic compounds and are also widely used as biocidal agents. This causes the problem of the presence of organotin compounds in the human food chain.[20] In addition, as for many drugs used in chemotherapy, they may have undesirable side effects of the pharmaceutical use of these compounds. Therefore understanding the interaction of organotin compounds with possible biological targets is highly desirable. In spite of these efforts, the mechanism of action of these drugs in the living cell is not well understood. The activity of these compounds led to the hypothesis that these drugs hydrolyze easily in aqueous media and transport the active part (R_2Sn or R_3Sn) inside the cells where it possibly reacts with DNA.[11] Some recent reviews point out the lack of solution equilibrium studies that could provide essential information on the bioactivity of di- or trialkyltin(IV) ions toward amino acids, peptides, and nucleotides.[2,3,7,9,11,13,14]

Amino acids are well-known as efficient biological metal ion binders; therefore their interaction with organotin cations possibly play an important role in the mechanism of the abovementioned toxic/antitumor effect. A few reports have discussed the coordination chemical behavior of different complexes formed by di- or trialkyltin(IV) ions toward amino acids in aqueous solution. We have also reported the complex formation of dimethytin(IV) cation with some nucleotides of purine and pyrimidine bases in aqueous solution and have shown that the purine and pyrimidine moieties are not involved in coordination, and the interactions are limited to the phosphate side of the ligands.[2,3] Continuing our studies on the species distribution of dimethyltin(IV), in this work we report on the complexation of the metal ion with tryptophan, tyrosine, and phenylalanine over a wide pH range in aqueous solution. ^{1}H NMR spectroscopy in D_2O-H_2O solutions was used to verify the different donor atoms and possible structures of the complexes.

EXPERIMENTAL SECTION

Chemicals. Dimethyltin(IV)dichloride, L-tryptophan, L-tyrosine, and L-phenylalanine were obtained from Fluka as reagent grade materials and were used without further purification. Sodium perchlorate was from Merck and was dried under vacuum at room temperature at least 72 h before use. NaOH solution was prepared from a titrisol solution (Merck). Perchloric acid was from Merck and was used as supplied. The aqueous stock solutions of the ligands were freshly prepared daily, and their concentrations were determined each time by titration with NaOH solution. All dilute solutions were prepared from double-distilled water with a conductance equal to (1.3 ± 0.1) μS.

Measurements. All measurements were carried out at 25 °C. The ionic strength was maintained to 0.1 mol·dm^{-3} with sodium perchlorate. A Jenway research pH-meter, model 3520, was used for the pH measurements. The hydrogen ion concentration was measured with a combined electrode (Jenway). The pH-meter was calibrated for the relevant H^+ concentration

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with a solution of 0.01 mol·dm^{-3} perchloric acid containing 0.09 mol·dm^{-3} sodium perchlorate (for adjusting the ionic strength to 0.1 mol·dm^{-3}). For this standard solution, we set pC_H ($-\log[H^+]$) = 2.00.[21] Junction potential corrections calculated from eq 1

$$pC_{H\ real} = pC_{H\ measured} + a + b[H^+]_{measured} \quad (1)$$

where a and b were determined by measuring the hydrogen ion concentration for two different solutions of perchloric acid or sodium hydroxide with sufficient sodium perchlorate to adjust the ionic media.

Procedure. A 50 mL acidic solution (0.1 mol·dm^{-3} $HClO_4$) of dimethyltin(IV) dichloride [(2.0 to 6.0)·10^{-3} mol·dm^{-3}] was titrated with an alkali solution (0.1 mol·dm^{-3} NaOH) of the ligands [(2.0 to 5.0)·10^{-3} mol·dm^{-3}] both of the same ionic strength. The absorbance and pC_H were measured after the addition of a few drops of the titrant, and this procedure extended up to the required pC_H. To exclude carbon dioxide from the system, a stream of purified nitrogen was passed through a sodium hydroxide solution and then bubbled slowly through the reaction solution. In all cases, the procedure was repeated at least three times and the resulting average values, and corresponding deviations from the average are shown in the text and tables.

The protonation constant of the ligands and their complexations with dimethyltin(IV) have been determined using a combination of spectrophotometric and potentiometric titration methods. The complex $M_xH_yL_z^{(2x+y-nz)}$ that formed is characterized by its stoichiometry (x:y:z). To determine the stability constant of the complexation, eqs 2 and 3 are defined by β_{xyz}:

$$xM^{2+} + yH^+ + zL^{n-} \rightleftarrows M_xH_yL_z^{(2x+y-nz)} \quad (2)$$

$$\beta_{xyz} = [M_xH_yL_z^{(2x+y-nz)}]/([M^{2+}]^x[H^+]^y[L^{n-}]^z) \quad (3)$$

The determination of the stability constant, β_{xyz}, based on the relation $A = f(pH)$,[22,23] was performed using the computer program Squad.[24] Absorbance, A, and pC_H were measured for solutions containing dimethyltin(IV) and each amino acid. Treatments of the spectrophotometric data [(250 to 300) nm with an interval of 0.5 nm], obtained during the titrations as a function of the H^+ concentration, were conducted with the computer program. The program allows the calculation of the stability constants for different stoichiometry models.

Spectroscopy Measurements. Spectrophotometric measurements were performed on a UV−vis Shimadzu 2100 spectrophotometer with a Pentium 4 computer and using thermostatted matched 10 mm quartz cells. The measurement cell was of flow type. A Masterflex pump allowed circulation of the solution under study from the potentiometric cell to the spectrophotometric cell, so the absorbance and pC_H of the solution could be measured simultaneously.

^{1}H NMR spectra of the metal ion and the complexes were recorded on a Bruker DRX-300 MHz spectrometer in H_2O−D_2O (1:1 by volume) using tetramethylsilane (TMS) as an external reference operating at room temperature. The concentrations of the NMR samples were 1 mM.

RESULTS AND DISCUSSION

Stepwise Acidity Constants of the Amino Acids. The protonation constants of the amino acids have been determined spectrophotometrically based again on the relation $A = f(pH)$. The measured absorbance, A [(250 to 300) nm in the interval of 0.5 nm], and pC_H from the spectrophotometric titration were conducted with the computer program Squad. The data in the computer program was fitted for eq 4 by minimizing the error squares sum of the experimental absorbances from the calculated ones.

$$H_{n-1}L^{n-2} + H^+ \rightleftarrows H_nL^{n-1}$$

$$K_n = [H_nL^{n-1}]/[H_{n-1}L^{n-2}][H^+] \quad (4)$$

where n (number of protons) could be 2 or 1 for the amino acids used. The computer program allows the calculation of the protonation constants with different stoichiometries. The number of experimental points (absorbance versus pC_H) was at least 40 (maximum 50) for each titration run. During the experiments, the solutions were stable, and the absorbance values did not change with time.

The results obtained using spectrophotometric and potentiometric pH titrations for the various acidity constants of the proton donors of the ligands are listed in Table 1 together with the values reported before in the literature.[11,25,26] The protonation constant values obtained in this work are in agreement with those reported before. It should be added that a third proton may be released from the phenolic group of tyrosine at pH > 10 which is not considered further in this work.

Hydrolysis of Dimethyltin(IV) Dichloride. The hydrolysis of $Me_2Sn(IV)^{2+}$ has been investigated in different media by some authors.[27,28] We performed earlier spectrophotometric titrations to obtain these data in various ionic strengths [(0.1 to 1.0) mol·dm^{-3}] $NaClO_4$ and NaCl media.[1] The hydrolysis constants of the hydrolyzed species are determined as before and are listed in Table 2. The detected species and their formation constants are in good agreement with those were reported earlier and show the strong tendency of dimethyltin(IV) to hydrolyze in aqueous solution to form various hydrolytic species.[27,28]

Table 1. Average Values of the Protonation Constants of the Amino Acids at 25 °C and Constant Ionic Strength (0.1 mol·dm^{-3} $NaClO_4$) together with the Values Reported in the Literature

amino acid	log K_2	log K_1	ref
tryptophan	2.25 ± 0.03	9.31 ± 0.05	this work
tyrosine	2.10 ± 0.09	9.05 ± 0.07	this work
phenylalanine	2.03 ± 0.06	9.18 ± 0.04	this work
tryptophan	2.35	9.33	25
tyrosine	2.34	9.11	26
phenylalanine	2.00	9.12	11

Table 2. Average Values of Hydrolysis Constants, β_{pq}, for $Me_2Sn(IV)$ Species in Aqueous Solution at 25 °C and Ionic Strength (0.1 mol·dm^{-3} $NaClO_4$), Where p and q Represent $Me_2Sn(IV)$ and Hydroxyl Ions, Respectively (Values Reported in the Literature Are Also Listed)

$-\log\beta_{11}$	$-\log\beta_{12}$	$-\log\beta_{13}$	$-\log\beta_{22}$	$-\log\beta_{23}$	ref
3.12 ± 0.03	8.43 ± 0.04	19.45 ± 0.09	4.86 ± 0.05	9.74 ± 0.08	this work
3.25	8.54	-	5.05	9.81	27
3.12	8.45	19.48	5.2	9.7	28

Table 3. Average Values of $\log \beta_{111}$, $\log \beta_{101}$, and $\log \beta_{1\text{-}11}$ at 25 °C and Constant Ionic Strength (0.1 mol·dm^{-3} $NaClO_4$), together with Some of the Values Reported in the Literature

complex species	$\log \beta_{111}$	$\log \beta_{101}$	$\log \beta_{1\text{-}11}$	ref
$(CH_3)_2Sn$-tyrosine	9.81 ± 0.09	6.57 ± 0.04	1.89 ± 0.03	this work
$(CH_3)_2Sn$-tryptophan	9.72 ± 0.10	6.48 ± 0.06	1.82 ± 0.04	this work
$(CH_3)_2Sn$-phenylalanine	9.61 ± 0.11	6.41 ± 0.06	1.75 ± 0.03	this work
$(CH_3)_2Sn$-glycine	11.03	7.99	2.4	7
$(CH_3)_2Sn$-histidine	13.23	7.96	1.56	7
$(CH_3)_2Sn$-alanyl-glycine	10.26	6.8	1.81	8
$(CH_3)_2Sn$-glycine	7.92	3.31	-	9
$(CH_3)_2Sn$-glycine	-	6.75	-	13

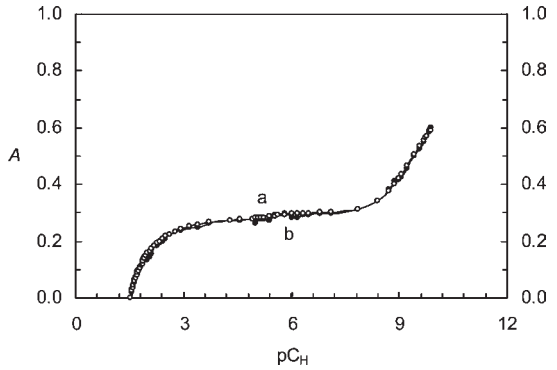


Figure 1. A typical graphical fitting for the complexation of dimethyltin(IV) with tyrosine at 25 °C and ionic strength (0.1 mol·dm^{-3} $NaClO_4$), (a) experimental and (b) calculated absorbances, respectively.

Complexation of the Amino Acids by Dimethyltin(IV). Considering eqs 2 and 3, different models including MHL, ML, $MH_{-1}L$, and several polynuclear and protonated species were tested by the program. As expected, polynuclear complexes were systematically rejected by the computer program, as also were MH_2L_2, MHL_2, and ML_2 (the charges are omitted for simplicity). The models finally chosen, formed by MHL, ML, and $MH_{-1}L$ for the amino acids besides the hydrolysis products of $Me_2Sn(IV)$, resulted in a satisfactory of numerical and graphical fitting. During the spectrophotometric titration of the amino acids by dimethyltin(IV) no precipitation was observed. The calculated average values of the stability constants for different experiments are listed in Table 3 together with the values of some homologous complexes reported in the literature for comparison.[7−9,13] With some differences, the formation constant values obtained in this work are in agreement with those reported before. The main differences are due to the different ligand and various experimental methods and the fact that a different background electrolyte has been employed to determine the values.

Figure 1 is shown as a typical example of graphical fitting for the observed and calculated absorbances from the computer program. In Figure 2 the equilibrium distribution of various species of $Me_2Sn(IV)$-tyrosine system is shown as a function of pC_H (as a typical example). The calculations are based on the stability constant values given in Table 3. The curves clearly demonstrate that an increase of pC_H is accompanied by formation of the diprotonated species and shows the complexes with a

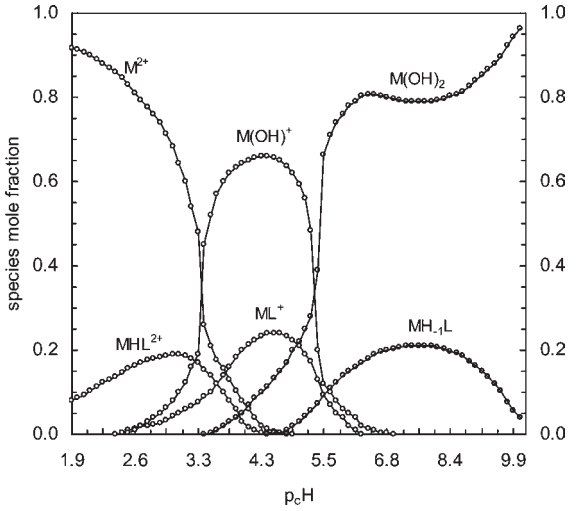


Figure 2. Species distribution diagram of the $Me_2Sn(IV)$-tyrosine system at 25 °C and ionic strength (0.1 mol·dm^{-3} $NaClO_4$).

1:1 metal ion-to-ligand ratio were formed. There was no evidence of the presence of polynuclear species in solution.

As can be seen from Table 3, the values of $\log \beta_{MHL}$, $\log \beta_{ML}$, and $\log \beta_{MH\text{-}1L}$ for the dimethyltin(IV) complexes with the studied amino acids are nearly identical due to the similarity in their structures, with some influence from the side groups. The inductive effects of the side groups in phenylalanine, tryptophan, and tyrosine are in increasing affinity order and so are apparently responsible for the small differences in the calculated stability constant values. This assumption is confirmed by the finding that the stability constant value of the complex with MHL, ML, and $MH_{-1}L$ stoichiometries formed by tyrosine has the highest values.

Figure 3 exhibits ^1H NMR of methyl signals of the studied systems at different pH values, and Figure 4 shows a plot of the $^2J(Sn-^1H)$ as a function of pH. As can be seen the $^2J(Sn-^1H)$ values increase in NMR titration with decreasing the pH. In the pH range (1.5 to 4.1), for tyrosine, MHL species is formed by the deprotonation of one proton from carboxylic group of the amino acid ($MHL^{2+} + H^+ \rightarrow M^{2+} + H_2L^+$). The species formed at different pH values were found to be in different protonation states. In acidic and alkaline pH range, two deprotonation processes take place for the studied systems (pH ≅ 2.8 to 5.7 and 4.6 to 9.5; 3.0 to 5.9 and 4.8 to 9.5; 3.1 to 6.1 and 4.9 to 9.5 for tyrosine, tryptophan, and phenylalanine, respectively) resulting in the formation of ML^+ and $MH_{-1}L$ species, $ML^+ + H^+ \rightarrow MHL^{2+}$ and $MH_{-1}L + H^+ \rightarrow ML^+$. The first reaction is due to the deprotonation of the amino group of the amino acids, and the second reaction is attributed to the deprotonation of a coordinated H_2O molecule in all cases, resulting in the formation of a mixed hydroxo complex. The evaluation of the titration curves show that only two hydrolyzed species, $M(OH)^+$ and $M(OH)_2$, are present in acidic and alkaline pH ranges, respectively; see Figure 2.

Lockhart and Manders studied the correlation of $^2J(Sn-^1H)$ and Me−Sn−Me angle in 25 methyltin(IV) compounds.[29] They proposed an empirical quadratic expression in terms of $^2J(Sn-^1H)$ to determine the C−Sn−C angle. It was found that these values provide useful information on the C−Sn−C bond angle of the compounds in a sample and indirectly on the possible

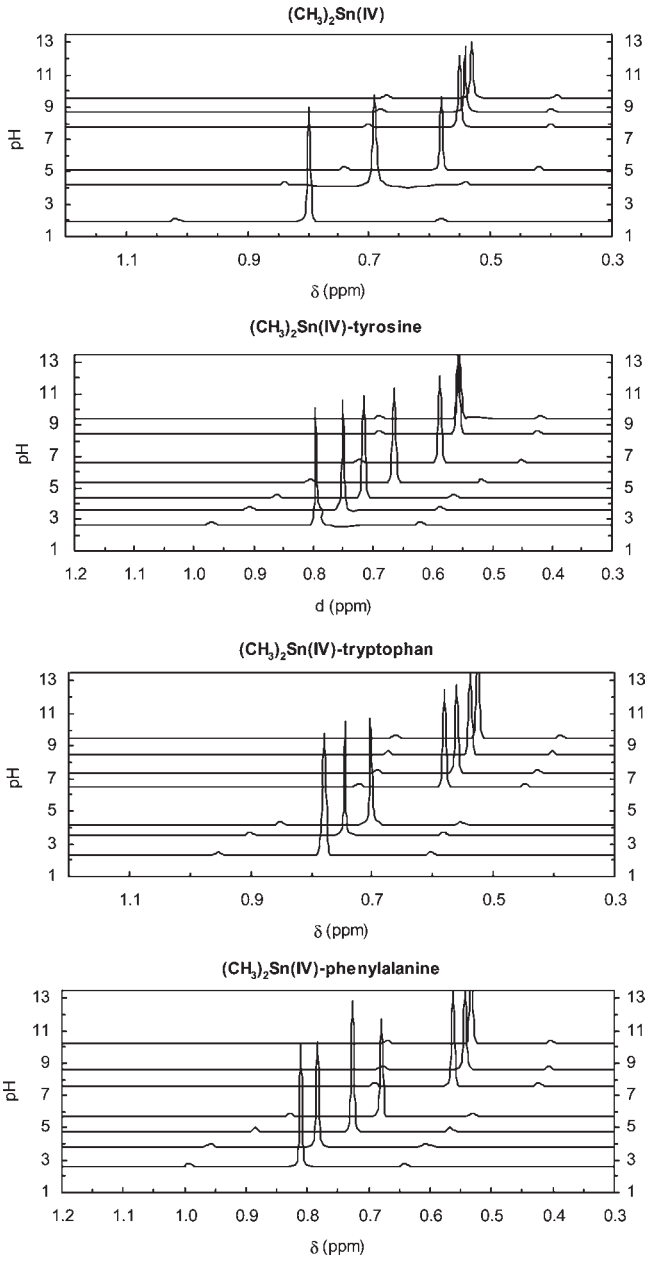


Figure 3. ^{1}H NMR spectra in the Me proton range for $Me_2Sn(IV)$ and the 1:1 $Me_2Sn(IV)$-tyrosine, tryptophan, and phenylalanine systems at different pH values.

coordination numbers and geometry around the Sn atom. Two bond coupling, $^{2}J(Sn-^{1}H)$, of the $Me_2Sn(IV)^{2+}$ alone and its complexes with the studied amino acids were determined via the ^{1}H NMR spectra at different pH values. It can be seen, in Figure 3, the signal of the methyl protons in $Me_2Sn(IV)^{2+}$ are sharp for all the systems and has almost the same chemical shift as in the solution of $Me_2Sn(IV)^{2+}$ alone. At higher pH the $^{2}J(Sn-^{1}H)$ values of $Me_2Sn(IV)$ are somewhat smaller than the complex systems, in Figure 4, possibly due to a slight distortion of C−Sn−C angle by the coordinated ligand.[29] Using the quadratic equation of Lockhart and Manders,[29] the average C−Sn−C bond angle at low pH (less than 4) and at higher pH values (more than 5) for the two species calculated from the coupling constant are nearly 175° and 131°, respectively. On this

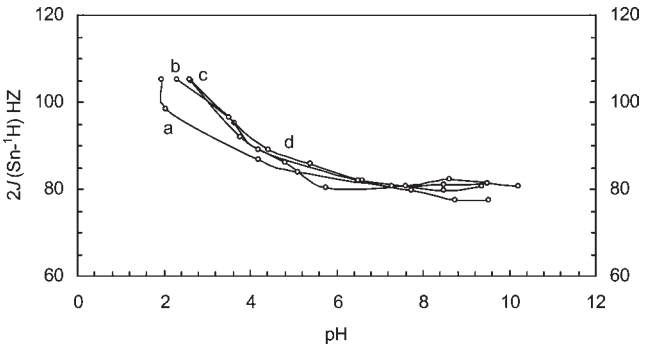
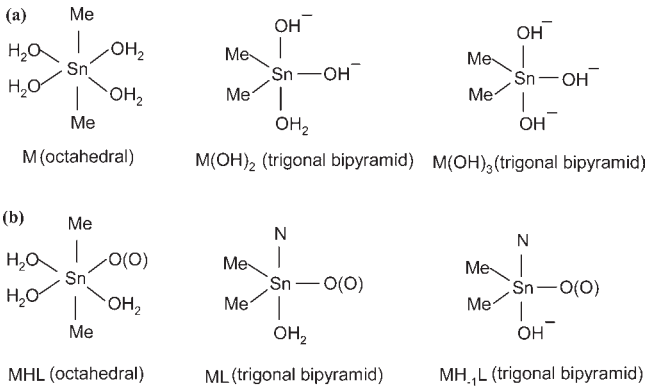


Figure 4. Measured coupling constants, $^{2}J(Sn-^{1}H)$, of Me protons in solutions of (a) $Me_2Sn(IV)$, (b) 1:1 $Me_2Sn(IV)$-tryptophan (c), 1:1 $Me_2Sn(IV)$-phenylalanine, and (d) 1:1 $Me_2Sn(IV)$-tyrosine as a function of pH.

Scheme 1. Proposed Structure of (a) the Hydrolyzed Species of Dimethyltin(IV) and (b) the Complexes of $(CH_3)_2Sn(IV)$-Amino Acids



basis the structures of $Me_2Sn(IV)^{2+}$ and their complexes with the amino acids are proposed as an octahedral at low pH and distorted trigonal bipyramidal arrangement at higher pH values as shown in Scheme 1.

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