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Interaction of Dimethyltin(IV) with *DNA* Constituents

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Summary. The interaction of dimethyltin(IV) (*DMT*) with some selected *DNA* constituents was investigated potentiometrically. The stepwise formation constants of the complexes were determined, and the concentration distribution of the various complex species was evaluated as a function of *pH*. The effect of dioxane on the protonation constants of the ligands and the formation constants of dimethyltin(IV) complexes are discussed. The thermodynamic parameters ΔH° and ΔS° were calculated from the temperature dependence of the equilibrium constants.

Keywords. Equilibrium studies; Effect of solvent and temperature; Dimethyltin(IV) dichloride; *DNA* constituents.

Introduction

Cancer chemotherapy based on metal complexes started at a clinical level in the late seventies with the use of *cis*-platin [1]. *cis*-Platin is still in use today in the form of carboplatin or iproplatin, which are characterized by lower toxicity and higher activity, against testicular and bladder tumors, ovarian carcinomas, head and neck cancers, *etc*. [2]. The mechanism of the antitumor action of *cis*-platin is believed to be due mainly to the formation of an intrastand crosslink with *DNA* [3, 4].

In an attempt to discover further metal based anticancer drugs with higher activity and lower toxicity, several hundreds of diorganotin(IV) complexes have been synthesized and tested [5]. High *in vitro* antitumor activity in a wide variety of human tumors has been observed [6].

Bearing in mind that information on the equilibria of organotin(IV) complexes with *DNA* constituents might provide further insight in their antitumor and cytotoxic activity, this work is directed to the study of the solution chemistry of organotin(IV) complexes with ligands of biochemical significance [7–14]. The present investigation aims to characterize the reaction of dimethyltin(IV) dichloride with inosine, 5′-*IMP*, 5′-*GMP*, adenine, adenosine, 5′-*AMP*, uracil, thymine, thymidine, cytosine, and cytidine.

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Results and Discussion

The acid dissociation constants of the ligands were determined under similar experimental conditions (ionic strength, temperature, solvent composition) as used for the study of organotin(IV) complex equilibria. The pK_a values obtained (Table 1) are in good agreement with those reported in water [15], and are lower than those obtained in dioxane-water mixtures (Table 3). This may be due to the increased basicity of the ligand donor groups when changing from pure water to dioxane-water mixtures.

The hydrolysis of the dimethyltin(IV) (*DMT*) has been investigated in a variety of media by several authors [16–19]. In this work, potentiometric titrations at different temperatures in dioxane-water mixtures of different composition were performed, and the species (1, 0, −1), (1, 0, −2), (1, 0, −3), (1, 0, −4), (2, 0, −2), (2, 0, −3), and (2, 0, −4) were detected[1]. Taking into account the species (1, 0, −4) and (2, 0, −4) that have not been reported in previous investigations [16–18] improved the quality of data fitting. The concentration distribution diagram for the hydrolysis of *DMT* is shown in Fig. 1. The species (1, 0, −1) and its dimeric form (2, 0, −2) prevail at $pH \approx 4$; the main species under physiological conditions ($pH = 6$–8) are (1, 0, −2) and (2, 0, −4).

A comparison of the potentiometric titration curves of *DNA* units in the presence and absence of *DMT* shows that the complex titration curves are located at significantly lower *pH* values than those of the ligands (Fig. 2), corresponding to the formation of a complex species through release of a hydrogen ion. The formation equilibria were characterized by fitting their potentiometric data to various models; the best results were obtained for complexes with stoichiometric coefficients (1, 1, 0) and (1, 2, 0). The model was tested by comparing the experimental titration data with theoretical curves calculated from the pK_a of the ligand and the stability constant of the formed complex. A good agreement between the experimental and theoretical data proved the validity of the model proposed.

The mode of interaction of dimethyltin(IV) with 5′-*IMP* and 5′-*GMP* has been investigated [18, 20, 21] and shown to depend on the *pH* value of the solution. At $pH = 2$–3, R_2Sn(IV) is involved in bonding with the phosphate group. Above $pH = 9$, R_2Sn(IV) reacts with O(2′) and O(3′) of the sugar unit of the nucleotide. In the present investigation, potentiometric data in the *pH* range below 9 were fitted. Consequently, bonding through the sugar unit may be ignored. The stability constant of the 5′-*IMP* complex is significantly higher than that its inosine counterpart, indicating that the phosphate group takes part in bonding with R_2Sn(IV). This is in agreement with previous results [20, 21].

It has been reported that a linear relationship between the stability constant of the complex and the acid dissociation constant of ligands with related structures holds for metal complexes [22]. The importance of this fact is founded in the possibility of estimating the stability of unknown complexes. Figure 3 demonstrates a relationship between $\log\beta(1, 1, 0)$ of the complexes and the pK_a values of *DNA* constituents without a phosphate group (curve A). This reveals that the *DNA* units

[1] The notation (*l, p, q*) refers to the stoichiometric coefficients of *DMT*, *DNA*, and H^+; cf. footnote to Table 1 and Refs. [16–18]

Table 1. Formation constants of dimethyltin(IV) complexes in water

Ligand[a]	$T/^{\circ}C$	l[b]	p[b]	q[b]	$\log\beta$[c]	S[d]
DMT	15	1	0	−1	−3.56 (0.01)	5.4×10^{-8}
		1	0	−2	−9.05 (0.01)	
		1	0	−3	−19.79 (0.04)	
		1	0	−4	−30.41 (0.04)	
		2	0	−2	−4.23 (0.01)	
		2	0	−3	−9.52 (0.01)	
		2	0	−4	−15.23 (0.01)	
Inosine		0	1	1	8.77 (0.01)	2.2×10^{-8}
		1	1	0	7.40 (0.04)	1.1×10^{-9}
		1	2	0	14.33 (0.02)	
5′-*IMP*		0	1	1	9.35 (0.02)	3.2×10^{-7}
		0	1	2	15.28 (0.04)	
		1	1	0	11.19 (0.03)	1.9×10^{-8}
		1	2	0	18.72 (0.06)	
DMT	20	1	0	−1	−3.31 (0.01)	6.1×10^{-8}
		1	0	−2	−8.64 (0.01)	
		1	0	−3	−19.27 (0.05)	
		1	0	−4	−39.71 (0.02)	
		2	0	−2	−3.77 (0.01)	
		2	0	−3	−8.91 (0.02)	
		2	0	−4	−14.44 (0.01)	
Inosine		0	1	1	8.67 (0.01)	2.4×10^{-8}
		1	1	0	7.72 (0.01)	7.0×10^{-10}
		1	2	0	14.64 (0.01)	
5′-*IMP*		0	1	1	9.25 (0.02)	3.2×10^{-7}
		0	1	2	15.17 (0.03)	
		1	1	0	11.51 (0.04)	3.2×10^{-8}
		1	2	0	19.02 (0.07)	
DMT	25	1	0	−1	−3.03 (0.01)	4.3×10^{-8}
		1	0	−2	−8.21 (0.01)	
		1	0	−3	−18.73 (0.03)	
		1	0	−4	−29.54 (0.02)	
		2	0	−2	−3.12 (0.01)	
		2	0	−3	−8.13 (0.02)	
		2	0	−4	−13.59 (0.02)	
Inosine		0	1	1	8.55 (0.01)	5.2×10^{-8}
		1	1	0	8.13 (0.03)	1.1×10^{-9}
		1	2	0	14.82 (0.03)	
Na_2HPO_4		0	1	1	6.79 (0.01)	9.5×10^{-10}
		1	1	0	6.41 (0.05)	
		1	2	0	10.94 (0.08)	3.9×10^{-8}
5′-*IMP*		0	1	1	9.14 (0.02)	3.5×10^{-7}
		0	1	2	15.07 (0.03)	
		1	1	0	11.90 (0.04)	1.8×10^{-8}
		1	2	0	19.37 (0.06)	
5′-*GMP*		0	1	1	9.44 (0.01)	4.9×10^{-8}

(*continued*)

Table 1 (*continued*)

Ligand[a]	$T/^{\circ}$C	l[b]	p[b]	q[b]	logβ[c]	S[d]
		0	1	2	15.57 (0.01)	
		1	1	0	12.34 (0.03)	1.3×10^{-8}
		1	2	0	20.13 (0.06)	
Adenine		0	1	1	9.65 (0.00)	1.6×10^{-8}
		0	1	2	13.85 (0.01)	
		1	1	0	10.01 (0.04)	2.2×10^{-8}
		1	2	0	17.70 (0.05)	
Adenosine		0	1	1	3.49 (0.01)	5.4×10^{-8}
		1	1	0	4.41 (0.07)	5.8×10^{-9}
		1	2	0	8.31 (0.06)	
5′-*AMP*		0	1	1	6.04 (0.01)	2.6×10^{-8}
		0	1	2	9.32 (0.02)	
		1	1	0	6.07 (0.04)	3.8×10^{-8}
		1	2	0	10.74 (0.06)	
Uracil		0	1	1	9.15 (0.00)	6.9×10^{-8}
		1	1	0	9.34 (0.01)	9.3×10^{-10}
		1	2	0	16.60 (0.01)	
Thymine		0	1	1	9.58 (0.00)	3.4×10^{-8}
		1	1	0	9.61 (0.01)	2.4×10^{-10}
		1	2	0	16.96 (0.01)	
Thymidine		0	1	1	9.50 (0.00)	2.9×10^{-8}
		1	1	0	9.52 (0.01)	3.5×10^{-10}
		1	2	0	16.83 (0.01)	
Cytosine		0	1	1	4.53 (0.00)	2.5×10^{-8}
		1	1	0	4.44 (0.04)	6.7×10^{-9}
		1	2	0	8.54 (0.01)	
Cytidine		0	1	1	4.10 (0.00)	2.1×10^{-8}
		1	1	0	3.77 (0.05)	1.7×10^{-9}
		1	2	0	7.69 (0.01)	
DMT	30	1	0	−1	−2.81 (0.01)	6.6×10^{-8}
		1	0	−2	−7.91 (0.02)	
		1	0	−3	−18.38 (0.05)	
		1	0	−4	−28.71 (0.02)	
		2	0	−2	−2.87 (0.02)	
		2	0	−3	−7.84 (0.04)	
		2	0	−4	−13.06 (0.04)	
Inosine		0	1	1	8.44 (0.00)	6.2×10^{-8}
		1	1	0	8.66 (0.07)	2.8×10^{-9}
		1	2	0	15.07 (0.07)	
5′-*IMP*		0	1	1	9.06 (0.01)	2.4×10^{-7}
		0	1	2	14.99 (0.03)	
		1	1	0	12.29 (0.06)	3.6×10^{-8}
		1	2	0	19.75 (0.09)	
DMT	35	1	0	−1	−2.49 (0.02)	6.9×10^{-8}
		1	0	−2	−7.54 (0.02)	
		1	0	−3	−17.95 (0.06)	
		1	0	−4	−28.20 (0.03)	

Table 1 (*continued*)

Ligand[a]	$T/^{\circ}C$	l^b	p^b	q^b	$\log\beta^c$	S^d
		2	0	−2	−2.27 (0.03)	
		2	0	−3	−7.11 (0.04)	
		2	0	−4	−12.34 (0.04)	
Inosine		0	1	1	8.38 (0.01)	1.2×10^{-8}
		1	1	0	8.99 (0.09)	1.5×10^{-9}
		1	2	0	15.32 (0.10)	
5′-*IMP*		0	1	1	8.96 (0.00)	2.5×10^{-8}
		0	1	2	15.17 (0.01)	
		1	1	0	12.71 (0.07)	3.2×10^{-8}
		1	2	0	20.14 (0.10)	

[a] 5′-*IMP*: inosine 5′-monophosphate, 5′-*GMP*: guanosine 5′-monophosphate, 5′-*AMP*: adenosine 5′-monophosphate; [b] *l*, *p*, and *q* are the stoichiometric coefficients of dimethyltin(IV), *DNA*, and H^+, respectively; [c] standard deviations in parentheses; [d] sum of square of residuals

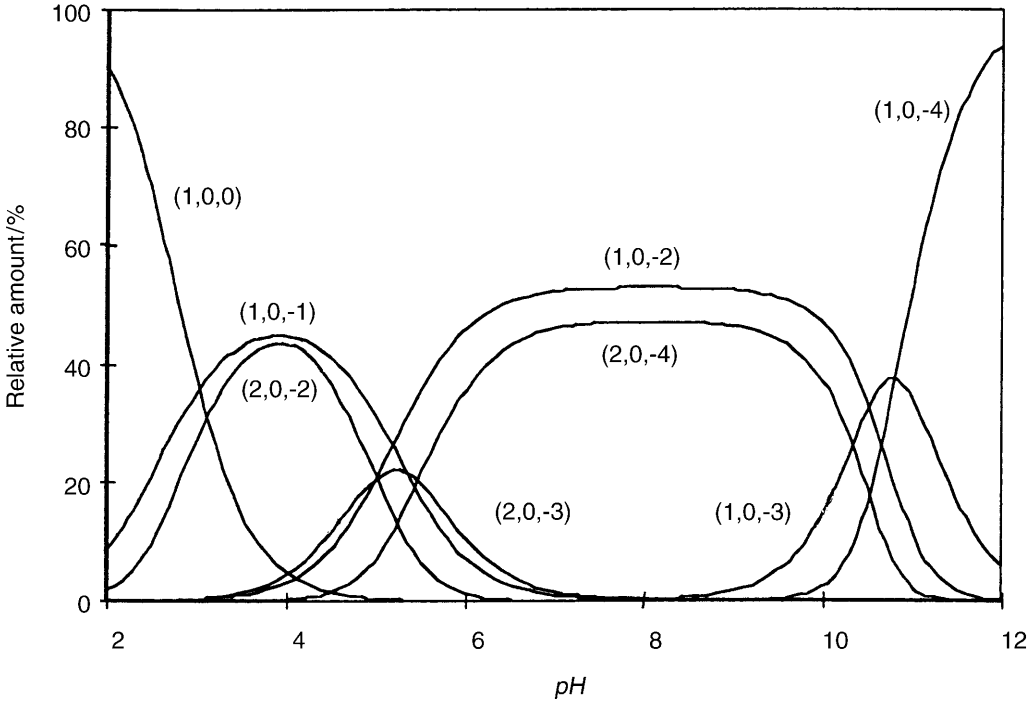

Fig. 1. Concentration distribution of complex species as a function of *pH* in the *DMT*/OH system ($[DMT] = 1.25\,\text{mmol}\cdot\text{dm}^{-3}$)

coordinate by their nitrogen base. However, the nucleotides 5′-*IMP* and 5′-*GMP* are not in accordance with the linear relationship, their stability constant values being located above the straight line. This may be explained on the premise that both phosphate group and nitrogen base take part in complex formation. Curve B in Fig. 3 demonstrates a linear relationship between $\log\beta(1, 1, 0)$ of *DNA* units containing a phosphate group and the corresponding overall dissociation constants values

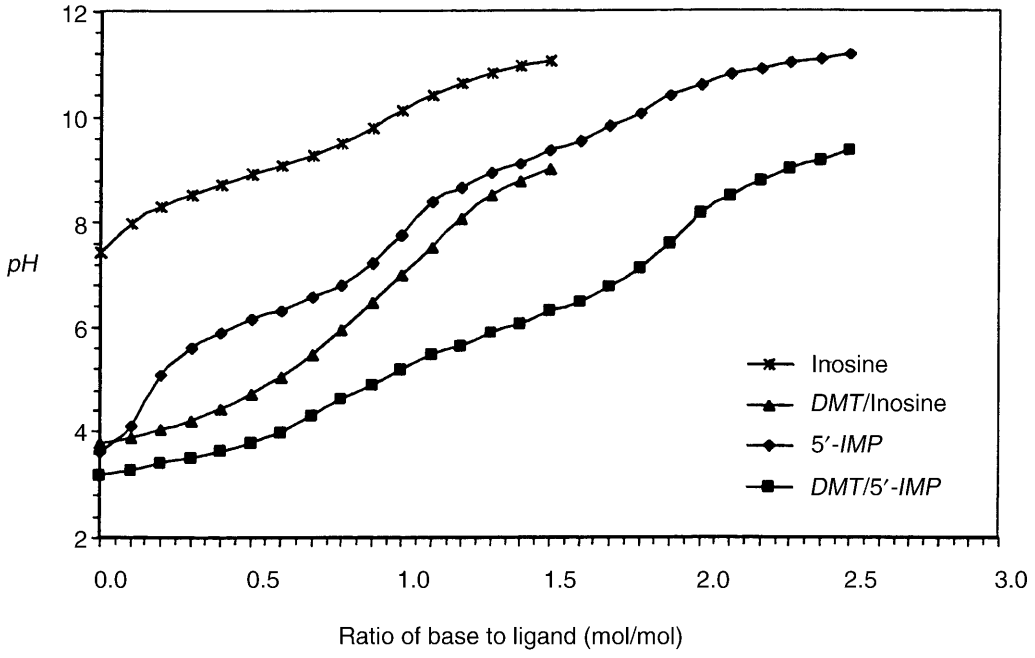

Fig. 2. Potentiometric titration curves of *DMT* in presence of inosine and 5′-*IMP*

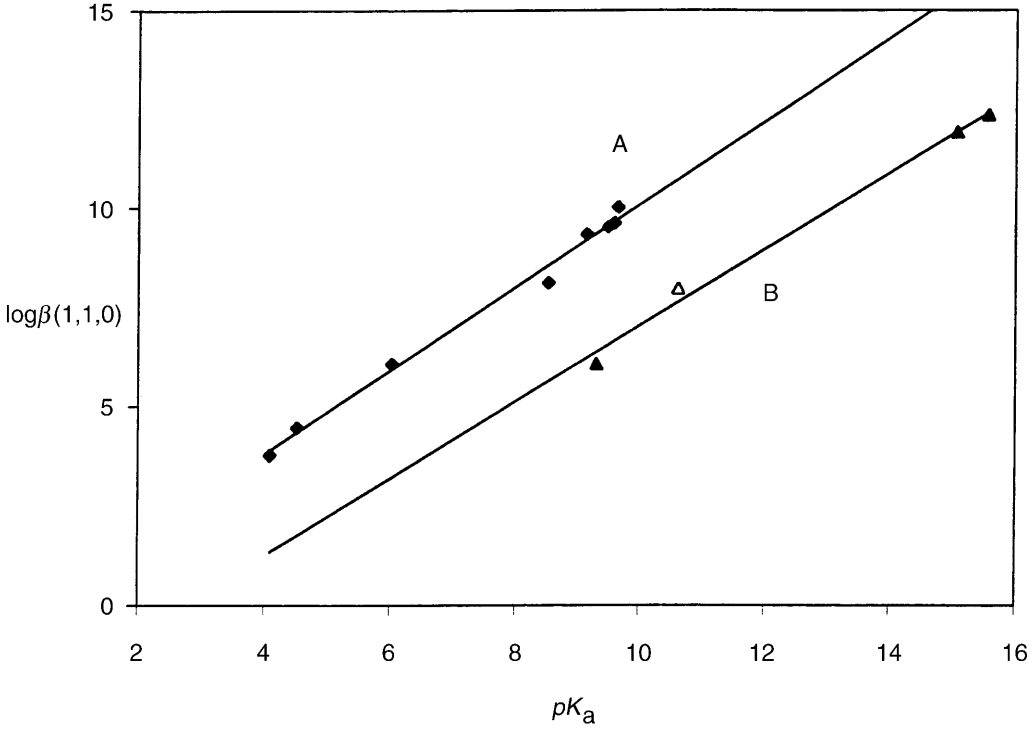

Fig. 3. Relationship between logβ(1, 1, 0) of the complexes and the pK_a of *DNA* units (curve A) or the sum of the pK_a values of the phosphate group and the base part of *DNA* constituents containing phosphate groups (curve B); the data of 5′-*ATP* (Δ) have been taken from Ref. [20]

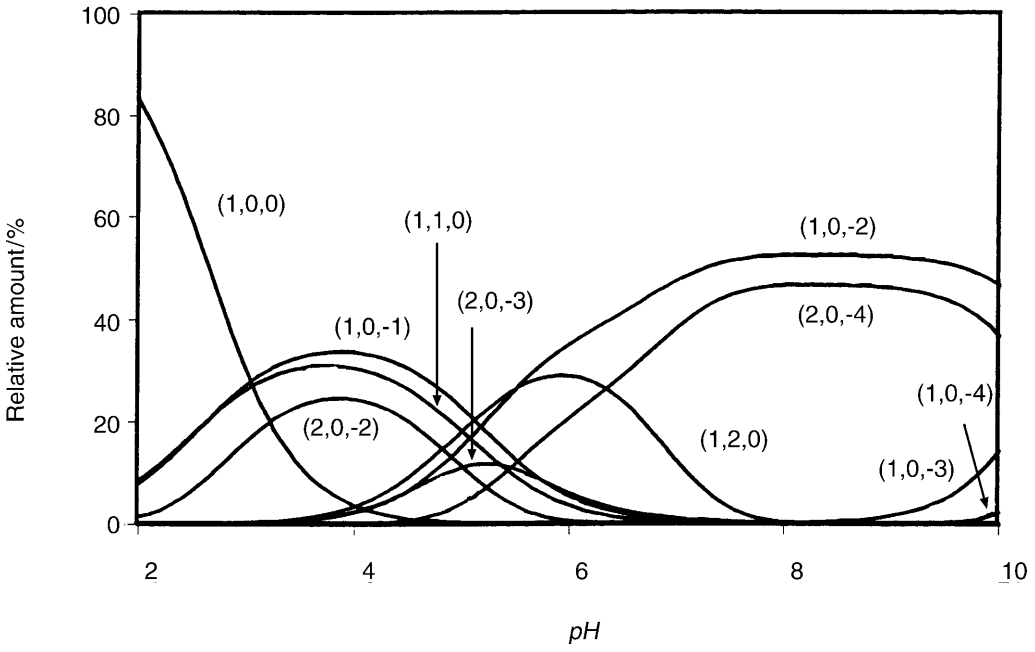

Fig. 4. Concentration distribution of complex species as a function of *pH* in the *DMT*/phosphate system ($[DMT] = 1.25\,\text{mmol}\cdot\text{dm}^{-3}$, $[Na_2HPO_4] = 2.5\,\text{mmol}\cdot\text{dm}^{-3}$)

(pK_a (phosphate) + pK_a (base)). This confirms that both phosphate group and base are involved in complex formation. Furthermore, the stability constants of *DNA* complexes containing a phosphate moiety are much higher than those of *DMT* with disodium hydrogen phosphate ($\log\beta(1, 1, 0) = 6.41$), thus yielding additional evidence for the above assumption. Nevertheless, in-depth structural investigations (*e.g.* multinuclear NMR measurements) will be required to elucidate the structure of the complexes.

Estimation of the concentration distribution of various complex species in solution provides a useful picture of *DMT* binding in biological systems. The concentration distribution diagrams (Figs. 4–9) lead to the results listed below.

a) *DMT* interacts with all ligands, forming species (1, 1, 0) at low *pH* and (1, 2, 0) at higher *pH* depending on the basicity of the base part of the *DNA* constituents.

b) The complex formation competes with the hydrolysis of *DMT*, the extent of competition depending on the basicity of the ligand. The highly basic 5′-*IMP* and 5′-*GMP* prevent hydrolysis. On the other hand, the less basic ligands as adenosine, cytosine, cytidine, and 5′-*AMP* do not (cf. Figs. 5 and 6). This is in agreement with previous study by *Gielen et al.* [21], where the interaction between diethyltin(IV) and both cytidine and 2′-deoxycytidine-5′-monophosphate does not prevent hydrolysis of *DMT*.

c) The interaction of *DMT* with inosine, uracil, thymine, and thymidine involves competition between the hydrolyzed species and the complexes (1, 1, 0) and (1, 2, 0) up to $pH \approx 8$ (Fig. 7). With adenine, the competition is extended to $pH \approx 9$ (Fig. 8).

d) The formation of 5′-*IMP* and 5′-*GMP* complex species (1, 1, 0) and (1, 2, 0) is accompanied by a decrease in the extent of *DMT* hydrolysis (Fig. 9). This is

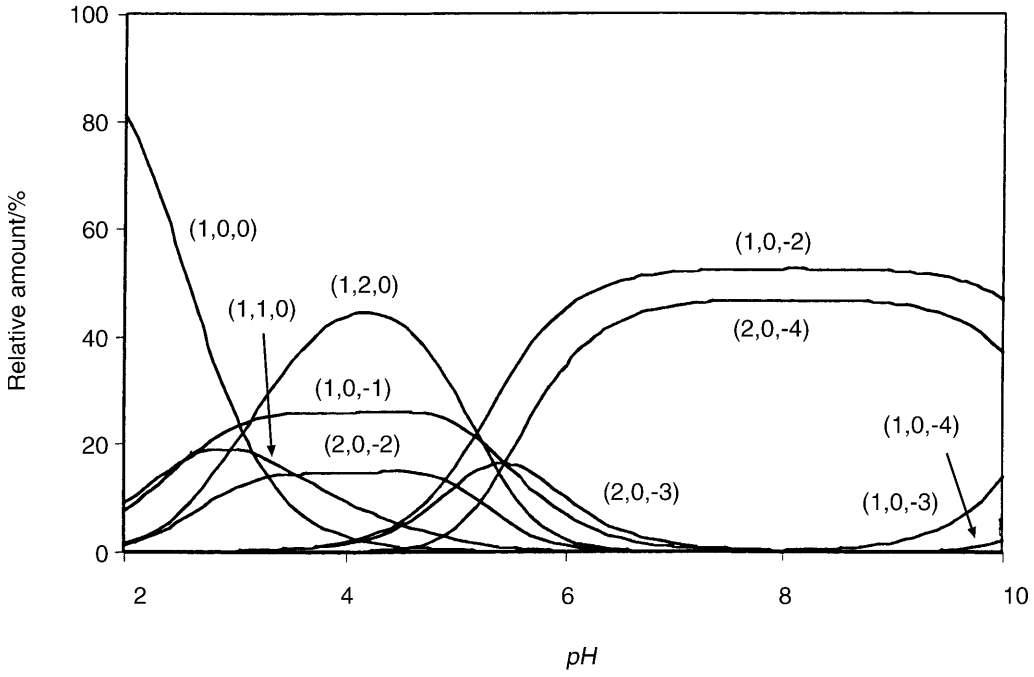

Fig. 5. Concentration distribution of complex species as a function of *pH* in the *DMT*/cytidine system ($[DMT] = 1.25\,\text{mmol}\cdot\text{dm}^{-3}$, $[\text{cytidine}] = 2.5\,\text{mmol}\cdot\text{dm}^{-3}$)

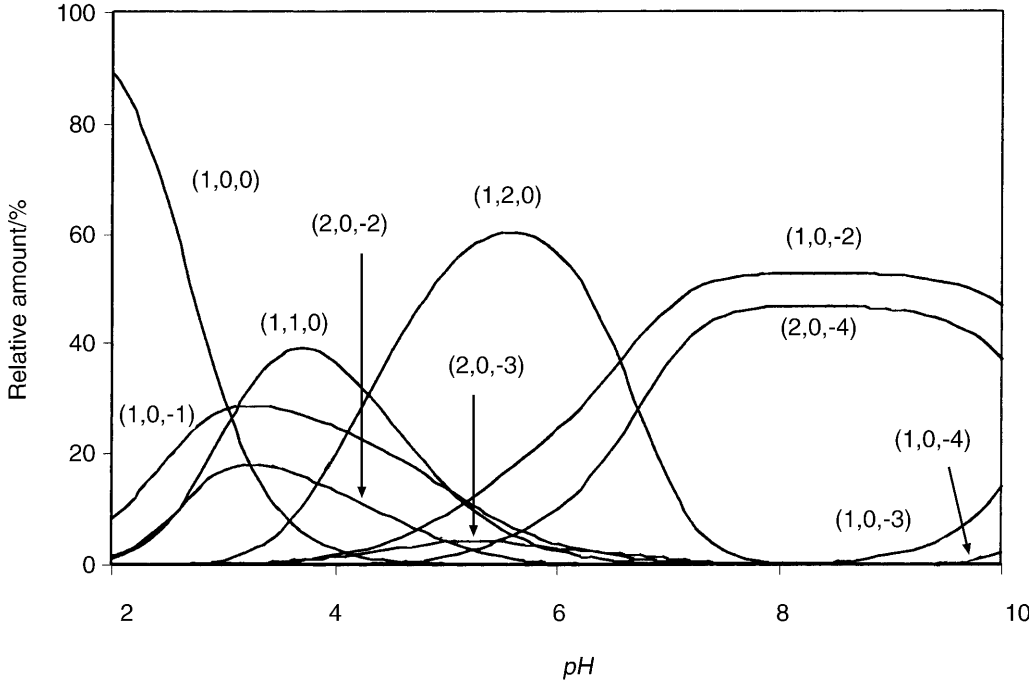

Fig. 6. Concentration distribution of complex species as a function of *pH* in the *DMT*/*5′-AMP* system ($[DMT] = 1.25\,\text{mmol}\cdot\text{dm}^{-3}$, $[5'\text{-}AMP] = 2.5\,\text{mmol}\cdot\text{dm}^{-3}$)

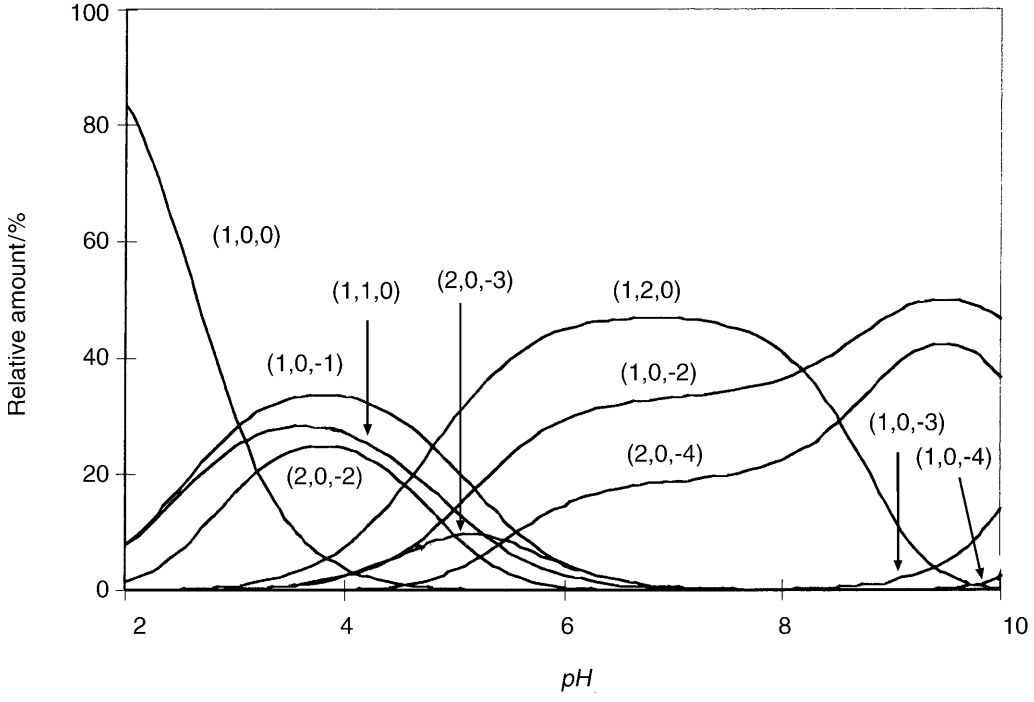

Fig. 7. Concentration distribution of complex species as a function of *pH* in the *DMT*/inosine system ($[DMT] = 1.25\ \text{mmol} \cdot \text{dm}^{-3}$, $[\text{inosine}] = 2.5\ \text{mmol} \cdot \text{dm}^{-3}$)

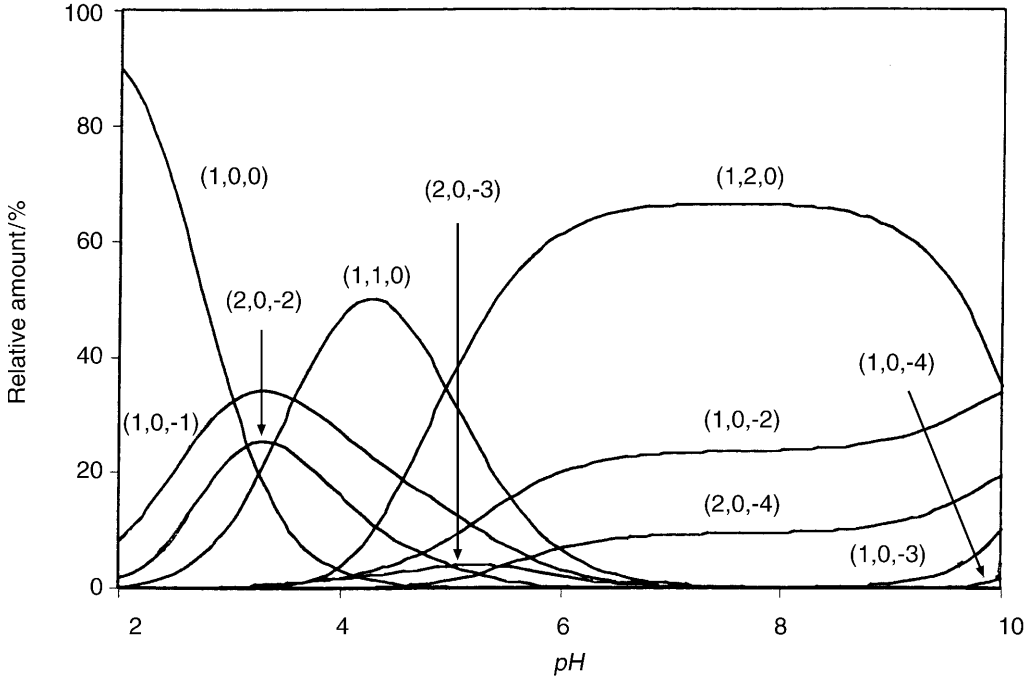

Fig. 8. Concentration distribution of complex species as a function of *pH* in the *DMT*/adenine system ($[DMT] = 1.25\ \text{mmol} \cdot \text{dm}^{-3}$, $[\text{adenine}] = 2.5\ \text{mmol} \cdot \text{dm}^{-3}$)

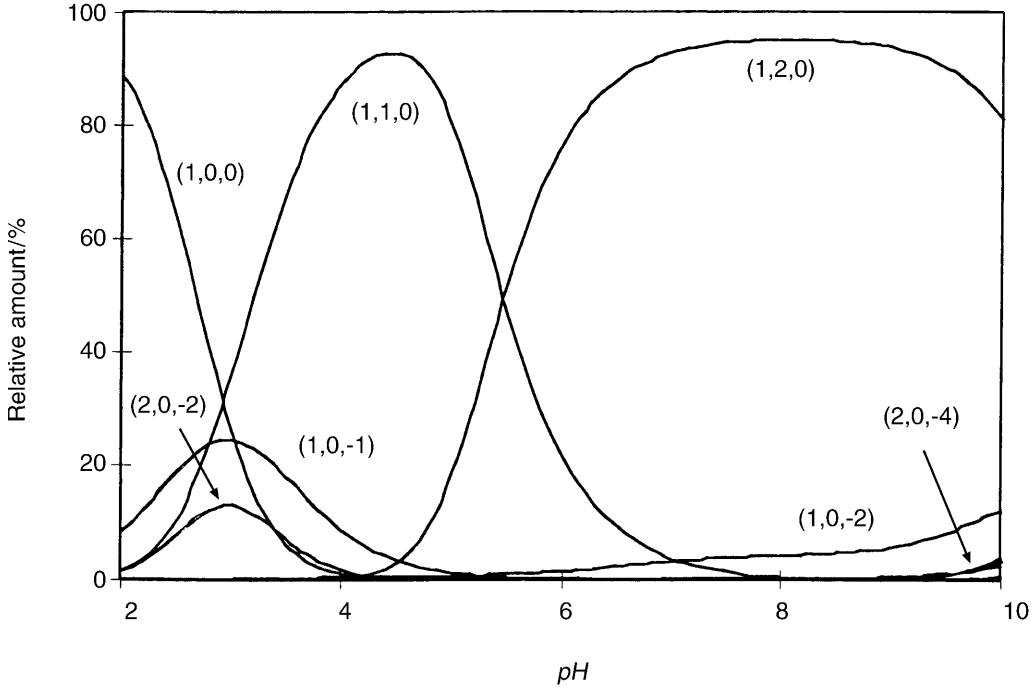

Fig. 9. Concentration distribution of complex species as a function of *pH* in the *DMT*/5′-*IMP* system ($[DMT] = 1.25\ \text{mmol} \cdot \text{dm}^{-3}$, $[5'\text{-}IMP] = 2.5\ \text{mmol} \cdot \text{dm}^{-3}$)

probably due to the presence of both the highly basic base part and the phosphate group. It has been reported that 5′-*GMP* does not prevent the hydrolysis above $pH \approx 7$ [20], probably because species (1, 2, 0), which predominates at this *pH* range, has not been considered in this investigation.

The pyrimidines uracil, thymine, and thymidine have only one basic nitrogen atom in the observed *pH* range (N(3)). Consequently, the pyrimidines ligate in the deprotonated form through this site. The thymine complex is more stable than that of uracil, most probably owing to the higher basicity of N(3) of thymine resulting from the electron-donating methyl group.

Both cytosine and cytidine undergo N(3) protonation under mildly acidic conditions as shown by NMR spectroscopy in solution and by X-ray crystallography in the solid state [23–26]. The pK_a values of the N(3) monocations of cytosine and cytidine are 4.53 and 4.10, respectively. The lower basicity of the nucleoside most probably results from the electron-withdrawing effect of the ribofuranosyl group which reduces the electron density in the cytosine ring. The formation of the complexes is, however, not accompanied with charge neutralization, which apparently results in relatively low stability constants of the corresponding species.

Protonated adenine undergoes proton dissociation from both N(1) and N(9). *Hodgson* [27] and *Marzilli* [28] have discussed complex formation both in solution and the solid state. Evidence has been provided for the point of attachment of adenine to a metal center; it has been frequently reported that N(9) is the coordination site in the dimethyltin(IV) complex. The pK_a of protonated adenosine refers to N(1)–H. This value is lower than that of N(1) of adenine, obviously again

due to the electron-withdrawing sugar moiety of adenosine. The *DMT* complex of adenosine is less stable than that of adenine, most probably because of the availability of the additional binding site N(9). 5′-*AMP* has two protonation sites (phosphate, $pK_a = 6.04$; N(1)–H, $pK_a = 3.28$). The *DMT* complex of 5′-*AMP* is considerably more stable than that of adenosine. Accordingly, the phosphate group is supposed to participate in the binding process.

The coordination geometry in dimethyltin(IV) has shown to be octahedral, the two methyl groups being collinear and located vertically following the rules of the group theory. The ligands are bound to tin at the equatorial sites. It should be recognized that further studies are necessary to elucidate the coordination geometry of organotin(IV) complexes, especially multinuclear NMR measurements. These aspects will be considered in future investigations.

Effect of temperature

The thermodynamic parameters ΔH° and ΔS° were obtained by a linear least-squares fit of ln*K vs.* $1/T$ leading to an intercept at $\Delta S^\circ/R$ and a slope of $-\Delta H^\circ/R$. The results obtained are summarized in Table 2 and interpreted as follows:

a) The formation constant of the hydrolyzed species of *DMT*, β(OH), can be calculated *e.g.* for species (1, 0, −1) as $\log\beta(\mathrm{OH}) = pK_w + \log\beta(1, 0, -1)$. The hydrolysis reactions are accompanied by an endothermic liberation of ordered water of hydration from the reactants and therefore by a significant increase in entropy. However, the ΔH° values in Table 2 have to be considered as the net summation of two opposing effects: the exothermic hydrolysis reaction and the endothermic liberation of ordered water of hydration.

Surprisingly, reaction 1 in Table 2, the formation of (1, 0, −1), is endothermic and displays a very large change in entropy ($\Delta S^\circ = 343.6\,\mathrm{J \cdot K^{-1} \cdot mol^{-1}}$) due to the release of first molecule of water of hydration. This contributes a negative value to the free energy change ($\Delta G^\circ = -62.2\,\mathrm{kJ \cdot mol^{-1}}$). Thus, the endothermic liberation of the strongly bound ordered water molecule exceeds the exothermic hydrolysis reaction. The formation of (1, 0, −2) (reaction 2) has a negative ΔH° ($-11.52\,\mathrm{kJ \cdot mol^{-1}}$); the entropy change is only $127.9\,\mathrm{J \cdot K^{-1} \cdot mol^{-1}}$, indicating that the second water molecule in not as strongly bound as the first one. Therefore, the exothermic hydrolysis reaction exceeds the endothermic liberation of the second water molecule. The net free energy change for reaction 2, $\Delta G^\circ = -49.6\,\mathrm{kJ \cdot mol^{-1}}$, is less negative than that of reaction 1. The formation of (1, 0, −3) (3) and (1, 0, −4) (4) is characterized by similar values of ΔH°, ΔS°, and ΔG°; the same holds for reactions 3 and 4 (formation of (2, 0, −3) and (2, 0, −4)). Reaction 5 (formation of (2, 0, −2)) involves hydrolysis and dimerization with the loss of four ordered water molecules. Although it has a positive ΔH°, it is accompanied by a very large entropy change ($689.5\,\mathrm{J \cdot K^{-1} \cdot mol^{-1}}$) due to loss of ordered water of hydration; therefore, the net contribution to ΔG° is negative ($\Delta G^\circ = -140.5\,\mathrm{kJ \cdot mol^{-1}}$).

b) The protonation reactions 8 and 11 at N(1) of ionsine and 5′-*IMP* are exothermic and of comparable ΔH°. Three factors affect the protonation reactions 8, 11, and 12: *i*) the neutralization reaction, which is an exothermic process, *ii*) desolvation of ions, which is an endothermic process, and *iii*) the change of the configuration and the arrangements of the hydrogen bonds around the free and the

Table 2. Thermodynamic parameters for the equilibria of dimethyltin(IV) complexes[a]

Equilibrium[b]	ΔH° / kJ · mol^{-1}	ΔS° / J · K^{-1} · mol^{-1}	ΔG° / kJ · mol^{-1}
DMT			
1) $M(H_2O)_4^{2+} + OH^- \rightleftharpoons M(H_2O)_3(OH)^+ + H_2O$	40.2 (0.5)	344 (2)	−62.2 (1.0)
2) $M(H_2O)_3(OH)^+ + OH^- \rightleftharpoons M(H_2O)_2(OH)_2 + H_2O$	−11.5 (0.2)	128 (1)	−49.6 (0.9)
3) $M(H_2O)_2(OH)_2 + OH^- \rightleftharpoons M(H_2O)(OH)_3^- + H_2O$	−21.4 (0.4)	−7 (1)	−19.3 (0.4)
4) $M(H_2O)(OH)_3^- + OH^- \rightleftharpoons M(OH)_4^{2-} + H_2O$	−20.9 (0.4)	−4 (1)	−19.6 (0.4)
5) $2M(H_2O)_4^{2+} + 2OH^- \rightleftharpoons M_2(H_2O)_4(OH)_2^{2+} + 4H_2O$	65.0 (0.8)	690 (7)	−140.5 (1.5)
6) $M_2(H_2O)_4(OH)_2^{2+} + OH^- \rightleftharpoons M_2(H_2O)_3(OH)_3^+ + H_2O$	−13.0 (0.3)	127 (1)	−50.7 (0.9)
7) $M_2(H_2O)_3(OH)_3^+ + OH^- \rightleftharpoons M_2(H_2O)_2(OH)_4 + H_2O$	−6.1 (0.1)	142 (1)	−48.5 (0.8)
Inosine			
8) $L^- + H^+ \rightleftharpoons LH$	−35.0 (0.5)	46 (1)	−48.8 (0.7)[c]
9) $M^{2+} + L^- \rightleftharpoons ML^+$	139.8 (1.1)	626 (6)	−46.8 (0.6)
10) $ML^+ + L^- \rightleftharpoons ML_2$	−60.7 (0.9)	−77 (1)	−37.9 (0.6)
5′-IMP			
11) $L^{3-} + H^+ \rightleftharpoons LH^{2-}$	−33.0 (0.4)	65 (1)	−52.2 (0.9)[c]
12) $LH^{2-} + H^+ \rightleftharpoons LH_2^-$	0.32 (0.01)	115 (1)	−33.8 (1.2)[c]
13) $M^{2+} + L^{3-} \rightleftharpoons ML^-$	129.6 (1.3)	664 (6)	−68.1 (0.8)
14) $ML^- + L^{3-} \rightleftharpoons ML_2^{4-}$	−8.5 (0.3)	115 (1)	−42.7 (0.7)

[a] For abbreviations, see footnote to Table 1; standard deviations are given in parentheses; [b] stepwise formation constants; [c] values from Ref. [14]

protonated ligands. The phosphate ion in *5′-IMP* is more solvated than its protonated form. Consequently, it contributes more to the endothermic process upon protonation, and the net ΔH° is only 0.32 kJ · mol^{-1}. Also, the phosphate ions form ordered hydrogen bonds with water molecules, which is confirmed by the large entropy change of $\Delta S^\circ = 114.6$ J · K^{-1} · mol^{-1}. This leads to a negative free energy change $\Delta G^\circ = -33.8$ kJ · mol^{-1}. Positive entropies due to the release of ordered water molecules and the breaking of hydrogen bonds have been observed by *Kramer-Schnabel et al.* for protonation and complexation reactions of organic monophosphates and copper ions [29].

c) Surprisingly the complexation reactions 9 and 13 between *DMT* and inosine and *5′-IMP* are endothermic with ΔH° values of 139.8 and 129.6 kJ · mol^{-1}, respectively. This is similar to what was found by *Kramer-Schnabel* [29] and can be interpreted as above by assuming that the enthalpy change is a net summation of two opposing effects, *i.e.* the exothermic complexation and the endothermic liberation of ordered water of hydration. This is confirmed by very large ΔS° values of 626.2 and 663.5 J · K^{-1} · mol^{-1} for reactions 9 and 13, respectively, giving negative ΔG° values (−46.8 and −68.1 kJ · mol^{-1} for inosine and *5′-IMP* complexes with *DMT*, respectively).

Effect of solvent

Traditionally, water has been considered as the solvent which best represents biological conditions. Although this is a general assumption, a lower polarity has been detected in some biochemical micro-environments, such as active sites of enzymes and side chains in proteins, sometimes hidden in low-dielectric cavities [30–34]. In these cases, the selection of other solvents seems recommendable in order to simulate the real properties of the medium. The results of a careful examination of the medium effect on the equilibrium constants is summarized in Table 3.

a) logβ(0, 1, 1) of inosine (N(1)–H) and logβ(0, 1, 1) and logβ(0, 1, 2) of 5′-*IMP* (N(1)–H) and (N(1)–H and P–OH) as well as the hydrolysis constants of *DMT* increase linearly with increasing amount of dioxane in the medium (Figs. 10 and 11). This may be correlated with the ability of a solvent of relatively low dielectric constant to increase the electrostatic forces between protons and ligand anions in the case of ligand dissociation and those between the proton and the hydrolyzed form of organotin(IV).

b) The variation of the stability constants of *DMT* complexes with inosine and 5′-*IMP* as a function of solvent composition is shown in Figs. 12 and 13. The stability constants of the 1:1 and 1:2 complexes (β(1, 1, 0) and β(1, 2, 0)) with inosine and the stability constant of the 1:1 complex (β(1, 1, 0)) with 5′-*IMP* increase with increasing amount of dioxane. On the other hand, β(1, 2, 0) of the 5′-*IMP* complex decreases with increasing dioxane proportion. The explanation may be that the 1:1 complex with 5′-*IMP* is negatively charged; consequently, the formation of the 1:2 complex does not involve neutralization of charges as is the case for the 1:2 complex with inosine.

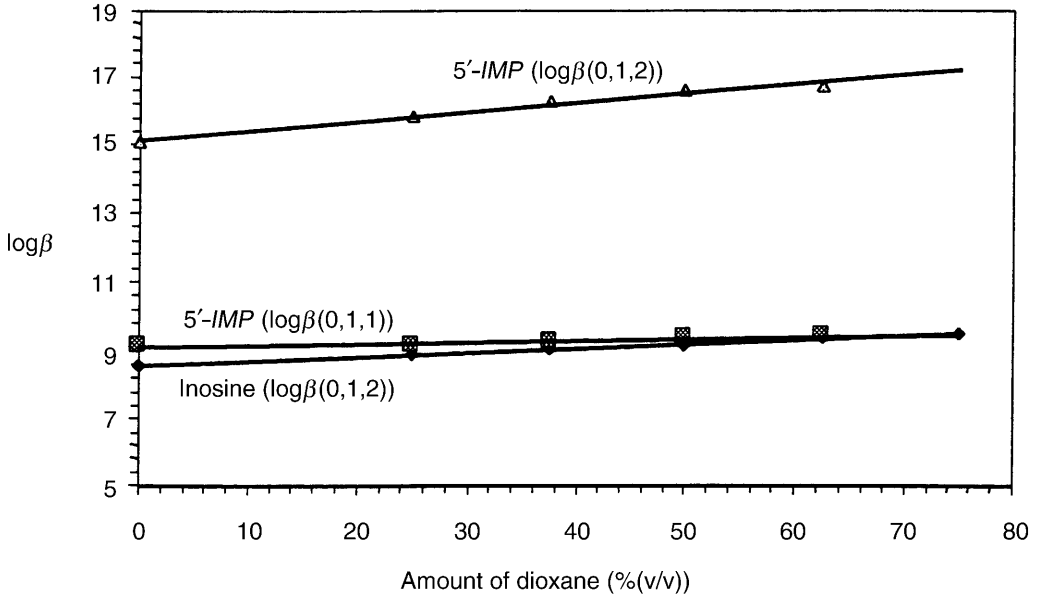

Fig. 10. Effect of dioxane on logβ of inosine and 5′-*IMP*

Table 3. Formation constants of *DMT* complexes in dioxane-water solutions of different compositions; for the meaning of symbols, cf. footnote to Table 1

System	Dioxane (%(v/v))	l	p	q	$\log\beta$	S
DMT	25	1	0	−1	−3.39 (0.00)	1.0×10^{-8}
		1	0	−2	−8.99 (0.01)	
		1	0	−3	−19.83 (0.01)	
		1	0	−4	−31.30 (0.01)	
		2	0	−2	−3.92 (0.01)	
		2	0	−3	−9.27 (0.04)	
		2	0	−4	−15.11 (0.03)	
Inosine		0	1	1	8.85 (0.00)	5.3×10^{-9}
		1	1	0	7.49 (0.03)	2.2×10^{-10}
		1	2	0	14.41 (0.02)	
5′-*IMP*		0	1	1	9.12 (0.00)	2.6×10^{-9}
		0	1	2	15.83 (0.01)	
		1	1	0	12.50 (0.04)	5.1×10^{-8}
		1	2	0	19.25 (0.08)	
DMT	37.5	1	0	−1	−3.53 (0.01)	1.4×10^{-8}
		1	0	−2	−9.23 (0.01)	
		1	0	−3	−20.18 (0.02)	
		1	0	−4	−31.83 (0.01)	
		2	0	−2	−4.17 (0.01)	
		2	0	−3	−9.61 (0.03)	
		2	0	−4	−15.59 (0.03)	
Inosine		0	1	1	9.04 (0.00)	2.7×10^{-9}
		1	1	0	7.68 (0.03)	4.4×10^{-10}
		1	2	0	14.62 (0.02)	
5′-*IMP*		0	1	1	9.24 (0.00)	9.3×10^{-10}
		0	1	2	16.28 (0.00)	
		1	1	0	12.73 (0.05)	6.0×10^{-8}
		1	2	0	19.30 (0.09)	
DMT	50	1	0	−1	−3.60 (0.01)	2.8×10^{-8}
		1	0	−2	−9.43 (0.01)	
		1	0	−3	−20.58 (0.02)	
		1	0	−4	−32.52 (0.02)	
		2	0	−2	−4.30 (0.01)	
		2	0	−3	−9.78 (0.04)	
		2	0	−4	−16.03 (0.04)	
Inosine		0	1	1	9.14 (0.01)	1.6×10^{-8}
		1	1	0	7.88 (0.03)	7.7×10^{-10}
		1	2	0	14.98 (0.02)	
5′-*IMP*		0	1	1	9.35 (0.00)	5.0×10^{-9}
		0	1	2	16.63 (0.01)	
		1	1	0	12.98 (0.05)	7.0×10^{-8}
		1	2	0	19.23 (0.09)	
DMT	62.5	1	0	−1	−3.68 (0.01)	8.7×10^{-8}
		1	0	−2	−9.62 (0.02)	
		1	0	−3	−20.89 (0.04)	

Table 3 (*continued*)

System	Dioxane (%(v/v))	*l*	*p*	*q*	log β	*S*
		1	0	−4	−33.26 (0.05)	
		2	0	−2	−4.47 (0.01)	
		2	0	−3	−9.90 (0.03)	
		2	0	−4	−16.51 (0.04)	
Inosine		0	1	1	9.36 (0.00)	5.1×10^{-9}
		1	1	0	8.13 (0.03)	1.5×10^{-9}
		1	2	0	15.31 (0.03)	
5′-IMP		0	1	1	9.39 (0.00)	9.9×10^{-10}
		0	1	2	16.72 (0.00)	
		1	1	0	13.21 (0.06)	9.3×10^{-8}
		1	2	0	19.07 (0.11)	
DMT	75	1	0	−1	−3.98 (0.02)	1.4×10^{-7}
		1	0	−2	−9.93 (0.05)	
		1	0	−3	−21.35 (0.09)	
		1	0	−4	−33.74 (0.08)	
		2	0	−2	−4.90 (0.01)	
		2	0	−3	−10.10 (0.01)	
		2	0	−4	−16.96 (0.02)	
Inosine		0	1	1	9.48 (0.00)	6.9×10^{-9}
		1	1	0	8.35 (0.04)	4.2×10^{-9}
		1	2	0	15.61 (0.03)	

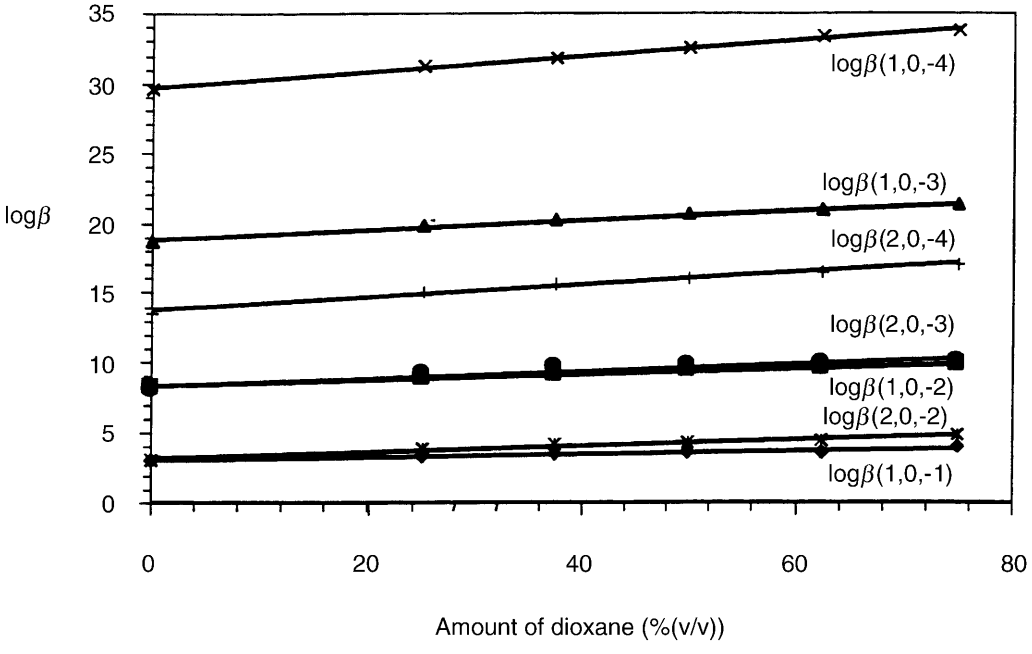

Fig. 11. Effect of dioxane on logβ of the system *DMT*/OH

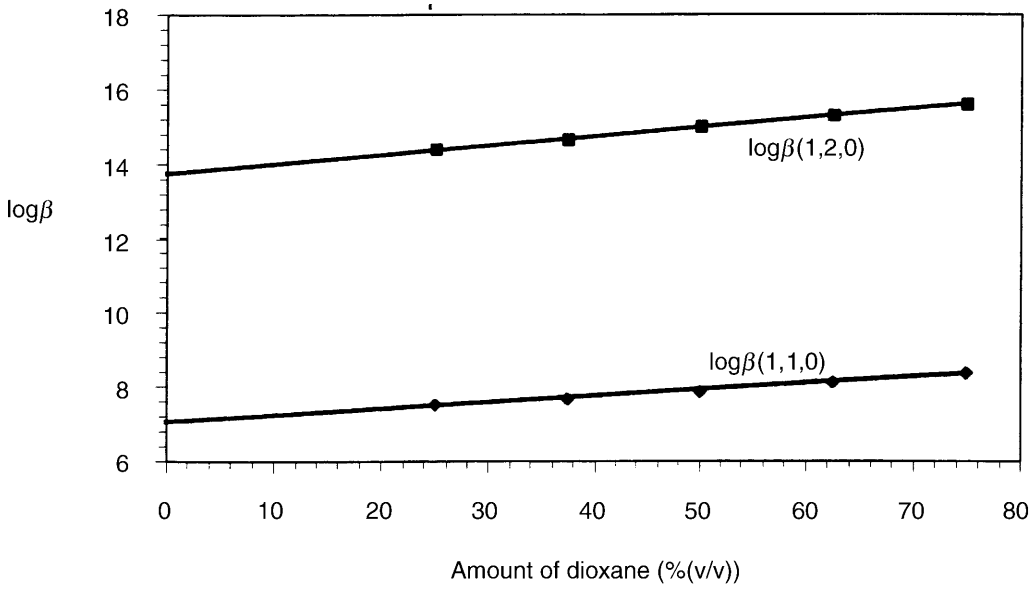

Fig. 12. Effect of dioxane on logβ of the system *DMT*/inosine

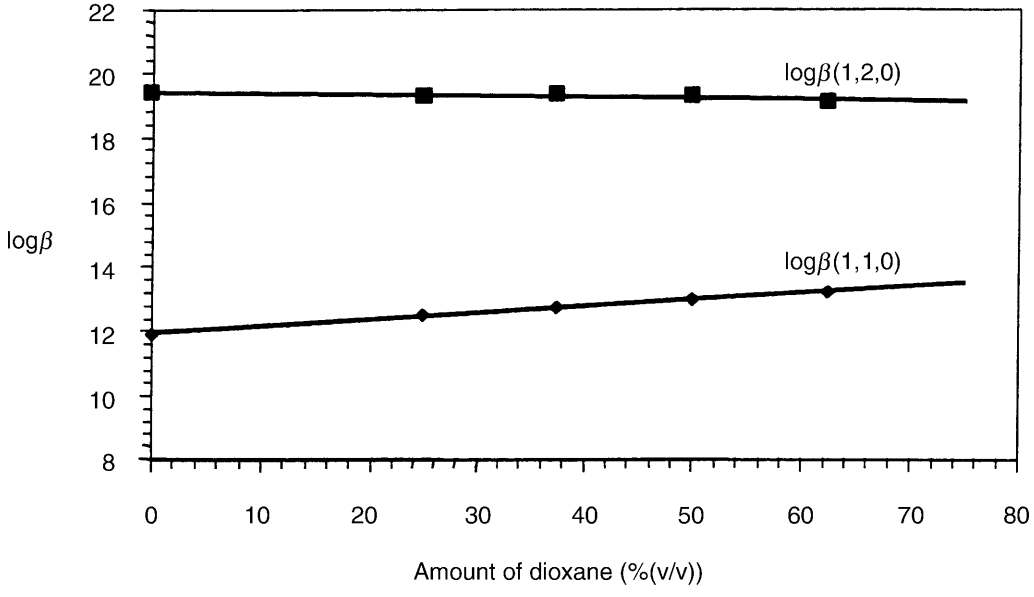

Fig. 13. Effect of dioxane on logβ of the system *DMT*/5′-*IMP*

Experimental

Materials and reagents

Dimethyltin(IV) dichloride (*DMT*) was obtained from Merck. The ligands (inosine, inosine 5′-monophosphate (5′-*IMP*), gauanosine 5′-monophosphate (5′-*GMP*), adenine, adenosine, adenosine 5′-monophosphate (5′-*AMP*), uracil, thymine, thymidine, cytosine, and cytidine) were purchased

from Sigma. 1,4-Dioxane was provided by Aldrich. NaOH stock solutions were prepared by diluting the content of BDH concentrated volumetric solution vials appropriately. These solutions were systematically checked by titration against potassium hydrogen phthalate.

Procedures and measuring techniques

The potentiometric titrations were performed using a Metrohm 686 titroprocessor equipped with a 665 dosimat (Herisau, Switzerland). The titroprocessor and the electrode were calibrated with standard buffer solutions prepared according to NBS specifications [35]. The titrations were carried out in a purified nitrogen atmosphere using a titration vessel described previously [36]. The temperature was maintained constant by a Colora ultrathermostat.

The protonation constants of the ligands were determined by titrating 40 cm^3 of a $2.5 \times 10^{-3}\,M$ ligand solution. The hydrolysis constants of *DMT* were determined by titrating 40 cm^3 of a $2.5 \times 10^{-3}\,M$ dimethyltin(IV) dichloride solution. The formation constants of organotin(IV) complexes were determined by titrating 40 cm^3 of a solution containing the ligand ($2.5 \times 10^{-3}\,M$) with dimethyltin(IV) solutions (1.00×10^{-2}, 5.00×10^{-3}, 1.25×10^{-3}, $6.25 \times 10^{-4}\,M$). Cytosine, cytidine, 5′-*IMP*, 5′-*GMP*, adenine, and 5′-*AMP* solutions were prepared in the protonated form by dissolution in an equimolar solution of nitric acid. The titrations were performed at different temperatures and in dioxane-H_2O solutions of different compositions. The ionic strength was adjusted to $0.1\,M$ by $NaNO_3$. pK_w values in dioxane-H_2O solutions were determined as described previously [37]. For this purpose, various amounts of standard NaOH solution were added to a $0.10\,M$ $NaNO_3$ solution. [OH] was calculated from the amount of base added, [H] was calculated from the *pH* value. The values obtained in this way for log[OH][H] ($\log K_w$) are −14.23, −14.50, −14.92, −15.12, and −15.63 for 25.0, 37.5, 50.0, 62.5, and 75.0% dioxane in H_2O, respectively.

The equilibrium constants were evaluated from titration data according to Eqs. (1) and (2).

$$l(M) + p(L) + q(\mathrm{H}) \rightleftharpoons (M)_l(L)_p(\mathrm{H})_q \tag{1}$$

$$\beta(l,p,q) = \frac{[(M)_l(L)_p(\mathrm{H})_q]}{[M]^l[L]^p[\mathrm{H}]^q} \tag{2}$$

The calculations were performed by means of an IBM 486 computer using the computer program MINIQUAD-75 [38]. The stoichiometries and stability constants of the complexes formed were determined by trying various possible composition models. The model selected gave the best statistical fit and was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals, as described elsewhere [38]. The fitted model was tested by comparing the experimental titration data points and the theoretical curve calculated from the values of acid dissociation constants of the ligands and the formation constants of the corresponding complexes. Tables 1 and 3 lists the formation constants together with their standard deviations and the sum of square of residuals as obtained from the program MINIQUAD-75. The concentration distribution diagrams were obtained using the program SPECIES [39].

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