

INTERACTION OF $\text{Et}_2\text{SnCl}_2(\text{phen})$ WITH NUCLEOTIDESLi Qingshan^{a,*}, Jin Nan^b, Yang Pin^b and Wan Jindong^b

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ABSTRACT

The syntheses of mixed-ligand complexes formulated as $\text{Et}_2\text{Sn}(\text{phen})(\text{AMP})\text{Cl}$ and $\text{Et}_2\text{Sn}(\text{phen})(\text{CMP})\text{Cl}$ as well as $\text{Et}_2\text{Sn}(\text{phen})(\text{CMP})\text{Cl}$ by reactions of the antitumor agent $\text{Et}_2\text{SnCl}_2(\text{phen})$ with equimolar amounts of nucleotides under biologically relevant conditions are described. The new complexes are characterized by microanalyses, UV, IR and ^1H and ^{31}P NMR spectra. These spectroscopic data suggest that one of the *cis*-chloro atoms in $\text{Et}_2\text{SnCl}_2(\text{phen})$ is substituted by a nucleotide and that Sn(IV) directly coordinates a phosphate group of the nucleotide.

INTRODUCTION

Since *cis*-diamminedichloroplatinum(II) (*cis*-platin) was first reported to exhibit a wide spectrum of antitumor activity¹, many metal complexes have attracted much attention as a new type of potential anticancer

drugs. Among them, some diorganotin(IV) complexes of the type $R_2Sn(N-N)X_2$ (where X is halide or pseudohalide, N-N is a chelating ligand) were found to be effective against P388 cells in mice².

A knowledge of tin-nucleoside or tin-nucleotide binding is fundamental to an assessment of the antitumor properties of organotin compounds. It has been generally assumed that the eventually coordinated organic ligand would facilitate the transport of the complexes across cell membranes, while the antitumor activity would be exerted by the dissociated diorganotin(IV) moieties³. These would interact with nucleic acids in a manner somewhat analogous to that shown by *cis*-platin which has been widely investigated⁴. In fact, the $R_2Sn(N-N)X_2$ type complexes were originally designed to structurally emulate *cis*-platin⁵, and there must be a preferable binding site on the nucleic acid, which can be tentatively characterized by suitable studies of model systems. So, the importance of detailed studies for the binding of $R_2Sn(N-N)X_2$ to nucleic acid constituents is evident for understanding the antitumor mechanism of this type compounds. But so far, to our knowledge, the interactions between $R_2Sn(N-N)X_2$ -type complexes and nucleotides or nucleosides are still largely unknown. In our previous work on this area⁶, we investigated the reaction between Et_2SnCl_2 (phen) and 5-fluorouracil which is structurally analogous to nucleoside. In this paper, we extend our investigation to the reaction of Et_2SnCl_2 (phen) with nucleotides in aqueous media and try to elucidate the aqueous coordination chemistry of this type of diorganotin(IV) drugs as well as its DNA binding model.

EXPERIMENTAL

All reagents and solvents were reagent grade and were used without further purification. Et_2SnCl_2 (phen) was prepared and purified according

to the literature method⁷. The nucleotides, adenosine 5'-monophosphate (AMP), cytidine 5'-monophosphate (CMP) and guanosine 5'-monophosphate (GMP) were purchased from Sigma and used without further purification. Carbon, hydrogen and nitrogen contents were determined by a Perkin-Elmer 240c instrument and chloride was determined with silver chloride. Tin was determined gravimetrically as SnO_2 . UV spectra were recorded on a Shimadzu UV-365 spectrophotometer. IR spectra were recorded on a Perkin-Elmer-1700 FT-spectrophotometer ($4000\sim 500\text{ cm}^{-1}$) with KBr discs and on a Perkin-Elmer-983 spectrophotometer with CsI plates ($500\sim 200\text{ cm}^{-1}$). ^1H and ^{31}P NMR spectra were recorded on a Bruker AM-500 MHz NMR spectrometer.

Synthesis of $\text{Et}_2\text{Sn}(\text{phen})(\text{AMP})\text{Cl}$ (I)

$\text{Et}_2\text{SnCl}_2(\text{phen})$ (1 mmol, 0.43 g) was dissolved in deionized water (50 mL) and AMP (1 mmol, 0.35 g) was dissolved in deionized water (50 mL). The two solutions were mixed and stirred at room temperature for 12 h, and then the pH of the solution was adjusted to 5.0 by adding 0.1 mol/L sodium hydroxide solution slowly and carefully with stirring. The reaction went on for 48 h. This slight-turbid solution was filtered. The filtrate was slowly concentrated to about 20 mL under low pressure at $50\sim 60\text{ }^\circ\text{C}$, and the white solid thus obtained was filtered, washed with ice-water, methanol and dry ether sequentially and finally dried over P_2O_5 in vacuum, yield 0.38 g (51.4 %). Calc. for $\text{Et}_2\text{Sn}(\text{phen})(\text{AMP})\text{Cl}$ ($\text{C}_{26}\text{H}_{31}\text{O}_7\text{N}_7\text{ClPSn}$, FW. 738.5): C, 42.25; H, 4.20; N, 13.28; Cl, 4.81; Sn, 16.07 %. Found: C, 41.60; H, 4.49; N, 12.91; Cl, 4.90; Sn, 16.21 %; m.p. $210.4\sim 213.0\text{ }^\circ\text{C}$ (dec).

Synthesis of $\text{Et}_2\text{Sn}(\text{phen})(\text{CMP})\text{Cl}$ (II)

This white complex was prepared from $\text{Et}_2\text{SnCl}_2(\text{phen})$ (1 mmol, 0.43 g) and CMP (1 mmol, 0.32 g) essentially as for complex (I)

at pH = 6.0, yield 0.46 g (64.5 %). Calc. for $\text{Et}_2\text{Sn}(\text{phen})(\text{CMP})\text{Cl}$ ($\text{C}_{26}\text{H}_{31}\text{O}_8\text{N}_5\text{ClPSn}$, FW. 714.5): C, 41.99; H, 4.34; N, 9.80; Cl, 4.97, Sn, 16.61 %. Found: C, 41.89; H, 4.75; N, 9.57; Cl, 4.82, Sn, 16.48 %; m.p. 205.3~206.3 °C (dec).

Synthesis of $\text{Et}_2\text{Sn}(\text{phen})(\text{GMP})\text{Cl}$ (III)

Solutions of $\text{Et}_2\text{SnCl}_2(\text{phen})$ (1 mmol, 0.43 g) in deionized water (50 mL) and GMP (1 mmol, 0.36 g) in 50 mL deionized water were mixed and stirred at room temperature for 12 h. When the pH was adjusted to 6.0 by adding 0.1 mol/L sodium hydroxide solution, a white precipitate was formed immediately. The reaction went on for 10 h, and then the precipitate was washed with ice-water, methanol and dry ether sequently, and then dried under vacuum, yield 0.62 g (82.4 %). Calc. for $\text{Et}_2\text{Sn}(\text{phen})(\text{GMP})\text{Cl}$ ($\text{C}_{26}\text{H}_{31}\text{O}_8\text{N}_7\text{ClPSn}$, FW. 754.5): C, 41.35; H, 4.11; N, 12.99; Cl, 4.71; Sn, 15.73 %. Found: C, 40.96; H, 4.19; N, 12.65; Cl, 4.78, Sn, 15.82 %; m.p. >300 °C (dec).

RESULTS AND DISCUSSION

The complexes (I) and (II) are slightly soluble in water. However, the complex (III) is neither soluble in water nor in the usual organic solvents, but is soluble in DMSO.

UV spectra of complexes (I) and (II) give absorption peaks at about 220 and 260 nm in deionized water, while under the same conditions, values of $\lambda_{\text{max}} = 224$ and 262 nm which are produced by a phenanthroline group are found for a solution of $\text{Et}_2\text{SnCl}_2(\text{phen})$. This indicates that complexes (I) and (II) still contain the phenanthroline ligand.

IR Data

IR spectral data and assignments for the nucleotides and new complexes are summarized in Tables I - III. It should be noted that our discussion about nucleotides as well as $\text{Et}_2\text{SnCl}_2(\text{phen})$ is largely based on the assignments made by Lord and Tomas⁹, Tajmir-Riahi and Theophanides¹⁰ and the normal coordinate analysis carried out by Tsuboi et al¹¹ and is in good agreement with the observations of these authors.

For the AMP derivative, no evidence is found for both N(1) and N(7) coordination to Sn(IV) by its IR spectrum. It has been suggested that N(1)-coordination causes a split of the absorption band¹⁰ at 1598 cm^{-1} . Such a split does not occur in the spectrum of complex (I), indicating that N(1) does not bond to Sn(IV). The bands at 1503 and 1470 cm^{-1} in the spectra of AMPNa_2 are assigned to the $\text{C}_8\text{-H}$ and $\text{N}_7\text{-C}_8$ stretching frequencies of the imidazole ring and these bands are neither shifted significantly nor exhibit great changes in their intensity. In addition, the bands at 1378 and 1300 cm^{-1} associated with vibrations of the imidazole ring also do not show considerable changes and these indicate that N(7) also does not coordinate Sn(IV).

For the CMP and GMP derivatives, N(7) coordination of GMP and N(3) coordination of CMP could also be ruled out by comparing IR data of free nucleotides with those of their complexes. Characteristic ring vibrations of the free nucleotides are very slightly affected, and thus, coordinations through N(7) of GMP and N(3) of CMP are unlikely. In all complexes the $\nu_{\text{Sn-N}}$ vibrations due to the Sn(IV) bonding through the nitrogen atom of the nucleotide are not obtained, because there is no band⁹ at about 235 cm^{-1} . Instead, the three new complexes still show only one $\nu_{\text{Sn-N}}$ band at about 418 cm^{-1} which is assigned to Sn(IV) bonding through the nitrogen atom of the phenanthroline ligand⁶. Moreover, the characteristic ring bands

TABLE I. IR Bands (cm^{-1}) for AMPNa_2 and its Complex.

Tentative assignment	AMPNa_2	$\text{Et}_2\text{Sn}(\text{phen})(\text{AMP})\text{Cl}$
$\nu \text{C}=\text{N} + \delta \text{NH}_2$	1690 vs	1680 m, 1680 s
$\nu \text{C}=\text{N} + \nu \text{C}=\text{C} + \delta \text{NH}_2$	1645 s	1640 vs
$\nu \text{C}=\text{N} + \nu \text{C}=\text{C}$	1598 s	1599 s
$\nu \text{C}_8-\text{N}_7 + \delta \text{C}_8-\text{H}$	1503 w	1505 m
$\delta \text{C}_8-\text{N}_9 + \nu \text{C}_8-\text{N}_9 + \delta \text{C}_8-\text{H}$	1470 s	1472 s
$\nu \text{N}_1-\text{C}_6-\text{N}_6$	1420 m	1425 s
ν pyrimidine ring	1378 m	1371 m
$\nu \text{C}_5-\text{N}_9 + \nu \text{C}_2-\text{N}_3 + \nu \text{C}_5-\text{N}_7$	1334 m	1330 m
$\nu \text{C}_9-\text{H} + \nu \text{N}_7-\text{N}_8$	1300 m	1300 m
$\delta \text{C}-\text{O}$ (sugar)	1145 s	1148 s
$\nu \text{PO}_3^{\ominus}$ deg.	1092 bs	1099 vs
$\nu \text{PO}_3^{\ominus}$ sym.	976 vs	1002 s, 961 m
$\nu \text{P}-\text{O}$	797 s	798 m
ν ring (phen)		727 s
$\nu \text{PO}_3^{\ominus}$ sym., deg.	540 m	539 m
$\nu \text{Sn}-\text{Cl}$		521 w
		509 w
$\nu \text{Sn}-\text{O}$		465 m
$\nu \text{Sn}-\text{N}$		418 m
$\nu \text{Sn}-\text{Cl}$		230 m

s = Strong; m = medium; b = broad; w = weak; v = very; ν = stretching; δ = bending; deg. = degenerate; sym. = symmetry.

of phenanthroline are shifted from 739 cm^{-1} in $\text{Et}_2\text{SnCl}_2(\text{phen})$ to about 725 cm^{-1} in these complexes. For $\text{Et}_2\text{SnCl}_2(\text{phen})$, there are two bands at 242 cm^{-1} and 220 cm^{-1} assigned to be Sn-Cl stretching vibrations⁴¹ which disappear after reaction giving, instead, a single stretching vibration for Sn-Cl in the $227 \sim 230 \text{ cm}^{-1}$ range for the complexes. These indicate that one of the *cis*-chloro atoms in $\text{Et}_2\text{SnCl}_2(\text{phen})$ has been replaced by nucleotides.

TABLE I. IR Bands (cm^{-1}) for CMPNa_2 and its Complex.

Tentative assignment	CMPNa_2	$\text{Et}_2\text{Sn}(\text{phen})(\text{CMP})\text{Cl}$
$\nu \text{C}=\text{O} + \delta \text{NH}_2$	1710 s	1698 s
$\delta \text{NH}_2 + \nu \text{C}=\text{N}$	1660 sh	1648 s
$\delta \text{N}-\text{H}$	1540 m	1518 s
$\nu \text{C}_4-\text{N}_3 + \nu \text{C}_4-\text{N}_4 + \nu \text{C}_2-\text{N}_3$	1495 s	1491 s
	1428 m	1425 m
$\nu (\text{C}-\text{C}) + \nu \text{C}-\text{N} + \delta \text{C}-\text{C}-\text{H}$	1377 m	1374 sh
	1333 w	1345 w
$\nu \text{C}_2-\text{N}_1 + \nu \text{C}_3-\text{N}_1 + \nu \text{C}_4-\text{N}_3$	1281 m	1284 m
$\nu \text{C}-\text{O}$ (sugar)	1163 s	1197 s
νPO_3^{2-} deg.	1081 bs	1111 s
		1062 s
νPO_3^{2-} sym.	987 s	985 s
		955 sh
$\nu \text{P}-\text{O}$	810 m	852 m
ν ring of phen		727 s
νPO_3^{2-} asym., deg.	583 m	587 m
$\nu \text{Sb}-\text{C}$		543 m
		508 m
$\nu \text{Sn}-\text{O}$		470 m
$\nu \text{Sn}-\text{N}$		418 m
$\nu \text{Sn}-\text{Cl}$		227 m

s = Strong; m = medium; b = broad; sh = shoulder; w = weak; v = very; ν = stretching; δ = bending; sym. = symmetry; deg. = degenerate.

TABLE III. IR Bands (cm^{-1}) for GMPNa_2 and its Complex.

Tentative assignment	GMPNa_2	$\text{Et}_2\text{Sn}(\text{phen})(\text{GMP})\text{Cl}$
$\nu \text{C}_6\text{-O} + \nu \text{C}_6\text{-C}_5$	1697 s	1688 vs
$\delta \text{NH}_2 + \nu \text{C}_2\text{-N}_2$	1652 sh	1632 vs
$\nu \text{C}_4\text{-N}_3 + \nu \text{C}_4\text{-C}_5 + \nu \text{C}_5\text{-N}_7$	1598 s	1604 sh
$\nu \text{C}_4\text{-N}_6 + \nu \text{C}_6\text{-O} + \nu \text{C}_2\text{-N}_1$	1531 m	1533 m
$\delta \text{C}_8\text{-H} + \nu \text{C}_8\text{-N}_7$	1480 m	1482 m
ν (pyrimidine ring)	1358 m	1360 m
$\nu \text{C}_8\text{-N}_1 + \nu \text{C}_5\text{-N}_7$	1254 sh	1251 m
$\delta \text{C}_8\text{-H} + \nu \text{C}_8\text{-N}_7$	1208 m	1197 m
$\nu \text{C-O}$ (sugar)	1123 m	1116 m
νPO_3^{2-} deg.	1076 bs	1086 vs 1016 sh
νPO_3^{2-} sym.	972 s	991 s 903 m
$\nu \text{P-O}$	805 m	815 m
ν phen		730 s
νPO_3^{2-} asym., def.	540 m	544 m
$\nu \text{Sn-C}$		525 m 508 m
$\nu \text{Sn-O}$		462 m
$\nu \text{Sn-N}$		420 m
$\nu \text{Sn-Cl}$		228 w

s = Strong; m = medium; b = broad; sh = shoulder; w = weak;
 ν = very; ν = stretching; δ = bending; sym. = symmetry; def. =
 deformation; deg. = degenerate.

By comparing the free nucleotides with their complexes, there are considerable changes in the $1100\sim 790\text{ cm}^{-1}$ region of their IR spectra which are assigned to the vibrations of phosphate groups including νPO_3^{2-} (deg.) in the $1076\sim 1092\text{ cm}^{-1}$ range, νPO_3^{2-} (sym.) in the $972\sim 987\text{ cm}^{-1}$ region and $\nu\text{P-O}$ at about 800 cm^{-1} . These bands are either shifted and split or decreased in intensities. These large perturbations suggest direct Sn-phosphate coordination^{10,12}. For all complexes, a new band appears in the $462\sim 470\text{ cm}^{-1}$ range which may be tentatively assigned to be the Sn-O stretching vibration.

As to $\nu(\text{NH}_2)$, $\delta(\text{NH}_2^+)$ and $\nu(\text{C=O})$ of the nucleotides, small shifts of these vibrations in their complexes may be due to the presence of different degrees of hydrogen bonding in the solid state¹³. So far, no crystal suitable for X-ray diffraction has yet been obtained, but tentative IR assignments of both symmetric and antisymmetric stretching vibrations for Sn-C are consistent with non-linear C-Sn-C, which indicates that the diethyltin coordination probably transposes a distorted octahedron which is the general case for six-coordinated diorganotin compounds. Thus, the nucleotide coordinates unidentately Sn(IV) through the phosphate group.

¹H and ³¹P NMR

¹H NMR spectral data provide further support for the structures of the expected compounds. Characteristic ¹H and ³¹P NMR spectra for the suggested compounds are listed in TABLE IV. For all new complexes, clear proton signals of the ligands are observed and the mole ratio of phenanthroline to Et groups and to nucleotides can be estimated by calculating the intensities of the relevant protons from ¹H NMR spectra. The calculation gives phenanthroline : ethyl : nucleotide = 1 : 2 : 1. Indeed, the ¹H NMR spectra agree with these predictions as shown by microanalyses, UV and IR spectra.

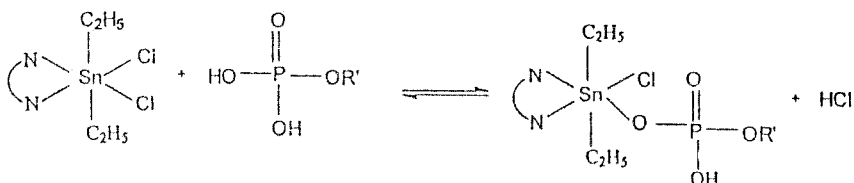
Coordination through N(7) of AMP and GMP as well as N(3) of CMP are ruled out, because no significant changes are observed in the complexes for the C(8)-H resonances of AMP and GMP as well as for the C(5)-H and C(6)-H resonances. Whilst the C(2)-H resonance for the AMP derivative also shows only slight changes, confirming that N(1) of AMP is not bound to Sn(IV). No binding of Sn(IV) to NH₂ or O of the ribose ring is directly observed by comparing the NH₂ and -O(2)-H resonances of free nucleotides with those of their complexes. Thus, according to ¹H NMR data along with the IR data discussed above, we deduce that the only binding site in the nucleotide is a phosphate group. Directly, the binding of Sn(IV) to phosphate is evident from the large upfield shift in the ³¹P signal of phosphate groups, in contrast to the free nucleotides (TABLE IV). The ³¹P NMR spectra of free AMP, CMP and GMP have signals at -0.28, 0.53 and 0.05 ppm, respectively, and these signals are greatly shifted to -5.42, -7.20 and -9.31 ppm for the corresponding complexes. Moreover, the coordination of the nucleotides to Sn(IV) causes broadening of the ³¹P signals. This result demonstrates that Sn(IV) is bound to a phosphate group.

Our experiments show that the reactions did not form any adducts unless the pH value of the solution was low and that the products could only be obtained by adjusting the pH between 3.0 and 7.0. The compositions of the white solids obtained are in good agreement with those suggested by the microanalyses, and are further supported by ¹H NMR, UV and IR spectra. The new complexes are very similar to our previously reported complex Et₂Sn(phen)(UF)Cl (UF is 5-fluorouracil)⁶ in which one of the *cis*-chloro atoms in Et₂SnCl₂(phen) is substituted by 5-fluorouracil in a basic catalytic process. The reactions in our case may also proceed as shown in Fig. 1.

TABLE IV. ^1H and ^{31}P NMR Data (δ , ppm) for the Complexes .

Complex	H (2)	H (5)	H (6)	H (8)	H (1')	NH_2	^{31}P
$\text{Et}_2\text{Sn}(\text{phen})(\text{AMP})\text{Cl}$	8.16 s			8.37 s	5.95 d ($J=4.7$ Hz)	7.20 s	-5.42 b
$\text{Et}_2\text{Sn}(\text{phen})(\text{CMP})\text{Cl}$		5.82 d ($J=2.9$ Hz)	7.85 d ($J=5.0$ Hz)		5.74 d ($J=6.9$ Hz)	7.06 s	-7.20 b
$\text{Et}_2\text{Sn}(\text{phen})(\text{GMP})\text{Cl}$				7.88 s	5.73 d ($J=6.9$ Hz)	6.34 s	-9.31 b
AMP	8.20 s			8.33 s	5.95 d ($J=5.0$ Hz)	7.30 s	-0.28 s
CMP		5.81 d ($J=3.0$ Hz)	7.82 d ($J=7.4$ Hz)		5.72 d ($J=7.3$ Hz)	7.05 s	0.53 s
GMP				7.88 s	5.71 d ($J=7.2$ Hz)	6.44 s	0.05 s

^1H chemical shift vs. TMS; ^{31}P chemical shift vs. 85% H_3PO_4 ; measured in $\text{DMSO}-d_6$. s = Singlet; d = doublet; b = broad.



$\text{R}' = \text{Adenosyl, Cytidine, Guanosine.}$

Fig. 1. Reaction Equation of $\text{Et}_2\text{SnCl}_2(\text{phen})$ with Nucleotides

To our knowledge, this is the first report of reactions between typical $R_2SnCl_2(N^{\wedge}N)$ -type antitumor organotin complexes, such as $Et_2SnCl_2(phen)$, and nucleotides under physiological conditions. The results suggest that one of the *cis*-chloro atoms in $Et_2SnCl_2(phen)$ could be preferentially substituted by nucleotide, and illustrate that Sn(IV) in $Et_2SnCl_2(phen)$ coordinates a phosphate of the nucleotide. Determination of the crystal structure and further studies of oligo- and polynucleotides are now in progress.

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