Anticancer Activity of Organotin Compounds. 2. Interaction of Diorganotin Dihalides with Nucleic Acid Bases and Nucleosides; the Synthesis of Adenine, Adenosine and 9-Methyladenine Adducts

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Abstract

The syntheses of the complexes formulated as $SnMe_2Cl_2(Ad)_2$ **(I)**, $SnMe_2Cl_2(Ado)_2$ **(II)**, $SnMe_2Cl_2$-$(9\text{-MeAd})_2$ **(III)** [Ad = adenine, Ado = adenosine, 9-MeAd = 9-methyladenine] as well as the more unexpected $SnPhCl_2(OH)(Ad)_2 \cdot 3H_2O$ **(IV)** and $SnPhCl_3(Ado)_2$ **(V)** by reaction of $SnMe_2Cl_2$ or $SnPh_2Cl_2$ with the appropriate bases in methanol is described. ^1H NMR studies suggest that coordination is through the N-7 position of the adenine base.

Introduction

A variety of metal complexes have now been shown to be antitumour agents – apart from those of the platinum group [1–3], some metallocene dihalides Cp_2MCl_2 [4], some organotin compounds of the type $SnR_2Cl_2 \cdot (\widehat{N}\widehat{N})$ where $\widehat{N}\widehat{N}$ is a chelating ligand [5] and very recently the $[FeCp_2]^+$ cation [6].

In our previous work in this area [5], we determined the structures of two active organotin compounds, $SnCl_2Et_2 \cdot (phen)$ and $SnCl_2(n\text{-}Bu)_2 \cdot (bipy)$ with the intention of determining the molecular parameters which were important for activity. We suggested that the most important conclusion was that the active species might be formed by dissociation of the dinitrogen ligand and replacement of at least one chloride ligand by a coordination site on DNA. The most recent crystallographic evidence on the binding of *cis*$[Pt(NH_3)_2(H_2O)(OH)]^+$ and related species to oligonucleotides and t-RNAs is that there is monodentate binding to the N-7 of a guanine residue, with a hydrogen bonded interaction to the keto oxygen (O-6) [1, 3]. Nothing is known about the interaction between tin and nucleic acids, nor the mode of action of the organotins as antitumour agents, so we have studied the interaction of diorganotin dihalides with nucleic acid bases and nucleosides to see how far the analogy can be pursued. In this paper we report the reactions between SnR_2Cl_2 (R = Me, Ph) with adenine, 9-methyladenine and adenosine. ^1H NMR studies suggest that coordination may be through N-7 of the adenine base. So far we have not succeeded in crystallizing samples suitable for X-ray analysis.

Experimental

Reagents

$SnMe_2Cl_2$ was prepared by the literature method [7] and was recrystallized or sublimed before use (m.p. 106 °C, lit. [8] 107.5–108 °C). $SnPh_2Cl_2$ was the generous gift of Dr. P. J. Smith (I.T.R.I, London) and was recrystallized from hexane before use (m.p. 42 °C, lit. [8] 42 °C). Adenine, adenosine, guanine, cytosine, thymine, theophylline, thymidine, uridine, uracil and cytidine were obtained from Sigma and used without further purification. 9-methyladenine was prepared by a literature method [9]. It was then recrystallized from an ethanol/water mixture and characterized by TLC and ^1H NMR. 9-methyladenine was selectively deuterated at H-8 by the method of Lippert [10], and was then recrystallized from D_2O.

All solvents were purified, dried over suitable reagents and redistilled before use.

Microanalyses were done by the Microanalytical Laboratory, University College, Dublin.

Instruments and Spectra

^1H NMR spectra were recorded on the Bruker WP80 NMR using dried d_6-DMSO (molecular sieve) and CD_3OD (CaH_2). IR spectra were recorded as KBr discs (occasionally as Nujol mulls using CsI plates) on the Perkin–Elmer 599. UV spectra were recorded in 'Uvasol' grade ethanol on the Perkin–Elmer 402.

Synthesis of $SnMe_2Cl_2(Ad)_2$ (I)

Adenine (0.273 g, 2.02 mmol) and dimethyltin dichloride (0.444 g, 2.02 mmol) were heated under

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reflux in methanol (120 cm^3) under nitrogen for 96 h. 80 cm^3 of methanol was then removed from the reaction mixture by distillation. The residual solution, on cooling to 5 °C for 1 h afforded a white solid which was filtered off (0.176 g) and identified as unreacted adenine. The volatiles were removed from the clear filtrate by distillation. The solid mass thus obtained was treated with 85 cm^3 ether in portions. The ether-soluble portion afforded 0.368 g of unreacted dimethyltin dichloride. The ether-insoluble portion was characterized as **(I)** (0.171 g, 35%), m.p. 230–270 °C (d). (Found: C, 28.94; H, 3.34; N, 29.01; Cl, 15.54; Sn, 24.32%. $C_{12}H_{16}N_{10}Cl_2Sn$ requires C, 29.40; H, 3.26; N, 28.59; Cl, 14.49; Sn, 24.24%; λ_{max} 215, 262 nm).

Synthesis of $SnMe_2Cl_2(Ado)_2$ (II)

Adenosine (1.262 g, 4.72 mmol) and dimethyltin dichloride (1.037 g, 4.69 mmol) were heated under reflux in methanol (250 cm^3) under nitrogen for 48 h. 50 cm^3 of the solvent was then removed from the reaction mixture by distillation. The concentrated mixture was then cooled to 5 °C for 1 h and then filtered, giving 0.427 g unreacted adenosine. The volatiles were removed from the clear filtrate by distillation. The residual white mass was washed thoroughly with 85 cm^3 ether in portions. The washings on evaporation afforded 0.690 g of unreacted dimethyltin dichloride. The white solid thus left was characterized as **(II)** (1.162 g, 66%), m.p. 168–205 °C (d). (Found: C, 35.63; H, 4.61; N, 19.09; Cl, 11.80; Sn, 15.32%. $C_{22}H_{32}N_{10}Cl_2O_8Sn$ requires C, 35.02; H, 4.21; N, 18.57; Cl, 9.42; Sn, 15.74%; λ_{max} 216, 263 nm).

Synthesis of $SnMe_2Cl_2(9\text{-}MeAd)_2$ (III)

9-Methyladenine (0.494 g, 3.32 mmol) and dimethyltin dichloride (0.729 g, 3.32 mmol) were heated under reflux in 100 cm^3 methanol for 98 h under nitrogen. 30 cm^3 of methanol was removed by distillation from the reaction mixture. On cooling to 5 °C for 1 h, the reaction mixture afforded 0.124 g of unreacted 9-methyladenine. The filtrate after separation of 9-methyladenine was evaporated to dryness. The solid residue was treated with 75 cm^3 ether in portions, the ether-soluble fraction afforded 0.450 g of unreacted dimethyltin dichloride. The ether-insoluble fraction was characterized as **(III)** (0.642 g, 74%) m.p. 245–255 °C (d). (Found: Sn, 22.82%. $C_{14}H_{20}N_{10}Cl_2Sn$ requires Sn, 22.92%; λ_{max} 215, 263 nm).

Synthesis of $SnPhCl_2(OH)(Ad)_2 \cdot 3H_2O$ (IV)

Adenine (0.308 g, 2.28 mmol) and diphenyltin dichloride (0.784 g, 2.28 mmol) were refluxed in 150 cm^3 methanol for 96 h. The volatiles were removed by distillation and the solid mass was washed thoroughly with 75 cm^3 ether in portions. The ether extract afforded 0.435 g of triphenyltin chloride (m.p. and m.m.p. 104 °C, lit. [8], m.p. 105.5–107 °C). The white solid after ether washing was characterized as **(IV)** (0.648 g, 99%), m.p. 150–200 °C (d). (Found: C, 32.05; H, 4.21; N, 24.38; Cl, 12.35; Sn, 20.35%. $C_{16}H_{20}N_{10}Cl_2O_3$ requires C, 32.55; H, 3.39; N, 23.74; Cl, 12.04; Sn, 20.12%; λ_{max} 218, 265 nm).

Synthesis of $SnPhCl_3(Ado)_2$ (V)

Adenosine (0.642 g, 2.40 mmol) and diphenyltin dichloride (0.835 g, 2.40 mmol) were refluxed in 200 cm^3 methanol for 48 h under nitrogen. The volatiles were removed by distillation and the residue thus obtained was washed thoroughly with 75 cm^3 ether in portion. The ether-soluble fraction afforded 0.452 g of triphenyltin chloride (m.p. and m.m.p. 104 °C, lit. [8], m.p. 105.5–107 °C). The ether-insoluble fraction was characterized as **(V)** (0.992 g, 99%), m.p. 142–200 °C (d). (Found: C, 36.67; H, 4.06; N, 15.84; Cl, 13.03; Sn, 14.35%. $C_{26}H_{31}N_{10}Cl_3O_8Sn$ requires C, 37.31; H, 3.70; N, 16.74; Cl, 12.73; Sn, 14.19%; λ_{max} 218, 265 nm).

Results and Discussion

Syntheses

The new compounds (Table I) have been characterized by elemental analysis, 80 MHz ^1H NMR, IR, UV and melting point. In each case the preparation was carried out with excess organotin (1:1 stoichiometry SnR_2Cl_2:Nu, where Nu = adenine, adenosine or 9-methyladenine), since this could readily be removed from the reaction mixture with hexane/ether. Careful monitoring of the weights of unreacted starting material recovered in each experiment always indicated 1:2 stoichiometry. The compounds cannot apparently be recrystallized without decomposition into their constituents, although they are stable as solids at room temperature and do not appear to be sensitive to air and moisture over periods of a few days.

The reaction of $SnPh_2Cl_2$ with Nu (Nu = adenine, adenosine) carried out in 1:1 stoichiometry results in the unexpected formation of $SnPh_3Cl$. The weight of material isolated (50% yield of $SnPh_3Cl$) suggests an almost quantitative disproportionation:

$$2SnPh_2Cl_2 + 4Nu \longrightarrow 2SnPh_2Cl_2 \cdot Nu_2 \quad (1)$$

$$2SnPh_2Cl_2 \cdot Nu_2 \longrightarrow SnPh_3Cl + SnPhCl_3 \cdot Nu_2 + 2Nu \quad (2)$$

We suggest the disproportionation of the complex in this way because there appears to be no literature report of the direct disproportionation of $SnPh_2Cl_2$ in our conditions. The weaker Lewis acidity of the Sn atom in $SnPh_3Cl$ and the steric bulk

TABLE I. ^{1}H NMR Data for New Compounds.[a]

Compound	δ (H-8)		δ (H-2)		δ(NH_2) d_6-DMSO	Other data[b]
	d_6-DMSO	CD_3OD	d_6-DMSO	CD_3OD		
$SnMe_2Cl_2(Ad)_2$ (I)	8.215	8.250	8.215	8.162	7.580	δ($SnMe_2$) 1.037 (*1.075*) $^2J(^1H-^{119}Sn)$ 113.30 (*94.60*) $^2J(^1H-^{117}Sn)$ 108.57 (*89.60*)
$SnMe_2Cl_2(Ado)_2$ (II)	8.416	8.412	8.274	8.237	7.658	δ($SnMe_2$) 1.050 (*1.086*) $^2J(^1H-^{119}Sn)$ 113.27 (*94.30*) $^2J(^1H-^{117}Sn)$ 108.57 (*89.60*) [d]
$SnMe_2Cl_2(9\text{-}MeAd)_2$ (III)	8.216	8.117	8.157	8.248	7.496	δ($SnMe_2$) 1.056 (*1.085*) $^2J(^1H-^{119}Sn)$ 113.22 (*94.43*) $^2J(^1H-^{117}Sn)$ 108.57 (*89.78*)
$SnPhCl_2(OH)(Ad)_2 \cdot 3H_2O$ (IV)	8.170	8.326	8.170	8.264	[e]	δ(Sn–Ph) 7.705, 7.730[c]
$SnPhCl_3(Ado)_2$ (V)	8.412	8.487	8.218	8.312	[e]	δ(Sn–Ph) 7.781, 7.431 (*7.850, 7.450*)[c,d]
Adenosine (≡Ado)	8.340	8.311	8.157	8.200	7.287	
9-methyladenine (≡9-MeAd)	8.083	8.061	8.157	8.215	7.137	
Adenine (≡Ad)	8.105	8.100	8.135	8.187	7.062	

Spectra recorded in saturated solutions of d_6DMSO and CD_3OD using internal TMS reference. All shifts are in p.p.m. downfield TMS. [a]Satisfactory integration of all spectra obtained. [b]Data in italics obtained in CD_3OD, otherwise d_6-DMSO. [c]Complex multiplets. [d]Data for ribose protons omitted. [e]Masked by phenyl protons.

presumably results in the complete dissociation of any adduct in this case. When Nu = adenine the product isolated has the composition $SnPhCl_2(OH)(Ad)_2 \cdot 3H_2O$, and we believe the difference in hydrolytic stability of the two adducts to be a consequence of the greater steric bulk of the adenosine ligands. The hydrolytic instability of $SnRCl_3$ is well known [11], and there are examples of stable $SnRCl_2(OH)$ compounds where R = Me, Et, Bu, Oct, in the literature [11, 12].

We have also attempted to synthesise organotin adducts of guanine, cytosine, thymine, uracil, theophylline, cytidine, thymidine and uridine by the method reported in this paper, but so far have isolated no adducts of any of these. In aqueous media we have isolated other organotin adducts of adenine and adenosine which we suspect to be polymeric [13]. Synthetic work on other aspects is continuing.

^{1}H NMR Data

Details of the spectra of (I)–(V) recorded in both d_6-DMSO and CD_3OD appear in Table I. (Resonances of the free bases and adenosine in our conditions are also reported). Binding sites of the adenine base should be suggested by the downfield shifts in the H-2 or H-8 proton on coordination to the adjacent nitrogens. There is a potential ambiguity, however, well described by Lippert [10] over the assignment of the shifted resonances since a large downfield shift on coordination to N-7 or N-9 of adenine will cause the H-8 resonance to be shifted below that of H-2. The ambiguity is also present for 9-methyladenine, but not adenosine, since H-8 has been shown to be the resonance at lower field in this case [14]. For 9-methyladenine, we resolved the potential ambiguity by selective deuteration of the ligand, to give the 6-ND_2, 8-D derivative [10]. The spectrum of partially deuterated (III) in either solvent, shows only the H-2 resonance, essentially unshifted by coordination in either solvent. We therefore believe that coordination through N-7 is most probable when the 9-position is blocked. The effect of changing solvent from D_2O to DMSO on the ^{1}H NMR H-8 resonance in Pt(II) complexes of 9-ethylguanine has also been studied in detail by Lippert *et al.* [15], although they could not satisfactorily account for the shifts. We note similar shifts between d_6-DMSO and CD_3OD, but likewise cannot account for them.

TABLE II. Relevant Infrared Absorptions.

Compound	ν(OH)	ν(NH$_2$)	ν(NH)	δ(NH$_2$)	ν(Sn–C)	ν(Sn–N)	ν(Sn–Cl)	Other ligand vibrations
$SnMe_2Cl_2(Ad)_2$ (I)		3292(sh)	3000–	1692(s)	567(m)	240(w)	295(m)	
		3097(sh)	2500(mb)		550(m)		280(m)	
$SnMe_2Cl_2(Ado)_2$ (II)		3320(s)		1670(s)	569(m)	235(w)	315(m)	
		3170(s)			555(m)		275(m)	
$SnMe_2Cl_2(9\text{-}MeAd)_2$ (III)		3270(s)		1685(s)	575(m)	238(w)	290(w)	
		3095(s)			554(m)		278(w)	
$SnPhCl_2(OH)(Ad)_2\cdot 3H_2O$ (IV)	3420–	3290(sh)	3000–	1685(s)	535(m)	232(w)	322(m), 312(m)	
	3200(wb)	3102(s)	2500(mb)	1655(s)			300(m), 296(m)	
							275(m)	
$SnPhCl_3(Ado)_2$ (V)		3355(sh)		1688(m)	560(m)	235(m)	312(m)	
		3152(s)		1648(m)			275(m)	
Adenine (≡Ad)		3295(s)	3010–	1675(s)				545(m)
		3105(s)	2500(mb)					340(m)
Adenosine (≡Ado)		3337(s)		1665(s)				590(m), 537(m), 522(m),
		3175(s)						415(m), 390(w), 350(w),
								320(w), 290(w)
9-methyladenine (≡9-MeAd)		3275(s)						543(m), 530(m)
		3100(s)		1672(s)				360(m), 245(m)

All spectra were recorded as KBr pellets in the range 4000–600 cm^{-1}, and as Nujol mulls on CsI windows in the range 600–200 cm^{-1}, s = strong, m = medium, w = weak, sh = shoulder, b = broad.

IR Data

Relevant IR data and assignments for the new compounds are presented in Table II. In the complexes $SnMe_2Cl_2\cdot(Nu)_2$ **(I–III)** the symmetric and antisymmetric stretches of the 6-NH_2 group [16] have been shifted to lower frequencies (Table II). The NH_2 deformation mode at about 1670 cm^{-1} [16] in neutral ligands has shifted to higher frequency in the complexes. Stretching frequencies ν(9N–H) are observed for **(II)** in the range 3100–2200 cm^{-1} [16]. Tentative assignments of the ν(Sn–C_2) [17–19, 22, 25], ν(Sn–Cl) [17–19, 21–23] and ν(Sn–N) [18, 20, 23] vibrations can be made, in reasonable agreement with published data on comparable systems.

In complexes **(IV)** and **(V)** the ν(NH_2) frequencies are lowered compared with the free ligands, and ν(9N–H) is also observed in **(IV)**. In **(IV)** and **(V)** there are two strong 6-NH_2 deformation modes, one lower and one higher than the free ligand values.

In **(IV)** a strong broad band at 3600 cm^{-1} (partly masked by the 6-NH_2 asymmetric stretch) is assigned to the ν(O–H) of coordinated Sn–OH [22, 24, 25], and probably also to the hydrogen-bonded water molecules [24].

Geometry of the Complexes

So far, crystals suitable for X-ray diffraction have not been obtained. Species **(I)–(III)** dissociate over long periods in solution in the absence of excess ligand; and whereas the more strongly accepting Sn atom in **(IV)** and **(V)** appears to suppress dissociation in solution, these compounds can only be obtained in microcrystalline form, although work in this area is continuing.

The complexes are all formulated as hexacoordinate, containing monodentate nucleobases, probably coordinating through N-7 (or N-9 where adenine is the ligand) as neutral ligands. This is the first report of the coordination of neutral nucleosides to organotin compounds, though coordination of anions is known [26]. Preliminary Mössbauer data [27] indicate that the dialkyl tin coordination is most probably at the *trans* positions of a highly distorted octahedron. Tentative IR assignments of both symmetric and antisymmetric stretching vibrations for both Sn–Cl and Sn–C are also consistet with non-linear C–Sn–C and Cl–Sn–Cl groupings.

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