DIORGANOTIN DERIVATIVES OF 2,6-DIHYDROXYMETHYLPYRIDINE AND OF 3-(*N*-PYRROLIDINO)-1,2-PROPANEDIOL: SYNTHESIS, CHARACTERIZATION AND *IN VITRO* ANTITUMOR ACTIVITY

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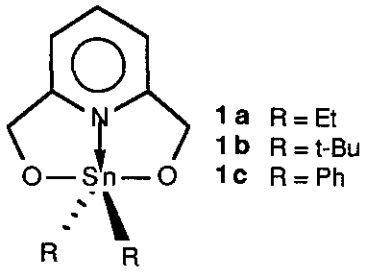
Abstract-Three diorganotin(IV) derivatives of 2,6-dihydroxymethylpyridine and 3-(*N*-pyrrolidino)-1,2-propanediol have been synthesized and characterized by Mössbauer spectroscopy, mass spectrometry and by ^{1}H, ^{13}C and ^{119}Sn nmr. Their activities *in vitro* against two human tumor cell lines, MCF-7 and WiDr, are compared to those of *cis*-platin and doxorubicin.

INTRODUCTION

Diorganotin derivatives of 2,6-pyridinedicarboxylic acid[1,2] were recently demonstrated to exhibit interesting *in vitro* antitumor activities against two human tumor cell lines, MCF-7, a mammary tumor, and WiDr, a colon carcinoma.[1,2] We were interested in comparing the activities of these compounds with those of their *reduced analogs*. To this purpose we prepared three diorganotin(IV) derivatives of 2,6-bis(hydroxymethyl)pyridine, the reduced analog of 2,6-pyridine dicarboxylic acid. The dibutyltin derivative of 3-(*N*-pyrrolidino)-1,2-propanediol was likewise prepared for comparison.

RESULTS AND DISCUSSION

The compounds prepared are described in Figure 1. They were obtained by reacting equimolar amounts of the diol with the diorganotin oxide in a mixture of toluene and ethanol, according to a procedure used for diorganotin derivatives of salicylic acids.[3]



1a R = Et
1b R = t-Bu
1c R = Ph

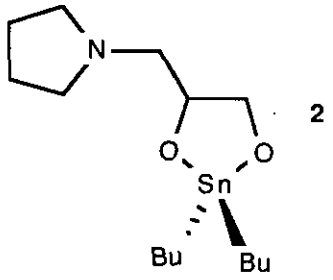


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Figure 1: Diorganotin(IV) derivatives of 2,6-dihydroxymethylpyridine and of 3-(N-pyrrolidino)-1,2-propanediol.

*Table 1: Melting points, recrystallization solvents, yields and Mössbauer parameters (I.S.: isomer shift relative to $Ca^{119m}SnO_3$; Q.S.: quadrupole splitting; Γ_1 and Γ_2: line widths) of compounds **1a**, **1b**, **1c** and **2***

Compd No	mp (°C)	recrystallization solvent	yield (%)	I.S. mm/s	Q.S. mm/s	Γ_1 mm/s	Γ_2 mm/s
1a	136-137	$CHCl_3$ + hexane	72	1.23	3.27	0.96	0.98
1b	128-130	$CHCl_3$ + hexane	69	1.21	2.12	0.81	0.85
1c	166-170	$CHCl_3$ + hexane	69	0.91	1.70	0.91	0.85
2	145-146	hexane	88	1.15	2.80	0.93	0.93

Compared with the Mössbauer parameters of homologous derivatives of dicarboxylic acids, the Q.S. and I.S. values of these diol derivatives are significantly lower:

	Q.S.	I.S.
2,6-pyridinedicarboxylic acid + Et_2SnO[1]	4.33	1.51
2,6-dihydroxymethylpyridine + Et_2SnO, **1a**	3.27	1.23

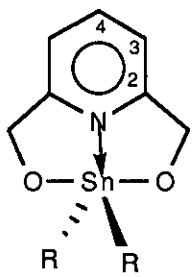
Although the difference might be attributed to the electron withdrawing power of the carbonyl group, the influence of particular structural arrangements cannot be ruled out since many association modes are known to occur in the solid state.[4]
Nevertheless, the four diol derivatives under interest show too high Q.S. values to be only four-coordinated.[5, 6] In the solid state, a fifth bond (N→Sn) is obvious in (**1a**), (**1b**) and (**1c**), whereas a fifth and/or sixth bond is probably intermolecular in (**2**).

Diorganotin(IV) derivatives of 2,6-dihydroxymethylpyridine, **1a**, **1b** and **1c**

^1H Nmr data

The proton nmr spectra of compounds **1a** to **1c** are given in Table 2.
The methyl and methylene protons of the ethyl group of compound (**1a**) have very similar chemical shifts, in analogy to ethyl derivatives of silicon, lead and cadmium.[7] Furthermore, the hydrogen atoms of each methylenic group in the enantiotopic ethyl groups are diastereotopic. As a consequence each ethyl spin system is of the ABC_3 type and exhibits a very complex pattern. Only the $^3J(^1H, ^{119/117}Sn)$ coupling constants could be evaluated.
The value of the $^3J(^1H,^{119}Sn)$ coupling constant (99 Hz) in compound (**1b**) is comparable to that of t-$Bu_2Sn(OCH_2CH_2)_2NMe$ (95 Hz)[8] and t-$Bu_2Sn(NMeCOCH_2)_2NMe$ (106 Hz)[9] but smaller than the value of ditertiobutyltin chloride (117 Hz).

*Table 2: ^1H Nmr data in $CDCl_3$ of compounds of the type $R_2Sn[2,6\text{-}(OCH_2)_2\text{-}C_5H_3N]$, **1a** to **1c**. Chemical shifts in ppm versus TMS as internal reference. The numbers between brackets are the $^nJ(^1H,^{117/119}Sn)$ coupling constants in Hz. The values between parentheses are the multiplicities of the resonance and the $^nJ(^1H,^1H)$ coupling constants in Hz [nr = non resolved]*
d: doublet; t: triplet; m: complex pattern; s: singlet; nr: non resolved
[b] overlapping with the meta and para protons of the phenyl groups
[c] overlapping with the ortho protons of the phenyl groups



1a R = Et
1b R = t-Bu
1c R = Ph

Protons	**1a**, R = Et	**1b**, R = t-Bu	**1c**, R = Ph
CH_3	0.86 - 1.35 (m) [$^3J \approx 144$]	1.24 [^3J = 95/99]	o-C_6H_5: 7.75 - 8.04 (m) [$^3J \approx 73$]
CH_2Sn	0.86 - 1.35 (m) [^2J: nr]	-	m & p-C_6H_5: 7.31 - 7.42 (m)
3-H	7.27 (d, ^3J =8)	7.33 (d, ^3J =8)	b
4-H	7.86 (t, ^3J =8)	7.92 (t, ^3J =8)	c
2-CH_2O	5.04 (s) [^3J = 15]	5.16 (s) [^3J = 11]	5.29(s) [^3J = 23]

^{13}C Nmr data

The ^{13}C nmr data of compounds **1a** to **1c** are shown in Table 3.

The resonances were assigned from the DEPT spectrum and from the signal intensity ratio of the carbon-3 to -4 signals of the aromatic carbons of the diolate ligand.

The values of the $^1J(^{13}C,^{119}Sn)$ coupling constants of compounds (**1a**) and (**1b**) allow to calculate a C-Sn-C angle of 122°,[10] a value close to that found for $Bu_2Sn(OCH_2CH_2)_2NMe$.[11] From an analogous relationship applicable to diphenyltin compounds,[12] an angle of 135° is obtained for compound (**1c**). The

error on these angles is of the order of 10°.

*Table 3: ^{13}C Nmr data of compounds **1a**, **1b** and **1c** in $CDCl_3$. Chemical shifts in ppm versus TMS as internal reference. The numbers between brackets are the $^{n}J(^{13}C,^{117/119}Sn)$ coupling constants in Hz. nr: non resolved*

Carbon	**1a**, R = Et	**1b**, R = t-Bu	**1c**, R = Ph
CH_3	9.5 [^{2}J = 35]	30.2 [^{2}J(nr)]	ipso-C: 141.4 [^{1}J = 900/936]
CH_2Sn	11.7 [^{1}J = 573/598]	-	ortho-C: 136.2 [^{2}J = 51]
CSn	-	37.9 [^{1}J = 579/606]	meta-C: 128.5 [^{3}J = 78]
			para-C: 129.7
C-2	160.4 [^{3}J = 50]	161.2 [^{3}J = 39]	159.7 [^{3}J = 64]
C-3	118.8	119.1	119.2
C-4	140.1	140.4	140.6
CH_2O	63.5 [^{2}J =32]	64.3 [^{2}J = 35]	63.3 [^{2}J = 29]

^{119}Sn Nmr data

The ^{119}Sn chemical shifts of compounds **1a** to **1c** are compatible with a pentacoordinate geometry.[13]

*Table 4: ^{119}Sn Nmr data of compounds **1a** to **1c** in $CDCl_3$. Chemical shifts in ppm versus tetramethyltin as external reference.*

No, R	**1a**, Et	**1b**, t-Bu	**1c**, Ph
$\delta(^{119}Sn)$ in ppm	-105.5	-237.2	-186.4

The differences between the experimental values can be ascribed to the nature of the organic group R linked to tin.[14]

Mass spectral data

The FAB mass spectra of compounds **1a** to **1c** are summarized in Table 5. The expected fragment-ions are observed for these compounds and confirm the fragmentation patterns proposed earlier[17]: the $(M+H)^+$ ion is intense; it looses RH or R_2 and fragmentates further into smaller ions.

*Table 5: Intensities of ions in the FAB mass spectra of compounds of the type C_5H_3N-2,6-$(CH_2O)_2SnR_2$, **1a** to **1c***

Fragment-ion	**1a**, R = Et	**1b**, R = t-Bu	**1c**, R = Ph
$Sn^{\cdot+}$	-	7	6
$HOSn^+$	-	16	4
RSn^+	11	7	16
C_5H_4N-2-$(CH_2O)Sn^+$	14	25	12
C_5H_3N-2,6-$(CH_2O)_2Sn^{\cdot+}$	26	85	57
C_5H_3N-2,6-$(CH_2O)_2SnR^+$	-	8	100
C_5H_3N-2,6-$(CH_2O)_2SnR_2H^+$	100	100	83

Di-n-butyltin(IV) derivative of 3-(*N*-pyrrolidino)-1,2-propanediol (**2**)

^1H and ^{13}C Nmr data

The ^1H and ^{13}C nmr spectra of compound (**2**) are given in Table 6.

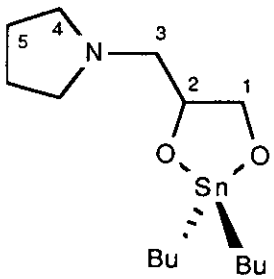
The ^1H signals of compound (**2**) are broad. This can be explained by the presence in solution of different oligomers in dynamic equilibrium[13] with a mean lifetime of the order of the proton nmr time scale.

The $^2J(^{13}C\text{-}^{117/119}Sn)$ coupling constants of C-1 and C-2 in compound (**2**) are not observed because of the low signal/noise ratio resulting from the high linewidth. Such a broadening is well known in ^{13}C spectra of similar compounds.[13]

Application of the equation of Howard[10] gives C-Sn-C angles of 143 ± 10° for

compound (**2**) that can be compared to the value of 132 ± 10° calculated for the di-n-butyltin derivative of 2-hydroxymethyl-6-methyl-3-pyridinol.[2] These values are close to those found by X-ray diffraction for similar dioxastannolanes.[7]

Table 6: ^{1}H and ^{13}C Nmr data of compound (2) in $CDCl_3$. Chemical shifts in ppm versus TMS as internal reference. The numbers between square brackets are the $^{n}J(^{1}H,^{117/119}Sn)$ or $^{n}J(^{13}C,^{117/119}Sn)$ coupling constants in Hz. The figures between parentheses are the multiplicities of the signal and the $^{n}J(^{1}H,^{1}H)$ coupling constants in Hz of the ^{1}H spectra. t: triplet; q: quartet; m: complex pattern; b: broad.



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	^{1}H-resonances	^{13}C-resonances
CH_3	0.92 (t,7)	13.9
CH_2	1.33 (tq,7,7)	27.4 [$^{3}J \cong 109$]
CH_2	1.57-1.68 (m) [^{3}J: nr]	27.9 [$^{2}J = 33$]
CH_2Sn	1.40 (t,7) [^{2}J: nr]	23.0 [$^{1}J \cong 660$]
1	3.74 (b)	66.7
2	3.06-3.20 (m)	71.4
3	2.3-2.4 (bm)	62.4
4	2.45-2.55 (b)	55.4
5	1.75 (b)	23.9

^{119}Sn Nmr data

The ^{119}Sn nmr spectrum of compound (**2**) in $CDCl_3$ exhibits many broad signals between -120 and -200 ppm; analogous patterns were already observed for diorganotin derivatives of diols[16] and were interpreted by dynamic equilibria

between several species in solution. This observation is in agreement with the ^{1}H nmr data of compound (**2**). The exact nature of these equilibria remains to be elucidated but the coalescences indicate a high degree of coordination lability at tin. These results are in strong contrast with the ^{119}Sn nmr spectrum of 2-hydroxymethyl-6-methyl-3-pyridinol which exhibits a single sharp resonance at -198.4 ppm,[2] as expected for **penta**coordinate tin atoms. In the latter compound, the generation of an additional coordination by oligomer formation is therefore likely. Such oligomerizations were evidenced for diorganostannylene derivatives of sugars.[7]

Mass spectral data

The FAB mass spectra of compound (**2**) is given below:
$(C_4H_8N\text{-}CH_2\text{-}CHOH\text{-}CH_2O)SnBu_2^+$: 30% ; $(C_4H_8N\text{-}CH_2\text{-}CHO\text{-}CH_2O)SnBu^+$: 14%; $C_2H_3O_2SnBu_2^+$: 100%; $(C_4H_8N\text{-}CH_2\text{-}CHO\text{-}CH_2O)SnH^+$: 35% ; Bu_2Sn^+: 64% ; $BuSn^+$: 25%; $HOSn^+$: 11% ; HSn^+: 37%; $Sn^{\cdot+}$: 15%.

In vitro antitumor tests

Compounds (**1a**) and (**2**) were tested *in vitro* against two human tumor cell lines, MCF-7, a mammary tumor, and WiDr, a colon carcinoma. Only the di-n-butyltin derivatives were selected because they are known to be more active than the diethyltin analogs in these models.[18] The results of these pre-screenings are given in Table 7.

Compound (**2**) exhibits activities as satisfactory as doxorubicin against MCF-7 and WiDr. Compound (**1a**) has a poor activity since it displays higher ID_{50} values than cis-platin against WiDr, even if it scores better than the latter reference compound against MCF-7.

*Table 7: ID_{50} values in ng/mL of compounds **1a** and **2** and of two reference compounds,[19] cis-platin and doxorubicin*

Compd	**1a**	**2**	cis-platin	doxorubicin
MCF-7	430	42	850[19]	63[19]
WiDr	1 276	79	624[19]	31[19]

EXPERIMENTAL PART

Syntheses

The synthesis of compounds (**1a**), (**1b**), (**1c**) and (**2**) is analogous to that of substituted or unsubstituted salicylic acids:[2,3] typically, 4.0 mmol of the appropriate diol dissolved in 150 ml of toluene and 50 ml of ethanol are added 4.0 mmol diorganotin oxide. The mixture is refluxed for 6 h and the ternary azeotrope water/ethanol/toluene is distilled off with a Dean-Stark funnel. The remaining solution is evaporated under vacuum. The crystallization solvents are given in Table 1.

Instruments

The Mössbauer spectra were recorded with the constant acceleration mode on an Elscint MVT4 Promeda counting instrument, with a $Ca^{119m}SnO_3$ source from Amersham. The probe was maintained at a temperature between 90 and 100 K, the source at room temperature. The digital data were treated with an iterative program and least square deconvoluted as a linear combination of Lorentzian functions.[10]

The mass spectra were recorded on a AEI MS 902S instrument coupled to a NOVA computer. Samples were introduced via the direct insertion probe.

The ^1H and ^{13}C nmr spectra were recorded at 270.13 and 67.93 MHz respectively on a Bruker AM 270 instrument. The ^{119}Sn nmr spectra were recorded at 186.5 MHz on a Bruker WM 500 instrument.

In vitro screening

Drug activity was determined using an automated *in vitro* technique described previously.[5] The samples were prepared by dissolving first the organotin compound in DMSO or in ethanol, and by diluting this solution with water.

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