Crystal and Molecular Structure and in Vitro Antiproliferative and Antitumor Activity of Two Organotin(IV) Carbohydrate Compounds

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3-*C*-[(Triphenylstannyl)methyl]-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (Ph_3SnCH_2 carbohydrate) and 3-*C*-(triphenylstannyl)-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (Ph_3Sn carbohydrate) were studied by diffraction methods, space groups $P2_12_12_1$, a = 6.073 (1), b = 13.091 (3), c = 37.739 (13) and a = 8.3219 (9), b = 11.876 (1), c = 29.575 (5) Å, respectively; both compounds have distorted tetrahedral coordination. The biological study of these compounds along with Ph_3SnCl shows the following: (a) With respect to their capacity to interfere with DNA, RNA, and protein synthesis of isolated fastly proliferating thymocytes, the triphenyltin carbohydrates are less active than Ph_3SnCl. Protein synthesis was found to be most sensitive with an IC50 value of approximately 0.3 μM for Ph_3SnCl, 3 μM for Ph_3Sn-carbohydrate, and greater than 5 μM for Ph_3SnCH_2-carbohydrate; (b) The in vitro activity toward the mouse tumor cell lines MOPC315, P815, SL2, and L1210, which was also performed for Bu_2SnCl_2, also showed that the two triphenyltin carbohydrates were less effective than Ph_3SnCl. From these results it is concluded that in vitro these Sn–C bonded triphenyltin carbohydrates are less active than Ph_3SnCl. Ph_3Sn carbohydrate is more active than Ph_3SnCH_2 carbohydrate, and this may be related with the long Sn–C (of the carbohydrate moiety) bond distance (2.225 (8) Å) for the former compound. This compound shows a biological activity which is in contrast to the normal inactivity of tetraorganotins and is the first active member of this family whose molecular structure is reported.

Introduction

Triphenyltin compounds are commercially used as industrial and agricultural biocides.[1] Due to their specific activity against two major plant diseases, the late blight on potatoes and the leaf spot in sugar beets, they have become important agricultural fungicides. It is to obtain products with improved biocidal activity that the recently synthesized triphenyltin carbohydrates were developed. Besides the high fungicidal and bactericidal properties, various aryl-substituted tin compounds have been shown to possess antitumor activity. In particular, diorganotin (alkyl and aryl) compounds have been studied for their antitumor activity which show promising results mainly in the P388 lymphocytic leukemia in mice.[2] At first, diorganotin compounds, with dihalide or dipseudohalide moieties as anionic residues, were studied for their antitumor activity.[3] More recently the antitumor activity of various organotin compounds in which the anionic residues are replaced by organic moieties O-, N- or S-bonded to the metal atom[4] has been studied. The structure–activity relationships of the various diorganotin compounds tested revealed that in vivo the diethyl- and diphenyltin derivatives were the most promising.[2] Strong antiproliferative effects in vitro toward fast proliferating thymocytes as well as some human lymphoid tumor cell lines have been demonstrated in di- and triphenyltin compounds.[5] In this report we describe the structural results on the recently synthesized triphenyltin carbohydrates along with their antiproliferative and antitumor activity, the latter effects being compared with those of triphenyltin chloride.

Results

(a) Crystallographic Study. The crystal structure of 3-*C*-[(triphenylstannyl)methyl]-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (henceforth named Ph_3SnCH_2 carbohydrate) is built up from well-separated discrete molecules. For 3-*C*-(triphenylstannyl)-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (named Ph_3Sn carbohydrate), the molecules are packed closer together with some intermolecular H–H distances that are similar to the sum of the corresponding van der Waals radii (2.40 Å),[6] e.g. H(of C(1))–H(of C(18) [$-^1/_2 - x, -y, ^1/_2 + z$]) = 2.28 Å; H(of C(7))–H(of C(12) [$-1 - x, -^1/_2 + y, ^1/_2 - z$]) = 2.36 Å; H(of C(6))–H(of C(11) [$-x, ^1/_2 + y, ^1/_2 - z$]) = 2.41 Å. Figures 1 and 2 show computer-generated drawings of the structures with H atoms omitted. In Ph_3SnCH_2 carbohydrate the two C(13) methylene H atoms have intramolecular contacts, one with H of C(5) (2.16 Å) and the other with H of C(2) (2.29 Å). Thus, it appears that rotation of the sugar unit around the C(3)–C(13) bond may be hindered. In Ph_3Sn carbohydrate H(of C(1))–H(of C(18)) is 2.59 Å and will be discussed later. Atomic coordinates (Tables SI and SII) and relevant structural parameters of both compounds (Table SIII) are given as supplementary material.

The metal coordination can be described as a distorted tetrahedral with three carbons arising from the three phenyls and the fourth, the methylene (C(13)) that links the sugar moiety in Ph_3SnCH_2 carbohydrate, and the 3C carbon atom of the sugar in Ph_3Sn carbohydrate which is conventionally named C(3). However the position of the O(3)H group should be taken into account because the

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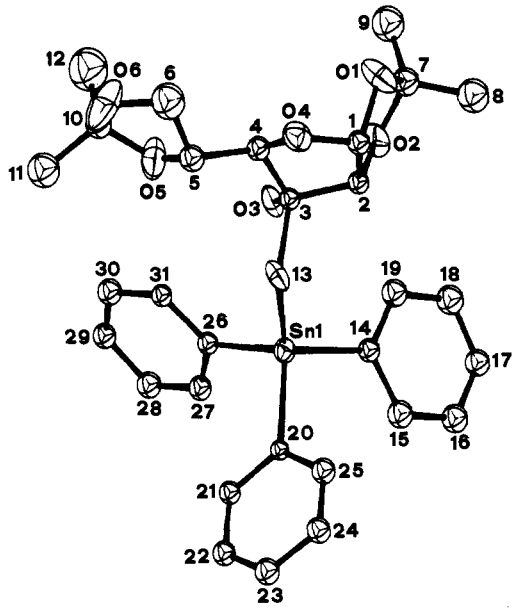

Figure 1. Computer-generated drawing of Ph_3SnCH_2 carbohydrate.

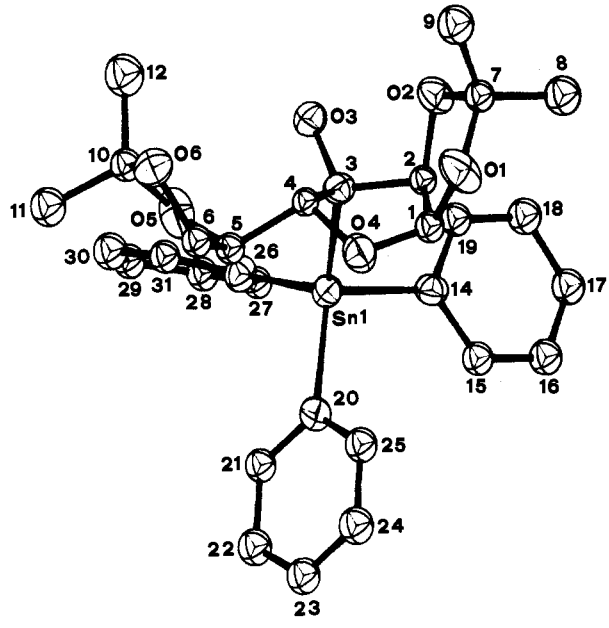

Figure 2. Computer-generated drawing of Ph_3Sn carbohydrate.

distance Sn–O(3) (see Figures 1 and 2) is 2.97 (1) Å (Ph_3SnCH_2 carbohydrate) and 2.898 (7) Å (Ph_3Sn carbohydrate). This is in between a normal covalent Sn–O bond, 2.07 Å,[6] and the corresponding sum of the van der Waals radii, 3.70 Å.[7]

These structures can be compared with the related compound 3-*C*-[(dibutyliodostannyl)methyl]-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose, referred to as Bu_2ISnCH_2 carbohydrate,[8a] which has one I and two *n*-butyl ligands instead of three phenyls as in Ph_3SnCH_2 carbohydrate. This compound has been described as having a distorted trigonal-bipyramidal arrangement with I and O (of the OH group) in axial positions and three *C*-alkyl atoms in the equatorial plane. Some relevant features of this structure are: Sn–O bond length of 2.68 Å; a long Sn–C (of *n*-butyl) bond distance (2.22 Å); and the bipyramidal axis angle (I–Sn–O) of 158°.[8a] Comparing these features to the present compounds, the latter equivalent atoms (C(20)–Sn–O(3)) form an angle of 161.3 (5)° (Ph_3SnCH_2 carbohydrate) and 140.4 (2)° (Ph_3Sn carbohydrate).

In order to describe the molecular geometry of the title compounds we have undertaken the following analysis. It is known that a perfect tetrahedron has six bond angles of 109.5° at the central atom. With the presence of different substituents the tetrahedron becomes distorted. Further we can consider a fifth ligand approaching the central atom of the tetrahedron and possibly stabilizing to a trigonal-bipyramidal configuration (TBP). Structures exist in between these two limits (tetrahedral and TBP). As we approach a TBP arrangement, the average of the six original tetrahedral angles tends to decrease. In Table SIV, given as supplementary material, some structural parameters for Sn–O bonded triphenyltins are listed. We present this table to see if the O atom at ca. 2.9 Å from Sn in our compounds is involved in bonding to the metal, in other words to see if the O can be a fifth ligand as was considered for Bu_2ISnCH_2 carbohydrate. The average value of the six bond angles is 109.4 (3.7)° and 109.4 (4.6)° for our compounds, which indicates in essence a tetrahedral arrangement for both compounds. From Table SIV it is evident that Sn–C (phenyls) bond distances are not sensitive to changes of coordination.

Investigating the Sn–ligand binding we see that the four Sn–C bond distances are equal (within esd) in Ph_3SnCH_2 carbohydrate (Sn–C(13) = 2.12 (2) Å, Sn–C(14) = 2.13 (2) Å, Sn–C(20) = 2.16 (2) Å, Sn–C(26) = 2.09 (2) Å). In Ph_3Sn carbohydrate, the three Sn–C (phenyl) bond distances are equal (Sn–C(14) = 2.15 (1) Å, Sn–C(20) = 2.166 (9) Å, Sn–C(26) = 2.13 (1) Å) but the sugar moiety is significantly farther than the phenyl groups (Sn–C(3) = 2.225 (8) Å). An explanation may be found by considering the previously mentioned repulsive interaction of the intramolecular H atoms (H(of C(1))–H(of C(18)) = 2.59 Å) that approaches the sum of the van der Waals radii (2.40 with reliability 0.10 Å[6]): a closer sugar unit implies a greater repulsion of these hydrogen atoms.

The three five-atom rings in the carbohydrate moiety of our complexes are all in the envelope (E) conformation. Generally this type of conformation has four atoms that lie approximately in a plane and the fifth one, the flap atom, at ca. 0.5 Å from the plane. As this atom comes closer to the plane, a twist (T) conformation becomes possible. For the T conformation it is useful to consider a plane made up of three successive ring atoms and to determine if the remainder two atoms are above and below the plane by approximately the same distance. These calculations to check the conformation of 3-*C*-R-1,2-5,6-di-*O*-isopropylidene-α-D-allofuranose compounds are reported in Table SV (deposited as supplementary material), and for comparison, 1,2-*O*-isopropylidene-α-D-allofuranose is also included. From Table SV a trend is apparent for the 1,2-dioxolane ring: the flap atom is closer to its plane than occurs for the other two rings. Bu_2ISnCH_2 carbohydrate shows the minimum value of the distance between the flap atom and the plane (d = 0.34 Å). If one considers the three-atom plane C(1)–C(2)–O(1), the deviations of the remainder atoms (O(2), C(7)) from this plane have opposite signs (–0.14 and 0.24 Å, respectively). Let us name Δ the difference of these absolute values, the absolute value is not the same (Δ = 0.10 Å); therefore the ring may be considered as having a hybrid conformation (E + T). The conformation for Ph_3Sn carbohydrate appears closer to the E-type because deviations from the plane C(1)–C(2)–O(1) are –0.12 Å (C(7)) and 0.30 Å (O(2)), with Δ = 0.18 Å. All of the other compounds in Table SV have the

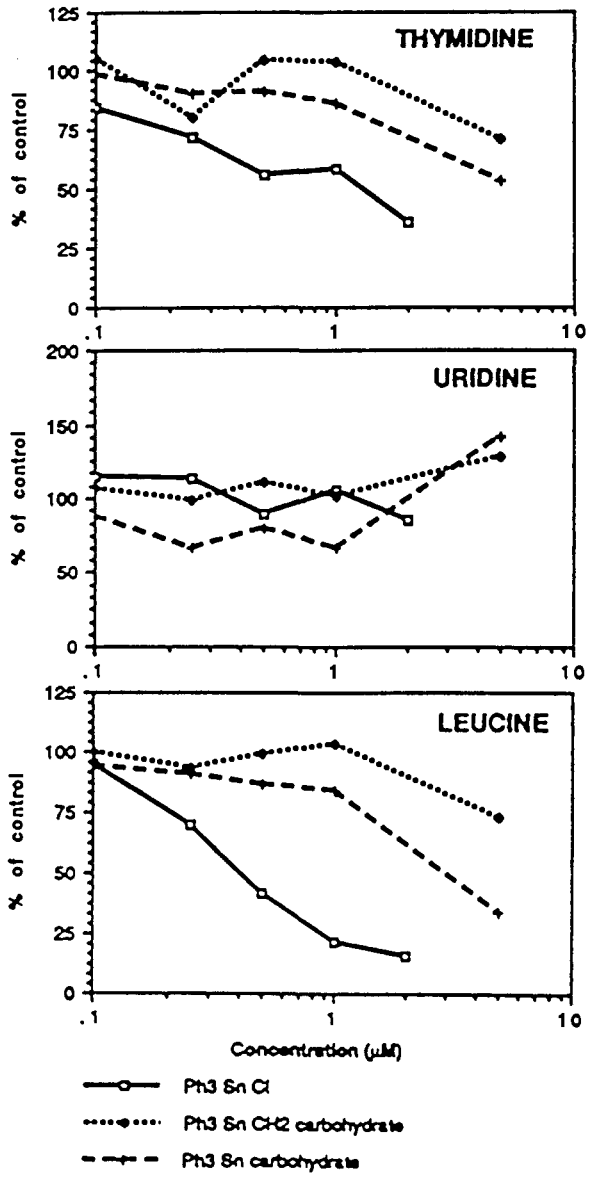

Figure 3. In vitro effects of the various triphenyltin compounds on the incorporation of [^{3}H]thymidine, [^{3}H]uridine and [^{14}C]-leucine of freshly isolated thymocytes. Results are expressed as the mean value of three experiments each performed in 4-fold.

envelope conformation. From Table SV C(4) and C(3) are favored flap atoms in the furanose ring; O(2), C(2), and C(7) are equally favored in the 1,2-dioxolane ring and C(6) has the highest occurrence as a flap atom in the 5,6-dioxolane ring.

(b) Biological Study. In Vitro Effects on Macromolecular Synthesis of Thymocytes. After a preincubation period of 30 min with the various triphenyltin compounds the incorporation of precursors for DNA, RNA, and protein synthesis of freshly isolated fast proliferating rat thymocytes was followed for an additional 1-h incubation period by adding [^{3}H]TdR, [^{3}H]Urd, and [^{14}C]-Leu, respectively. As shown in Figure 3 the incorporation of precursors for DNA and protein synthesis was concentration dependently reduced by Ph_3SnCl, whereas the RNA synthesis was not affected up to the highest dose level tested of 2 μM Ph_3SnCl. Protein synthesis was found to be most sensitive and reduced by approximately 80% at a level of 1 μM Ph_3SnCl, at which level DNA synthesis was inhibited by almost 50%. Ph_3Sn carbohydrate as well as Ph_3SnCH_2 carbohydrate were less effective than Ph_3SnCl. Up to 1 μM both compounds did not affect the macromolecular synthesis of thymocytes. At the highest dose level tested 5 μM, the DNA and protein synthesis were inhibited, with Ph_3Sn carbohydrate being somewhat more active than Ph_3SnCH_2 carbohydrate. Although up to 1 μM of both triphenyltin carbohydrates RNA synthesis was not affected the [^{3}H]Urd incorporation was increased at a level of 5 μM.

In Vitro Antitumor Activity. The in vitro effects of Ph_3SnCl and the triphenyltin carbohydrates were determined by their interference with the proliferation of four different mice tumor cell lines, as measured by the incorporation of [^{3}H]thymidine, after a 24-h incubation period. For comparative purposes di-*n*-butyltin dichloride was also tested. It was apparent from the results presented in Figure 4 that an almost equal concentration-dependent decrease of the DNA synthesis was observed with all tested compounds in the MOPC315, P815, SL2, and L1210 tumor cell lines. Ph_3SnCl and Bu_2SnCl_2 displayed the highest activity, which resulted in a complete inhibition of the [^{3}H]TdR incorporation of the various tumor cell lines at a dose level of 0.5 μM. A very steep effect curve was found between 0.1 and 0.5 μM of these organotin compounds. Ph_3SnCH_2 carbohydrate did not show an effect in any of the tumor cell lines up to the highest dose level tested of 5 μM. In contrast, Ph_3Sn carbohydrate dose-dependently decreased the [^{3}H]TdR incorporation of the various tumor cell lines, except for the L1210 tumor cell line in which DNA synthesis was only inhibited above 1 μM. In Table II the IC50 values of the various compounds are summarized and clearly demonstrate that both Ph_3SnCl and Bu_2SnCl_2 are almost equally and most active with IC50 values of approximately 0.2 μM. Ph_3Sn carbohydrate with IC50 values ranging from 0.5 to 0.7 μM is more active than Ph_3SnCH_2 carbohydrate which was inactive up to 5 μM.

Discussion

With regard to the actual mode of action of antitumor-active organotins no explanations are available from results obtained with tumor cells. On the basis of studies of Crowe et al.,[3b] it seems unlikely that organotin compounds will interact with DNA by cross-linking Sn with suitable oriented nitrogen bases as appears to explain the antitumor activity of cis-platin and its analogs.[9] Our current knowledge on cellular effects of organotins is mainly restricted to studies with cells of the immune system, because of the predominant immunotoxic effects of various di- and trisubstituted organotin homologues.[10] Besides direct cytotoxicity, di- and trisubstituted organotins affect cell energetics, membrane-associated functions, and macromolecular synthesis. As antitumor effects might result from effects on macromolecular synthesis, in particular protein synthesis,[11] the recently synthesized triphenyltin carbohydrates were screened for their activity on DNA, RNA, and protein synthesis of thymocytes. Compared to Ph_3SnCl the triphenyltin carbohydrates were less effective with regard to inhibition of DNA and protein synthesis. At a level of 1 μM Ph_3SnCl, when DNA and protein synthesis were reduced to approximately 50 and 20%, respectively, both triphenyltin carbohydrates did not show any effect. Only at a concentration of 5 μM DNA and protein synthesis were inhibited, with Ph_3SnCH_2 carbohydrate being less active than Ph_3Sn carbohydrate. At that dose level DNA and protein synthesis of thymocytes is almost abolished with Ph_3SnCl (data not shown). In accordance with previous observations with trialkyl-

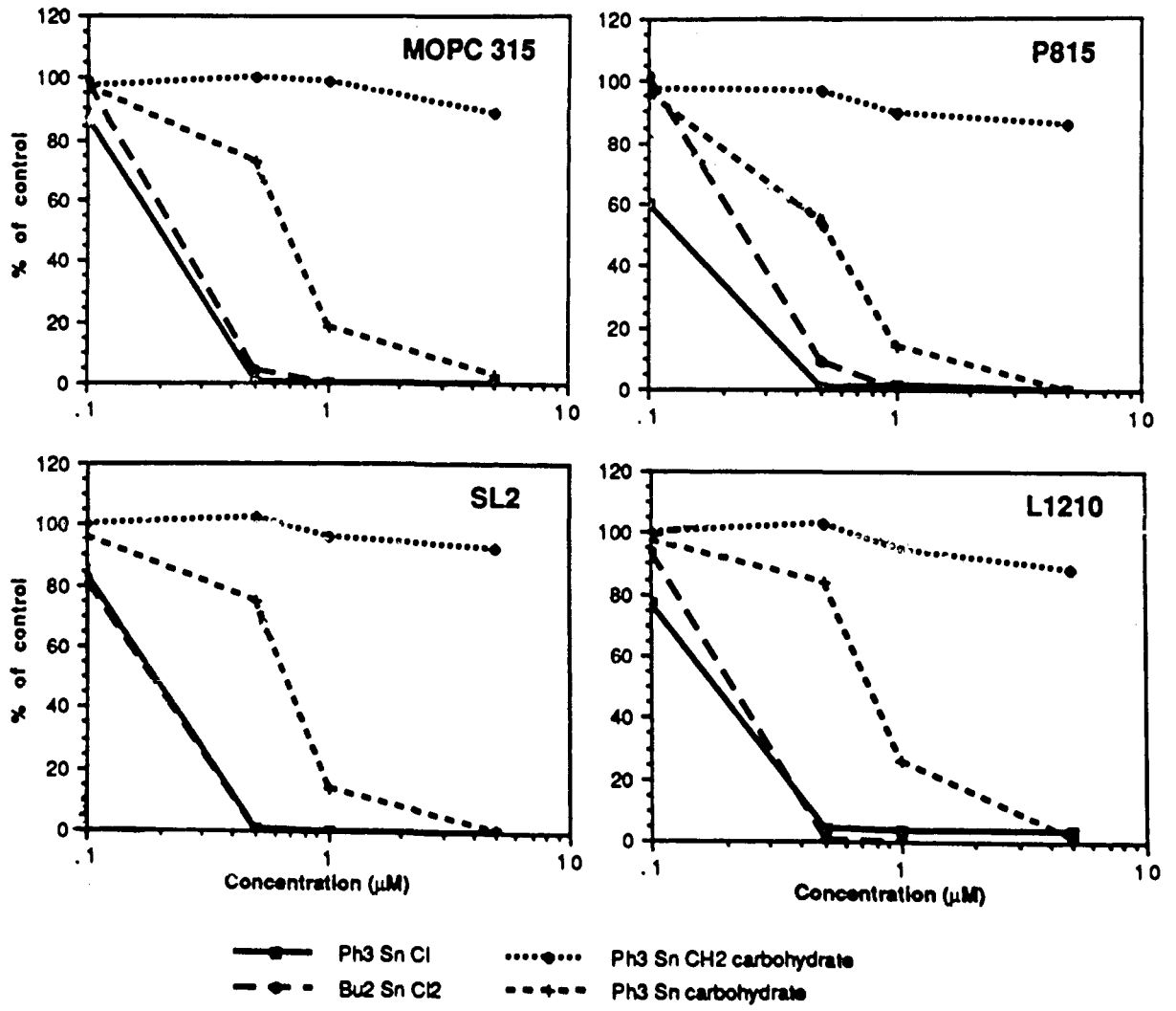

Figure 4. In vitro effects of the various triphenyltin compounds and Bu_2SnCl_2 on the [^{3}H]thymidine incorporation of four different mice tumor cell lines after a 24-h incubation period. Results are expressed as the mean value of two experiments each preformed in 4-fold.

Table I. Summary of Crystal Data and Intensity Collection

	Ph_3SnCH_2 carbohydrate	Ph_3Sn carbohydrate
formula	$C_{31}H_{36}O_6Sn$	$C_{30}H_{34}O_6Sn$
formula weight	623.34	609.31
a (Å)	6.073 (1)	8.3219 (9)
b (Å)	13.091 (3)	11.876 (1)
c (Å)	37.739 (13)	29.575 (5)
α (deg)	90	90
β (deg)	90	90
γ (deg)	90	90
volume (Å)^3	3000 (1)	2922.9 (7)
$F(000)$	1280.00	1248.00
μ	68.67	70.38
space group	$P2_12_12_1$	$P2_12_12_1$
Z	4	4
crystal dimensions (mm)	0.25 × 0.15 × 0.15	0.30 × 0.25 × 0.20
temperature	298 K	
radiation	Mo	Cu
data collection mode	ω-scan	
scan speed (deg/min)	2	
scan range (deg)	0.8	1.1
background counts	1/4 of scantime at 0.45 from the center of scan range	1/4 of scantime at 0.6 from the center of scan range
2θ limits (deg)	3, 60	3, 138
reflections collected	5246	3151
reflections used, $I > 3\sigma(I)$	1653	2530
R_f, R_w	0.062, 0.076	0.045, 0.060

substituted organotin compounds,[5] [^{3}H]Urd incorporation was also less sensitive for the triphenyltin compounds. At up to 1 μM of the various triphenyltin compounds, [^{3}H]-Urd was not affected, whereas at 5 μM of the triphenyltin

carbohydrates [^{3}H]Urd incorporation was somewhat increased. Previous studies revealed that with disubstituted organotins [^{3}H]Urd incorporation is dose relatedly stimulated,[11] whereas trisubstituted organotins in general inhibit [^{3}H]Urd incorporation, being a less sensitive parameter than the [^{3}H]TdR and [^{14}C]Leu incorporation. In this context the unexpected observations of an increased [^{3}H]Urd with the two triphenyltin carbohydrates cannot be explained, but seems to be coincidental. A more sensitive concentration range between 1 and 10 μM of the respective compounds should be studied to evaluate the relevance of this finding.

The triphenyltin carbohydrates also exhibited a lower in vitro antitumor activity if compared to Ph_3SnCl. The various compounds tested showed an almost equal concentration-dependent decrease of [^{3}H]TdR in the various tumor cell lines. As demonstrated by the IC50 values (Table II) Ph_3SnCl and Bu_2SnCl_2 were most effective, followed by Ph_3Sn carbohydrate and Ph_3SnCH_2 carbohydrate. The observation that Ph_3SnCl was somewhat more active than Bu_2SnCl_2 is in accordance with their previously observed in vitro activities toward various tumor cell lines of human origin.[5] Ph_3Sn carbohydrate demonstrated a higher activity in the in vitro antitumor assay if compared to its inhibitory activity on the macromolecular synthesis of thymocytes. The longer incubation period of 24 h in the antitumor assay versus 1.5 h in the thymocyte proliferation assay will be of importance in this respect. As discussed previously,[2,11] there are many indications to assume that the R_2Sn^{2+} and R_3Sn^+ moieties, in which R stands for various possible alkyl or aryl groups, are the ultimate reactive species of the di- and trisubstituted organotin halide or pseudohalide compounds as well as Sn–O, Sn–N, and Sn–S bonded ones, respectively. Since Sn–C bonds are considered to be more hydrolytically stable than Sn–halide, Sn–O, Sn–N, and Sn–S bonds, our triphenyltin carbohydrates (mainly Ph_3SnCH_2 carbohydrate) resemble tetraorganotin compounds which are considered to be biologically inactive, unless they are converted to triorganotin compounds by hydrolysis, metabolism,[12] or other mechanism. Based on our results both triphenyltin carbohydrates may be considered almost stable in the short-term study, whereas in the antitumor assay of 24 h, Ph_3Sn carbohydrate seems to be less stable, resulting in a higher concentration of the Ph_3Sn^+ species and consequently, a higher activity.

In summary, the active compound, Ph_3Sn carbohydrate, has a longer tin–sugar(C) bond than those of the tinphenyl C atoms. This lengthening may facilitate the conversion to Ph_3Sn^+ in comparison with the less active Ph_3SnCH_2 carbohydrate compound since it is known that a longer bond is a weaker bond. This structural feature (an easily cleaved Sn–C bond) appears not to be taken into account in previous antitumoral activity studies of organotins and is the major finding of this study. As reported above, the activity of di- and triorganotins is high against fast growing tumors, like leukemias. One may associate this action with a fast separation of the anionic moieties. Instead, the suspected Sn–C cleavage in Ph_3Sn carbohydrate produces a slow process, as shown by its lower activity in comparison with other organotins. One can speculate also that the lower activity of this compound may generate less undesirable side effects shown by organotins. This might be a way to decrease the high toxicity of organotin compounds while retaining the high antitumor activity.

Table II. IC50 Values of the Various Triphenyltin Compounds and Bu_2SnCl_2 in Four Different Mice Tumor Cell Lines, As Determined by the Reduction of the [^{3}H]Thymidine Incorporation after a 24-h Incubation Period[a]

	IC50[a] (μM)			
compound	MOPC315	P815	SL2	L1210
Ph_3SnCl	0.20	0.13	0.19	0.18
Ph_3Sn carbohydrate	0.67	0.54	0.66	0.76
Ph_3SnCH_2 carbohydrate	>5	>5	>5	>5
Bu_2SnCl_2	0.23	0.26	0.20	0.22

[a] IC50 = 50% reduction of the [^{3}H]thymidine incorporation after a 24-h incubation period.

Experimental Section

Synthesis of these triphenyltin carbohydrates has been described elsewhere.[8] Glucose was reacted to give 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose.[13,14] This keto-sugar was subsequently reacted with the organotin moieties Ph_3SnCH_2-Li or Ph_3SnLi yielding the addition products 3-*C*-[(triphenylstannyl)methyl]-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose, Ph_3SnCH_2 carbohydrate, melting point 102–104 °C, and 3-*C*-(triphenylstannyl)-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose, Ph_3Sn carbohydrate, melting point 118.5–120 °C, respectively. Crystalline samples of both triphenyltin carbohydrates were furnished by J. Wardell, University of Aberdeen, Scotland.

Collection and Reduction of X-ray Data. Data of the title compounds were collected with a Nicolet R3 diffractometer. Monitoring of four standard reflections ([0, 0, 4], [0, 0, −4], [1, 0, 7], [−1, 0, −7], Ph_3SnCH_2 carbohyrate, and [0, 1, 2], [0, −1, −2], [−1, 1, 0], [1, −1, 0], Ph_3Sn carbohydrate), taken every 100 reflections, indicated no decay during data collection. A ψ-scan of some suitable reflections with the χ angle close to 90° showed absorption phenomena only for Ph_3Sn carbohydrate (reflections [3, 0, 1] and [2, −1, 0]), and the corresponding correction was applied. Data were also corrected for Lorentz and polarization effects. Table I summarizes crystal parameters and details of data collection.

Solution and Refinement of the Structures. The structures were solved using Patterson methods and Fourier techniques to obtain all the non-H atoms. Refinement of atom parameters was by least-square procedures. The function minimized was $\Sigma w(|F_o| - |F_c|)$ with weights[15] of the type $w = a + F_o + cF_o^2$, where a and c are of the order of $2F_o$(min) and $2/F_o$(max). In the final cycles of least squares the H atoms were included at fixed positions, d(C–H) = 0.96 Å, C–C–H angle = 120 or 109.5°. Due to the low number of refined reflections in both compounds it was necessary to choose only certain atoms to be refined anisotropically. These were Sn, O, and C(13) for Ph_3CH_2 carbohydrate. Since the Ph_3Sn carbohydrate refinement has more available reflections, the ipso carbon of each phenyl ring was also refined anisotropically. These carbon atoms were chosen because of their proximity to the presumed active site of these compounds, the Sn environment. As can be seen in Figure 1 O(5), O(6), and C(6) have high displacement parameters, indicating that some disorder is likely to be present. However a Fourier difference map did not show additional peaks in the area. In addition, C(8), C(9), C(11), and C(12) also show high displacement parameters, but this is not unusual for terminal methyls groups. Calculations were performed by running the CAOS program[16] on an Eclipse Data General computer. Atomic scattering factors and anomalous dispersion terms were taken from the *International Tables for X-ray Crystallography*.

Test Compounds. The organotin compounds used in this study, Ph_3SnCH_2 carbohydrate and Ph_3Sn carbohydrate, were furnished by J. Wardell, University of Aberdeen; triphenyltin chloride (Ph_3SnCl) and di-*n*-butyltin dichloride (Bu_2SnCl_2) were kindly provided by H. A. Meinema, Institute for Applied Chemistry, TNO, Utrecht, the Netherlands. The triphenyltin carbohydrates, Ph_3SnCl and Bu_2SnCl_2, used in the various in vitro assays were dissolved in absolute ethanol just before addition to the cell suspensions. The final ethanol concentration was 0.1%, which did not affect any of the test systems.

Animals. Specific pathogen-free Wistar-derived male rats, Balb/c and DBA/2 male mice were purchased from the Central Institute for Breeding of Laboratory Animals, Harlan-CPB, Austerlitz, The Netherlands. The animals were housed in plastic cages maintained at room temperature of (23 ± 2 °C) and a relative humidity of 50–60% with a 12-h light/dark cycle. Diet (Hope Farms, Woerden, The Netherlands) and tap water were constantly available.

Tumor Transplantation. The L1210 (lymphoid leukemia), P815 (mastcell tumor), and SL2 (T cell leukemia) tumor cells were obtained from the Department of Pathology, University Hospital Utrecht, The Netherlands, and maintained in DBA/2 mice. The IgA plasmacytome MOPC315 was obtained from the Department of Immunology, University Hospital Utrecht, The Netherlands, and maintained in Balb/c mice. Transplantation of the various tumor cells was performed using a 7-day schedule. Therefore the tumor cells were harvested from the ascites (see below), washed, counted, and subsequently injected intraperitoneally in an amount of 3.10^5 MOPC315, 1.10^6 P815, 4.10^5 SL2, and 1.10^6 L1210 tumor cells in 8–12 week old mice.

Preparation of Tumor Cell and Thymocyte Suspensions. The tumor cells were isolated from the tumor-bearing mice at the 7th day after transplantation by flushing the peritoneal cavity with 4 mL of RPMI 1640 medium. The isolated tumor cells were washed twice in the same buffer by centrifugation at 1000 rpm for 10 min. To remove cell clumps, the cells were drawn through a 25-gauge needle after the last washing and finally suspended in RPMI 1640.

Thymocytes were isolated from the thymus glands immediately after decapitation of the rats. After removal of the adjoining lymph nodes the thymus glands were sampled in cold Dulbecco's phosphate buffered saline supplemented with 2 mM glucose (PBS/glu, pH 7.4). The glands were minced with sterile scissors in fragments and were then gently pressed through a nylon sieve with a pore diameter of 220 μm. The cells were washed twice with PBS/glu, and to remove cells clumps, the suspensions were drawn through a 25-gauge needle. The tumor cells and thymocyte suspensions were counted with an electronic particle counter (Coulter Counter, Model ZF, Coulter Electronics, Dunstable, Bedforshire, England), and their viability was determined with a saturated solution of trypan blue in 0.45% NaCl, supplemented with 50% heat inactivated homologues serum for trypan blue exclusion. At the start of the experiments the viability was always more than 96%.

Cell Culture Experiments. Nucleoside and Amino Acid Incorporation. In short-term experiments of 1.5 h the effects of equimolar concentrations of the various Ph_3Sn compounds were studied on the basal DNA, RNA, and protein synthesis of freshly isolated thymocytes. Suspensions of 2.10^7 thymocytes in PBS/glu were incubated in a shaking water bath at 37 °C in the presence of graded concentrations of the various organotin compounds. After a 30-min preincubation period 1 μCi/mL [^{3}H]-thymidine ([^{3}H]TdR; final concentration 20 nM), 1 μCi/mL ([^{3}H]-Urd, final concentration 20 nM) or 50 nCi/mL [^{14}C]leucine ([^{14}C]Leu, final concentration 145 μM) was added to the thymocyte suspensions. At 30 and 60 min after label addition samples were taken in 4-fold and the cells were harvested on fiber filters (Cell Culture harvester, Skatron, Lierbyen, Norway) using a 5% solution of trichloroacetic acid. The filters were dried and transferred to scintillation vials containing 5 mL of scintillation fluid (β-count, Baker, Deventer, The Netherlands), and the radioactivity was counted in a Kontron MR 300 liquid scintillation counter.

Proliferation Assay. In a 24-h culture assay the effects of the various Ph_3Sn compounds and Bu_2SnCl_2 were studied on the proliferation of the four different tumor cell lines by determination of the [^{3}H]thymidine incorporation. After isolation and washing of the tumor cells (see above) they were preincubated for 4 h at a concentration of 2.10^5 cells/mL in RPMI 1640 medium supplemented with 10% fetal calf serum and 1 mM glutamine in an incubator with a humid atmosphere of 5% CO_2 in air. After this 4-h preincubation period the cells were washed again and concentrated to a suspension of 4.10^5 cells/mL. From the tumor cell suspensions 100 μL were added into wells of 96-well microtiter plates. Stock solution of the various Ph_3Sn compounds and Bu_2SnCl_2 in ethanol were diluted in complete RPMI 1640, of which 100 μL was added to the tumor cells to obtain the desired final concentration. The microtiter plates were subsequently incubated at 37 °C in a CO_2 incubator for 24 h. After the first hour of culture, 0.5 μCi [^{3}H]TdR was added, and after an additional 8-h incorporation period the tumor cells were harvested and the radioactivity counted as described above.

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Supplementary Material Available: Atomic coordinates (Tables SI and SII), bond distances and angles (Table SIII), structural parameters in Sn–O triphenyltins (Table SIV), conformational data for the sugar moiety (Table SV), listings of displacement parameters (Tables 1S, 2S), and hydrogen coordinates (Tables 3S, 4S) of both compounds (11 pages). Ordering information is given on any current masthead page.

References

(1) (a) Piver, W. T. Organotin Compounds. Industrial Applications and Biological Investigation. *Environ. Health Perspect.* **1973**, *4*, 61–79. (b) van der Kerk, G. J. M. Organotin Chemistry: Past, Present and Future. In *Organotin Compounds: New Chemistry and Applications*; Zuckerman, J. J., Ed.; American Chemical Society: Washington, 1976; pp 1–25.

(2) Crowe, A. J. The Chemotherapeutic Properties of Tin Compounds. *Drugs Future* **1987**, *12*, 255–275.

(3) (a) Crowe, A. J.; Smith, P. J.; Atassi, G. Investigations into the Antitumor Activity of Diorganotin Compounds. I. Diorganotin Dihalides and Di-pseudohalide Complexes. *Chem. Biol. Interact.* **1980**, *32*, 171–178. (b) Crowe, A. J.; Smith P. J.; Atassi, G. Investigations into the Antitumor Activity of Organotin Compounds. 2. Diorganotin Dihalide and Dipseudohalide Complexes. *Inorg. Chim. Acta* **1984**, *93*, 179–184.

(4) (a) Barbieri, R.; Pellerito, L.; Ruisi G.; Lo Giudice, M. T. The Antitumor Activity of Diorganotin(IV) Complexes with Adenine and Glycylglycine. *Inorg. Chim. Acta* **1982**, *66*, L39–40. (b) Saxena, A.; Tandon, J. P. Antitumor Activity of some Diorganotin and Tin(IV) Complexes of Schiff Bases. *Cancer Lett.* **1983**, *19*, 73–76. (c) Haiduc, I.; Silvestra, L.; Gielen, M. Diorganotin Compounds: New Organometallic Derivatives Exhibiting Antitumor Activity. *Bull. Soc. Chim. Belg.* **1983**, *92*, 187–189. (d) Huber, F.; Roge, F.; Carl, L.; Atassi, G.; Spreafico, F.; Filippeschi, S.; Barbieri, R.; Silvestri, A.; Rivarola, E.; Ruisi, G.; Di Bianca F.; Alonzo, G. Studies on the Antitumor Activity of Di- and Triorganotin(IV) Complexes of Amino Acids and Related Compounds, of 2-Mercaptoethanesulfonate, and of Purine-6-thiol. *J. Chem. Soc., Dalton Trans.* **1985**, 523–527. (e) Ruisi, G.; Silvestri, A.; Lo Giudice, M. T.; Barbieri, R.; Atassi, G.; Huber, F.; Graatz K.; Lamartina, L. The Antitumor Activity of Di-n-butyltin(IV) Glycylglycinate, and the Correlation with the Structure of Dialkyltin(IV) Glycylglycinates in Solution Studied by Conductivity Measurements and by Infrared, Nuclear Magnetic Resonance, and Mossbauer Spectroscopic Methods. *J. Inorg. Biochem.* **1985**, *25*, 229–245. (f) Gielen, M.; Willem, R.; Mancilla, T.; Ramharter J.; Joosen, E. Strategy for the Development of Novel Organotin Anticancer Agents. *Silicon, Germanium, Tin Lead Compd.* **1986**, *9*, 349–365. (g) Penninks, A. H.; Bol-Schoenmakers, M.; Gielen, M.; Seinen, W. A Comparative Study with Di-n-butyltin Dichloride and Various Sn-O Bonded Di-n-butyltin Derivatives on the Macromolecular Synthesis of Isolated Thymocytes and the in vitro and in vivo Antitumour Activity. *Main Group Metal Chem.* **1989**, *12*, 1–15.

(5) Penninks, A. H.; Punt, P. M.; Bol-Schoenmakers, M.; van Rooijen, H. J. M.; Seinen, W. Aspects of the Immunotoxicity, Antitumor Activity and Cytotoxicity of Di- and Trisubstituted Organotin Halides. *Silicon, Germanium, Tin Lead Compd.* **1986**, *9*, 367–380.

(6) Pauling, L. In *The Nature of the Chemical Bond*; Cornell University: Ithaca, New York, 1960; pp 246–261.

(7) Swisher, R. G.; Vollano, J. F.; Chandrasekhar, V.; Day, R. O.; Holmes, R. R. Pentacoordinated Molecules. 54. Pentacoordinated Structures of Triphenyltin Esthers of Anthranilic Acid, o-Dimethylaminobenzoic Acid, and p-Aminobenzoic Acid Formed by Intramolecular Carboxylate Group Coordination. *Inorg. Chem.* **1984**, *23*, 3147–3152.

(8) Cox, P. J.; Doidge-Harrison, S. M. S. V.; Howie, R. A.; Nowel, I. W.; Taylor, O. J.; Wardell, J. L. C-Stannylated Carbohydrate Derivatives. Part 3. 1,2-5,6-di-O-isopropylidene-3-C-(organostannyl)methyl-α-D-allofuranose Derivatives. Crystal and Molecular Structure of 3-C-(Dibutyliodostannyl)-methyl-1,2:5,6-di-O-isopropylidene-α-D-allofuranose. *J. Chem. Soc., Perkin Trans.* **1989**, 2017–2022. (b) Taylor, O. J.; Wardell, J. L.; Mazhar, M. C-Stannylated Carbohydrate Derivatives. Part 2. Effect of the Structure of the Sugar on the Reactivities of (Triphenylstannyl)carbohydrate Derivatives towards Iodine. *Main Group Met. Chem.* **1989**, *12*, 107–115.

(9) Prestayko, A. W. Cisplatin: a Preclinic View. In *Cisplatin: Current Status and New Developments*; Prestayko, A. W., Crooke, S. T., Carter, S. K., Eds.; Academic Press: New York, 1980; pp 1–7. (b) Sherman, S. E.; Gibson, D.; Wang, A. H. J.; Lippard, S. J. X-ray Structure of the Major Adduct of the Antitumor Drug Cisplatin with DNA: Cis-[Pt(NH_3)$_2$d(pGpG)]. *Science* **1985**, *230*, 412–417.

(10) (a) Seinen, W.; Penninks, A. H. Immunosuppression as a Consequence of a Selective Cytotoxic Activity of Certain Organometallic Compounds on Thymus and Thymus-dependent Lymphocytes. *Ann. N.Y. Acad. Sci.* **1979**, *320*, 499–517. (b) Snoeij, N. J.; Penninks, A. H.; Seinen, W. Biological Activity of Organotin Compounds - An Overview. *Environ. Res.*, **1987**, *44*, 335–353. (c) Boyer, I. J. Toxicity of Dibutyltin, Tributyltin, and other Organotin Compounds to Humans and to Experimental Animals. *Toxicology* **1990**, *55*, 253–298.

(11) Penninks, A. H.; Bol-Schoenmakers, M.; Seinen, W. Cellular Interactions of Organotin Compounds in Relation to their Antitumor Activity. In *Tin-based Antitumor Drugs*; Gielen, M., Ed.; Nato ASI series, Series H: Cell Biology; Springer-Verlag: Berlin, 1990; Vol. 37, pp 169–170.

(12) Cremer, J. E. The Biochemistry of Organotin Compounds. The Conversion of Tetraethyltin into Triethyltin in Mammals. *Biochem. J.* **1958**, *68*, 685–692 (b) Iwai, H.; Wada, O. Dealkylation of Tetraalkyltin Compounds in the Intestinal Mucosa of Rabbits. *Ind. Health* 1981, *19*, 247–253.

(13) Schmidt, O. T. Hydride Reduction of 6-Deoxy-α-D-glucose-lithiumaluminum. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Wolfrom, M. L., Eds.; Academic Press: New York, 1963; Vol. 1, pp 198–201.

(14) Jones, G. H.; Moffat, J. G. Oxidation of Carbohydrates by the Sulfoxide-carbodiimide and Related Methods. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Wolfrom, M. L., Eds.; Academic Press: New York, 1972; Vol. 6, pp 315–322.

(15) Cruickshank, D. J. Errors in Least-squares Methods. In *Computing Methods in Crystallography*; Rollet, J., Ed.; Pergamon Press: Oxford, U. K. 1965; pp 112–116.

(16) Cerrini, S.; Spagna, R. Caos Program, *4th European Crystallographic Meet.*, Oxford, U.K., 1977, pp 7.