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Synthesis, antitumor activity and crystal structure of the triphenyltin derivative of *exo*-7-oxa-bicyclo[2,2,1]hept-5-ene-3-*N*-*p*-tolylamide-2-acid

Jinshan Li,* Yongqiang Ma, Runchang Liu and Jing Li
National Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, People's Republic of China

The synthesis and crystal structure of $Ph_3SnO_2CCHCHCH{:}CHCH(O)CHCONHC_6H_4CH_3{\cdot}CH_2Cl_2$ are reported. The monomer units are bridged by the carbonyl oxygen of the amide group, thus forming a polymer in which each tin atom is best described as having a distorted five-coordinate geometry. There is a relatively strong intramolecular hydrogen bond between the amide hydrogen and the ether oxygen. The *in vitro* antitumor activities of the title compound against HL-60, BGC-823, Bel-7402, SKOV3, KB and Hela tumor lines are reported. The title compound shows a distinct advantage when the metal (tin) is introduced into the acid.

Keywords: organotin carboxylate; crystal structure; antitumor activity

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1 INTRODUCTION

The study of organotin compounds is of current interest owing to their wide range of applications,[1–3] such as in biocides and as homogeneous catalysts in industry. Recently, the potential antitumor activity of organotin carboxylates has been widely studied.[4–6] As is well known, cantharidin (*exo*-2,3-dimethyl-7-oxa-bicyclo[2,2,1]heptane-2,3-dicarboxylic anhydride) and its derivatives have good biological activities, for example they have both anticonvulstant[7] and antitumor activities.[8] In order to investigate whether incorporating an organotin into *exo*-7-oxa-bicyclo[2,2,1]hept-5-ene-3-*N*-*p*-tolylamide-2-acid improves its antitumor activities with reduction in toxicity, the title compound was prepared and its antitumor activity determined. At the same time, we were also interested in studying the bonding in the title compound.

* Correspondence to: Jinshan Li, National Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, People's Republic of China.

2 EXPERIMENTAL

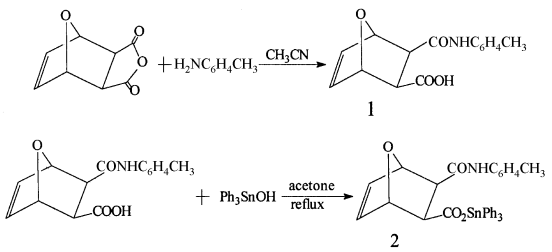
2.1 General

All operations were performed in an atmosphere of dry argon using Schlenk and vacuum techniques. All solvents were dried by standard methods and distilled prior to use. Elemental analyses were determined on a Yanaco CHN Corder MT-3 elemental analyzer. IR spectra were recorded on a Bruker Equinox 55 spectrometer in KBr discs or in $CHCl_3$ solution. ^{1}H NMR spectra were measured on a Bruker AC-200 spectrometer in $CDCl_3$ solution with TMS as internal standard. Mass spectra (EI) were recorded on an HP-5988A at 70 eV; the ionization temperature was 200 °C.

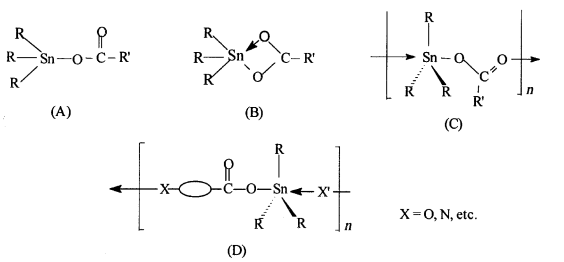
2.2 Synthesis

Ph_3SnOH was prepared by a standard method.[7] *exo-cis*-7-Oxa-bicyclo[2,2,1]hept-5-ene-3-amide-2-acid (compound **1**), m.p. 192–194 °C, was prepared by stirring a CH_3CN solution of *exo-cis*-7-oxa-bicyclo[2,2,1]hept-5-ene-2,3-dicarboxylic anhydride with *p*-methylaniline; the crude product was recrystallized from ethanol–petroleum ether (85% yield; Scheme 1).

A mixture of triphenyltin hydroxide (0.55 g) and compound **1** (0.41 g) in 1:1 molar ratio was refluxed in acetone for 6 h. After that time a white solid



Scheme 1



Scheme 2

formed, which was separated by filtration and crystallized from CH_2Cl_2–petroleum ether; yield 0.76 g (81.7%); m.p. 101–102 °C. Anal. Found C, 58.22; H, 4.17; N, 2.04. Calc. for $C_{33}H_{29}NO_4Sn\cdot CH_2Cl_2$: C, 57.74; H, 4.42; N, 1.98%. ^{1}H NMR ($CDCl_3$): δ 2.19 (s, 3H, CH_3), 2.86–2.98 (q, 2H, COCHCHCO), 5.11–5.43 (d, 2H, CHOCH), 6.66–6.78 (q, 2H, CH═CH), 6.95–7.06 (q, 4H, C_6H_4), 7.29–7.56 (m, 15H, C_6H_5), 8.12 (s, 1H, CONH). IR: (i) KBr: ν_{asy}(COO) 1649, ν_{sym}(COO) 1351, $\Delta\nu$(COO) 298, ν(CONH) 1629, ν(N—H) 3319 cm^{-1}; (ii) $CHCl_3$: ν_{asy}(COO) 1651, ν_{sym}(COO) 1358, $\Delta\nu$(COO) 293, ν(CONH) 1678, ν(N—H) 3342 cm^{-1}. MS (positive ions, relative intensity): *m/z* 351 (Ph_3Sn^+, 11), 272 [$(M\text{-}Ph_3Sn)^+$, 1], 77 (Ph^+, 100).

2.3 Crystallography

Diffraction data for compound **2** were obtained at 293 K on a Bruker Smart 1000 diffractometer (graphite-monochromatized Mo Kα radiation, λ = 0.710 73 Å). Of the total 18 227 reflections recorded, 6586 reflections (R_{int} = 0.0526) were used for the structure determination and refinement. The crystal class, orientation matrix and accurate unit-cell parameters were determined by standard procedures. The intensities were corrected for absorption using the SADABS program. The structure was solved by heavy-atom methods and refined by a full-matrix least-squares procedure based on F^2. Non-hydrogen atoms were refined with anisotropic thermal parameters.

Crystal data: $C_{33}H_{29}NO_4Sn\cdot CH_2Cl_2$; 0.30 × 0.25 × 0.20 mm^3; monoclinic; space group $P2_1/c$; unit cell dimensions: a = 12.3712(10), b = 18.4488(15), c = 14.1062(11) Å; β = 91.137(2) °, V = 3218.9(4) $Å^3$; Z = 4; D_c = 1.459 Mg m^{-3}; $F(000)$ = 1432; final R indices $[I > 2\sigma(I)]R_1$ = 0.0462, wR_2 = 0.1358; μ = 0.997 mm^{-1}.

2.4 Antitumor activity determination

2.4.1 Cell lines

The KB cell lines and Hela cell lines were obtained from the Institute of Cancer, Tianjin. Other cell lines were derived in the National Research Laboratories of Natural and Biomimetic Drugs of Peking University. The cell lines were maintained in a continuous logarithmic culture in RPMI 1640 medium supplemented with 10% fetal calf serum and in 5% CO_2 atmosphere.

2.4.2 Compound 2 assay

Compound **2** was tested for cytotoxic activity *in vitro* according to the MTT[9] or SRB[10] methods. The following cell lines were used for screening: human immature granulocyte leukemia (HL-60), human hepatocellular carcinoma (Bel-7402), human gastric carcinoma (BGC-823), human colo carcinoma (HCT-8), human ovarian carcinoma (SKOV-3), human nasopharyngeal carcinoma (KB) and human cervical carcinoma (Hela). The cell lines were transferred into the 96-wells cell culture plate against which the compounds were tested at three different concentrations (10, 1, 0.5 μM). 50 μl of 0.1% MTT or SRB (Sigma) were added to each well. A 48 h continuous drug exposure protocol was used. The cytotoxicity was determined by expressing the mean optical densities for drug-treated cells at each concentration as a percentage of that of untreated cells. Results are presented in Table 4.

3 RESULTS AND DISCUSSION

Compound **1** was prepared under mild condition (Scheme 1). Previous workers[7] had failed to prepare compound **1** in benzene under reflux and also found that the reaction product was the half

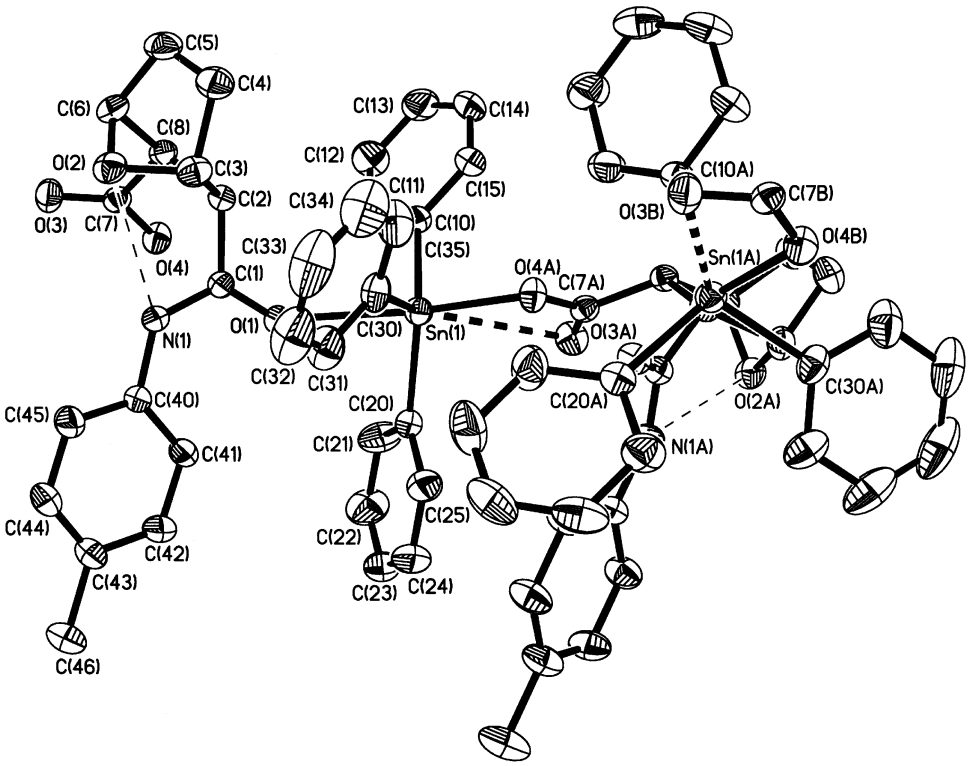


Figure 1 Molecular structure and crystallographic numbering scheme for compound **2**.

amide of maleic acid. This reaction does not occur in methanol or ethanol, and an anti-Diels–Alder reaction was found in toluene under reflux. However, in CH_3CN the reaction occurs readily on stirring at room temperature. Compound **2** is a white crystalline solid with a sharp melting point. It is easily soluble in organic solvents such as chloroform and dimethyl sulfoxide, but it is not stable in CH_2Cl_2 solution. The solution changes from colorless to yellow and the yellow solid from

Table 1 Selected bond distances and bond angles of compound **2**

Bond	Distances (Å)	Bond	Angles (°)
Sn(1)—O(4A)	2.106(3)	O(4A)—Sn(1)—O(1)	170.06(10)
Sn(1)—C(10)	2.130(4)	O(4A)—Sn(1)—C(20)	107.72(13)
Sn(1)—C(20)	2.119(4)	O(4A)—Sn(1)—C(10)	95.51(13)
Sn(1)—C(30)	2.132(4)	O(4A)—Sn(1)—C(30)	89.96(14)
Sn(1)—O(1)	2.542(3)	C(20)—Sn(1)—C(10)	122.39(16)
Sn(1)—O(3A)	3.079(3)	C(20)—Sn(1)—C(30)	114.00(18)
O(1)—C(1)	1.231(4)	C(10)—Sn(1)—C(30)	118.03(17)
O(3)—C(7)	1.219(5)	C(20)—Sn(1)—O(1)	79.26(12)
C(7)—O(4)	1.298(5)	C(10)—Sn(1)—O(1)	86.27(12)
N(1)—C(1)	1.335(5)	C(30)—Sn(1)—O(1)	80.60(13)
N(1)—O(2)	2.856(5)	C(1)—O(1)—Sn(1)	130.9(2)
N(1)—H(1)	0.860	N(1)—C(1)—C(2)	114.8(3)
H(1)—O(2)	2.180	O(2)—C(3)—C(2)	100.6(3)
		C(7)—O(4)—Sn(1)	117.4(2)
		O(2)—H(1)—N(1)	135.27

Table 2 Fractional coordinates ($\times 10^4$) and thermal parameters ($Å^2 \times 10^3$) of non-hydrogen atoms for compound **2**

	x	*y*	*z*	$U_{(eq)}$
Sn(1)	2356(1)	2188(1)	6476(1)	41(1)
O(1)	2892(2)	2840(1)	8002(2)	43(1)
O(2)	1945(2)	5032(1)	8428(2)	52(1)
O(3)	1899(3)	4315(2)	10 607(2)	57(1)
O(4)	1866(2)	3170(2)	10 117(2)	50(1)
N(1)	3503(2)	3897(2)	8623(2)	43(1)
Cl(1)	6632(5)	1695(3)	629(5)	271(3)
Cl(2)	8561(6)	1263(4)	830(7)	206(3)
Cl(3)	7583(13)	649(7)	1891(6)	338(9)
C(40)	4626(3)	3758(2)	8752(3)	42(1)
C(41)	5072(3)	3075(2)	8680(4)	55(1)
C(42)	6185(4)	2997(3)	8851(4)	66(1)
C(43)	6838(4)	3584(3)	9085(4)	65(1)
C(44)	6371(4)	4251(3)	9134(4)	67(1)
C(45)	5276(4)	4338(2)	8969(4)	59(1)
C(46)	8043(4)	3484(4)	9258(5)	92(2)
C(1)	2731(3)	3467(2)	8261(3)	38(1)
C(2)	1630(3)	3818(2)	8153(3)	39(1)
C(3)	1710(3)	4570(2)	7641(3)	50(1)
C(4)	563(4)	4792(3)	7371(4)	63(1)
C(5)	116(4)	4990(2)	8157(4)	60(1)
C(6)	959(3)	4881(2)	8928(3)	49(1)
C(7)	1677(3)	3858(2)	10 010(3)	41(1)
C(8)	1074(3)	4051(2)	9088(3)	39(1)
C(9)	7500(10)	1531(7)	1614(6)	301(14)
C(10)	838(3)	1999(2)	7119(3)	43(1)
C(11)	733(4)	1935(3)	8082(3)	59(1)
C(12)	−260(4)	1806(4)	8477(4)	76(2)
C(13)	−1167(4)	1746(3)	7911(5)	77(2)
C(14)	−1079(4)	1804(3)	6968(4)	72(2)
C(15)	−104(4)	1934(3)	6556(4)	59(1)
C(20)	3772(3)	1608(2)	6875(3)	46(1)
C(21)	3862(4)	1260(3)	7735(4)	74(2)
C(22)	4825(5)	947(4)	8031(5)	90(2)
C(23)	5704(5)	978(3)	7486(5)	84(2)
C(24)	5646(4)	1308(4)	6633(5)	85(2)
C(25)	4680(4)	1628(3)	6333(4)	66(1)
C(30)	2660(4)	3241(2)	5918(3)	54(1)
C(31)	3647(5)	3570(3)	6063(4)	75(2)
C(32)	3844(7)	4266(4)	5708(6)	104(2)
C(33)	3043(10)	4619(4)	5223(5)	113(3)
C(34)	2087(8)	4304(4)	5055(5)	104(2)
C(35)	1877(6)	3613(3)	5414(4)	78(2)

the solution showed a different melting point and IR spectra.

3.1 IR

The IR spectra of the title compound have been recorded in the range of 4000–400 cm^{-1}. It is well known that there are mainly four kinds of structure for triorganotin carboxylates;[11,12] the four-coordinate structure for the monomer (A), the *cis* five-coordinate structure (B), the polymeric structure due to the carboxylate group (C) and the polymeric structure due to a donor bridging atom in the R′ part of the monomer unit (D) (see Scheme 2).

The IR data of the triorganotin carboxylates are most instructive in comparing solid- and solution-state structures. Extensive work using IR spectra has confirmed that most compounds of the type R_3SnO_2CR' are coordination polymers in the solid phase. In solution, or in the liquid phase, the compounds are monomeric.[13–14] For compound **2**, $\delta\nu$(COO) in solid and in a solution of $CHCl_3$ are almost the same, and this result indicates that the carboxylate may be in form A. However, ν(CONH) in the solid is shifted to 1629 cm^{-1} from 1678 cm^{-1} in $CHCl_3$. These results show that the polymeric form D bridged by C═O of CONH in the solid (confirmed by X-ray analysis) is disrupted in solution to give the monomer A. The N—H stretching frequency in the solution is higher than in the solid. However, the N—H stretching frequencies, both in the solid and in the solution, are shifted higher from the free ligand (compound **1**, ν(N—H) 3278 cm^{-1}). We interpret these features in terms of intramolecular hydrogen bonding in the crystal of compound **2**.

3.2 Crystal structure

A colorless single crystal was obtained from CH_2Cl_2–petroleum ether (60–90 °C). The structure of the asymmetric unit with atom numbering of compound **2** is shown in Fig.1. Selected bond lengths and angles are given in Table 1. Final fractional atomic coordinates for the non-hydrogen atoms are listed in Table 2. Triorganotin(IV) carboxylates are generally five-coordinate carboxylate-bridged polymers in which the repeat units are propagated in a zig-zag or helical manner in the crystal lattice.[15–17] However, the structure of the title compound consists of polymeric chains of the asymmetric unit in which triphenyltin moieties are bridged by the carbonyl oxygen of amide from the adjacent molecule, rather than the carbonyl oxygen of the carboxylate. The tin atoms have a distorted trigonal bipyramid environment; in apical positions there are atoms O(4) of the carboxylate group and O(1) of the carbonyl oxygen of amide from an adjacent unit. At the same time it may be inferred

Table 3 Selected geometric parameters in structures of some triorganotin carboxylates

Compound	Sn—O(1) (Å)	Sn…D (Å)	T[a]	Δ(Sn)[b] (Å)	Ref.
$Ph_3SnO_2CCH_3$	2.19	2.35(O)	C		18
$Ph_3SnO_2C(CH_2)_2COPh$	2.27	2.25(O)	C	−0.015	14
$Ph_3SnO_2CC_5H_4N$	2.14	2.57(N)	D	0.12	19
$Ph_3SnO_2CC_6H_4$-2-OH	2.08	3.03(O)	C	0.38	20
$Ph_3SnO_2CC_6H_4$-2-Cl	2.20	2.38(O)	C	0.027	21
$Me_3SnO_2CH_2NH_2$	2.14	2.57(N)	D	0.12	22
$Me_3SnO_2CCH(OH)Ph$	2.15	2.57(O)	D	0.22	15
	2.15	2.54(O)		0.18	
$Ph_3SnO_2CCH_2NHCONH_2$	2.14	2.35(O)	D	0.13	13
—[c]	2.11	2.54(O)	D	0.29	This work
—[d]	2.06–2.12	2.63–3.11		0.39–0.63	15
—[e]	2.14–2.25	3.11–3.66		0.08–0.21	15

[a] Structures for triorganotin carboxylates.
[b] Deviation of Sn atom from the equatorial plane.
[c] Compound **2**.
[d] In monomeric triorganotin carboxylates.
[e] In polymeric carboxylates with bridging carboxylate groups.

that this polymeric structure is more likely than an intramolecular chelate structure, since the latter would imply a seven-membered chelate ring of doubtful stability.

The O(1)—Sn(1)—O(4) bond angle is only 170.1(10)°. The geometry at tin, however, is distorted, as is evident from the oxygen–tin–carbon angles [O(4)—Sn(1)—C(20) 107.72(13), O(4)—Sn(1)—C(10)95.51;C(20)—Sn(1)—O(1)79.26(12), C(30)—Sn(1)—O(1) 80.60(13)°], which should be 90° in trigonal bipyramidal geometry. The distortion is also seen in the sum of the angles subtended in the equatorial plane (354.4°); and the tin atom is displaced out of the equatorial plane by 0.2920 Å towards the ester oxygen [O(4A)]. This may be attributed to the amide nitrogen lone-pair electron being still available to hydrogen bond, so the electron shift, which increases the basicity of the amide oxygen, is less than expected, and the result is that the bridging is not as strong as in the other cases shown in Table 3. The tin geometry lies between four- and five-coordinate, as shown by the ΔSn values in Table 3. The distortion can be attributed to the weak Sn—O(3A) interaction [Sn—O(3A) 3.079 Å]. This is well within the sum of the two van der Waals' radii (3.70 Å)[21], although it is long in comparison with both the covalent and bridging coordinate Sn—O bonds (see Table 3). The electronegativity of the carbonyl oxygen [O(3A)] of the carboxylate is greater than the ether oxygen [O(2)]. However, the hydrogen bond happens at O(2), and this makes the formation of Sn—O(3A) chelation possible. So the geometry at tin should be described as distorted five-coordination trigonal bipyramid and perhaps tending to a distorted octahedron. In the crystal lattice CH_2Cl_2 is

Table 4 Inhibition ratio against some tumor lines of compounds **1** and **2** *in vitro*

	Inhibition ratio 10 μM (1 μM)						
Compound	HL-60	HCT-8	BGC823	Bel-7402	SKOV3	KB	Hela
1	17.74 (12.98)	18.35 (18.76)	−5.90 (1.08)	−6.29 (−7.12)	−10.51 (−12.59)	17.54 (17.92)	3.65 (3.36)
2	93.40 (62.64)	87.60 (42.46)	93.97 (13.28)	96.04 (7.03)	88.82 (6.09)	97.27 (49.14)	90.90 (10.60)
$Ph_3SnO_2CCH_3$	93.84 (2.99)	68.35 (−4.99)	85.07 (10.44)	3.59 (−6.69)	—	98.74 (4.17)	98.18 (19.85)

disordered, and the occupances of Cl(2) and Cl(3) are both 0.5.

There is a relatively strong intramolecular hydrogen bonding interaction between the amide hydrogen [N(1)—H] and the ether oxygen O(2), forming a six-membered ring (the six atoms of this ring are coplanar within ±0.047 Å); this makes the weak effect of Sn—O(3A) more likely. In $Ph_3SnO_2CCH_2NHCONH_2$,[11] however, the polymer units are held together by intermolecular hydrogen bonding interactions between the amide hydrogen [N(1)—H] and the carboxylate carbonyl oxygen [O(2)] of an adjacent molecule. The N(1)—O(2) distance of compound **2** is 2.856(5) Å, which is less than the sum of the van der Waals' radii of 3.11 Å.[11]

4 ANTITUMOR ACTIVITY

Compounds **1** and **2** were screened *in vitro* for their antitumor activity, determining the growth inhibition ratio against HL-60, BGC-823, Bel-7402, SKOV3, KB and Hela cell lines. Table 4 lists the antitumor activities of the two compounds. The results of bioassay showed that the title compound exhibits good inhibition activity against tumor lines *in vitro*, showing higher antitumor activity than compound **1**. In comparison with $Ph_3SnO_2CCH_3$, compound **2** shows a higher inhibition ratio in 1 μM except in Hela tumor lines. These results are promising. Further studies on antitumor activity of the product are under way.

5 SUPPLEMENTARY MATERIAL

Crystallographic data for the structure analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC no. 141852. Copies of this information may be obtained free of charge from: The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44-1223-336033; Email: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk.

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