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Synthesis, characterization and antitumour activity of 7,7-di-n-butyl-5,9-dioxo-6,8-dioxa-7-stanna-spiro[3,5]nonane, a di-n-butyltin(IV) analog of "paraplatin", and of a series of di-*n*-butyltin(IV) derivatives of mono- and disubstituted malonic acids

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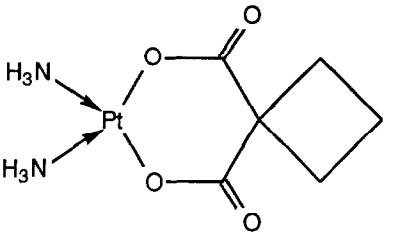
Abstract.

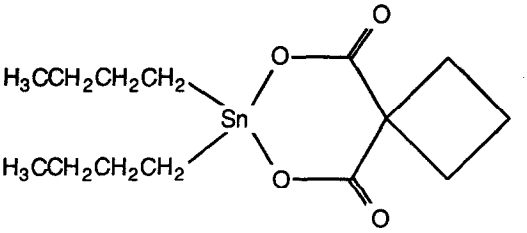
Cis-diammine[1,1-cyclobutanedicarboxylato]platinum(II) ("carboplatin" or "paraplatin") is a therapeutic agent clinically used against some cancers. Because several diorganotin(IV) derivatives do exhibit antitumour properties, we have prepared and characterized 7,7-di-n-butyl-5,9-dioxo-6,8-dioxa-7-stanna-spiro[3,5] nonane, compound **1**, a di-n-butyltin(IV) analog of "paraplatin".To determine if the spiro structure or/and the presence of the four-membered ring is important for the antitumour activity, we have also prepared and characterized a series of di-n-butyltin(IV) derivatives of mono- and disubstituted malonic acids.
Compound **1** did not show any significant anti-tumour activity *in vivo* against L1210 leukemia in mice.

Introduction

Cis-diammine[1,1-cyclobutanedicarboxylato]platinum(II) ("carboplatin" or "paraplatin")



is a therapeutic agent clinically used against some cancers [1].
Because several diorganotin(IV) derivatives do exhibit antitumour properties [2], we have prepared and characterized 7,7-di-n-butyl-5,9-dioxo-6,8-dioxa-7-stanna-spiro- [3,5]nonane, compound **1**, a di-n-butyltin(IV) analog of "paraplatin":



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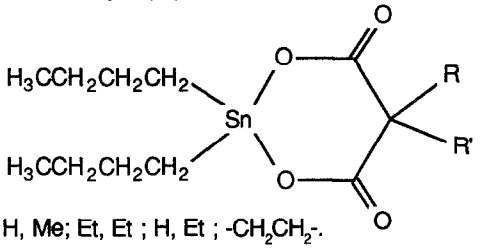
To find out whether the spiro structure or/and the presence of the four-membered ring influence the antitumour

activity, we have also prepared a series of di-n-butyltin(IV) derivatives of mono- and disubstituted malonic acids:



where R, R´ are respectively Me, Me ; H, Me; Et, Et ; H, Et ; $-CH_2CH_2-$.

Results and discussion.

Synthesis of 7,7-di-*n*-butyl-5,9-dioxo-6,8-dioxa-7-stanna-spiro[3,5]nonane.

7,7-Di-*n*-butyl-5,9-dioxo-6,8-dioxa-7-stanna-spiro[3,5]nonane, compound **1**, has been prepared by reacting di-*n*-butyltin oxide and 1,1-cyclobutanedicarboxylic acid in a 1:1 ratio

Synthesis of a series of di-*n*-butyltin(IV) derivatives of mono- and disusbtituted malonic acids

The synthesis of di-*n*-butyltin(IV) derivatives (compounds **2** - **7**) of mono- and disusbtituted malonic acids was performed similarly. Synthesis data and melting points are given in table 1.

When the reaction is run in an ethanol / toluene mixture, the formation of the eventually expected ternary azeotrope ethanol / toluene / water is never observed. When the reaction is run in an ethanol / benzene mixture, the ternary azeotrope ethanol / benzene / water is distilled off. However, after recrystallization from a not especially dried solvent, similar compounds were obtained that gave the same NMR spectra in $CDCl_3$ and that were NMR pure. Only in the case of the compound **4**, the melting points measured for the compounds prepared in toluene or benzene are different, but surprisingly the melting point of a mixture of the two solids was identical to the higher one.

Compound R	R'	#	Solvent	Melting point (ºC)
CH_3	CH_3	**2**	toluene / ethanol benzene / ethanol	146-148 143-147
C_2H_5	C_2H_5	**4**	toluene / ethanol benzene / ethanol	124-125 146-150
$-CH_2CH_2CH_2-$		**1**	toluene / ethanol	160-162
CH_3	H	**3**	toluene / ethanol	66 - 69
C_2H_5	H	**5**	toluene / ethanol benzene / ethanol	65 - 70 63 - 67
$-CH_2CH_2-$		**7**	toluene / ethanol	249-252(dec.)

Table 1: Melting points of di-*n*-butyltin(IV) derivatives of mono- or disubstituted malonic acids

^{1}H and ^{13}C NMR Spectroscopy.

The experimental values of the ^{1}H NMR chemical shifts and coupling constants of compounds **1** to **5** are given

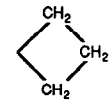
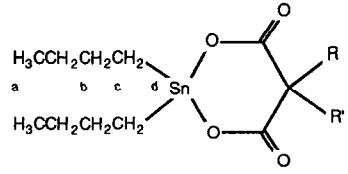
Proton R / R'	CH_3 / CH_3 δ; [J] **2**	CH_3 / H δ; [J] **3**	C_2H_5 / C_2H_5 δ; [J] **4**	C_2H_5 / H δ; [J] **5**	 δ; [J] **1**
a	0.918 [7.3] t	0.916 [7.3] t	0.916 [7.3] t	0.915 [7.3] t	0.916 [7.3] t
b	1.389 [7.3] se	1.385 [7.3] se	1.390 [7.3] se	1.385 [7.3] se	1.392 [7.3] se
c, d	complex pattern 1.751-1.664	complex pattern 1.668-1.751	complex pattern 1.729-1.684	complex pattern 1.750-1.677	complex pattern 1.764-1.683
R	1.466 s	1.440 [7.3] d	CH_3:0.855 [7.5] t CH_2:1.981 [7.5] q	CH_3:1.001 [7.5] t CH_2:1.936 [7.5] q_5	 1.932 [7.8] q_5
R'		3.504 [7.3] q		3.341 [7.5] t	 2.550 [7.8] t

Table 2: ^1H NMR spectra of 

δ: ppm; [J]: Hertz

q: quartet; q_5: quintet; t: triplet; se: sextet; s: singlet

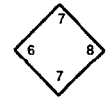
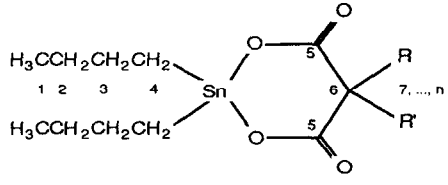
Carbon R / R'	CH_3 / CH_3 δ; [|J|] **2**	CH_3 / H δ; [|J|] **3**	C_2H_5 / C_2H_5 δ; [|J|] **4**	C_2H_5 / H δ; [|J|] **5**	 δ; [|J|] **1**
1	13.56	13.50	13.56	13.50	13.56
2	26.47 [91]	26.35 [96]	26.62 [92]	26.41[n.v.]	26.39 [93]
3	26.80 [32]	26.58 [33]	26.97 [30]	26.67 [33]	26.72 [31]
4	25.26 [536, 562]	25.32 [559]	25.44 [535, 560]	25.44 [534]	25.29 [536]
5	183.13	180.17	182.17	179.53	181.95
6	50.05	46.19	58.93	53.70	52.94
R, R'	23,12	7: 14,26	7: 24,22 8: 8,71	7: 22,82 8: 12,24	7: 29,78 8: 16,30

Table 3: ^{13}C NMR spectra of 

[n.v.]: not visible
* not attributed
δ: ppm; [J]: Hertz

in table 2.All the signals can easily be assigned for compounds **1** to **5.** Compound **7** is too insoluble to record its NMR spectrum. ^{13}C NMR chemical shifts and carbon-tin coupling constants of compounds **1** to **5** are given in table 3. All the signals can easily be assigned. All spectra were also recorded with the DEPT pulse sequence and confirmed this assignement, except for compound **6** (R, R' = Ph, H).

Characterization of compound 6.

Instead of the expected methine proton, the ^1H NMR spectrum of **6** (m.p. 60-63°C) exhibits a singlet at 3.469 ppm that integrates for two protons, showing the presence of a methylene group. The ^1H NMR spectrum of **6** is also different from the other ones because the sextet observed between 1.385 and 1.392 ppm for the CH_2 next to the methyl group of the butyl chains for the other compounds has become a complex pattern appearing between 1.156 and 1.294 ppm for compound **6.** This can be explained if the butyl groups are diastereotopic and if the protons of the methylene groups thereof are also diastereotopic. Other complex patterns are found for the two other methylenes between 1.536 and 1.413, and the methyl group of the butyl substituents absorbs at 0.830 ppm (with a coupling constant of 7.3 Hz). The phenyl group appears as a complex pattern between 7.2 and 7.3 ppm. The presence of a methylene carbon appearing in the ^{13}C NMR spectrum at 43.87 ppm instead of the expected methine group is confirmed by the ^{13}C NMR DEPT spectrum of compound **6.** The butyl signals are seen at 13.43, 27.23, 27.53 for the $CH_3CH_2CH_2$ of the butyl groups respectively, abd the methylene of the butyl groups bound to the tin appears as two lines at 26.79 and 26.73 ppm, respectively, which confirms the diastereotopic character of the butyl substituents of compound **6.** The carbonyl carbon absorbs at 177.43 ppm and the ipso, ortho, meta and para protons of the phenyl ring appear at 135.82, 129.25, 128.42 and 126.64 ppm. respectively.
The most reasonable explanation for these experimental facts is that **6** is a compound resulting from the reaction of phenylacetic acid (decarboxylated phenylmalonic acid) with di-*n*-butyltin oxide, eventually through the formation of di-n-butyltin bis(phenylacetate) followed by a hydrolysis yielding the corresponding oxide.

$Bu_2SnO + 2\ HOOC\text{-}CH(Ph)\text{-}COOH = Bu_2Sn(OCOCH_2Ph)_2 + H_2O + 2\ CO_2$

$2\ Bu_2Sn(OCOCH_2Ph)_2 + H_2O = Bu_2(PhCH_2COO)Sn\text{ - }O\text{ - }Sn(OCOCH_2Ph)Bu_2 + 2\ PhCH_2COOH$

The molecular weight determined by cryoscopy in benzene for **6** is 1,500 ± 300. The structures shown in figure 1 are compatible with these experimental results.

Mössbauer spectroscopy.

The Mössbauer parameters obtained for compounds **1** to **7** are summarized in table 4.

R	CH_3	C_2H_5	$-CH_2CH_2CH_2-$	CH_3	C_2H_5	C_6H_5	$-CH_2CH_2-$
R'	CH_3	C_2H_5		H	H	H	
#	**2**	**4**	**1**	**3**	**5**	**6**	**7**
Q.S.	3.66	3.88	3.64	4.22	3.96	3.08	4.61
I.S.	1.48	1.54	1.47	1.50	1.54	1.25	1.44

Table 4 : Isomer shift (mm/s relative to calcium stannate) and quadrupole splitting (mm/s) observed for compounds **1** to **7**

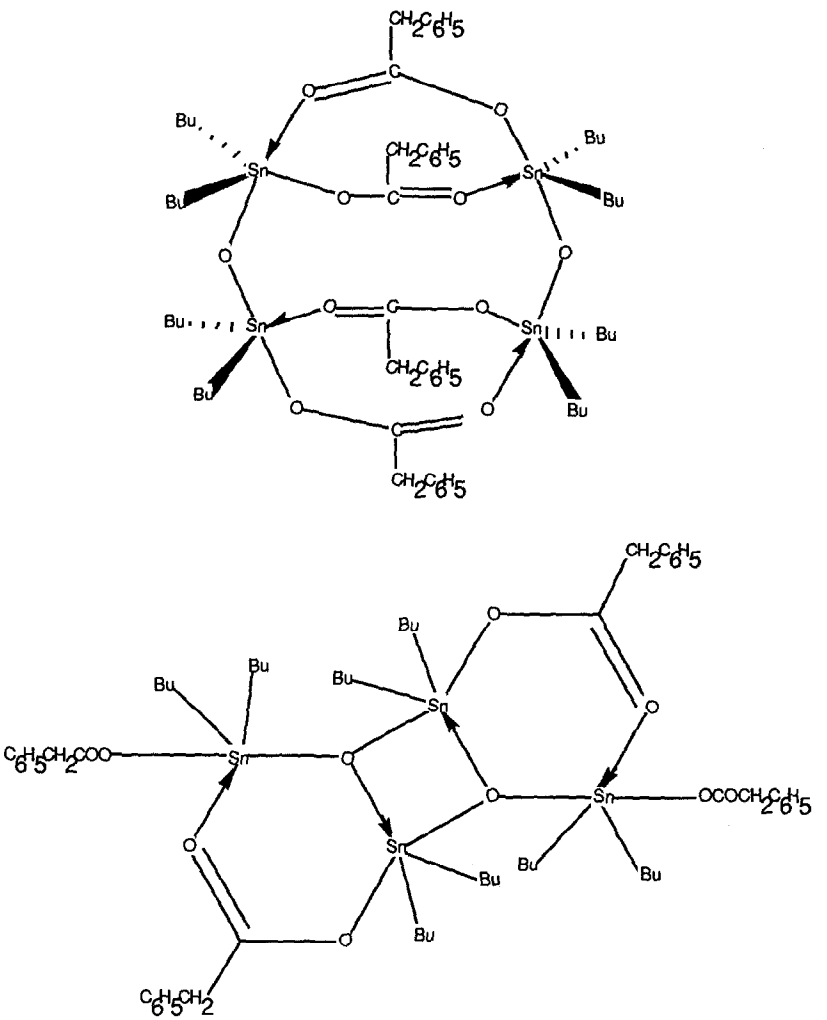


Figure 1: Proposed structures for compound **6**.

For all compounds, the isomer shift is equal to about 1.5 mm/s, which is the expected value for diorganotin(IV) derivatives.The values observed for the quadrupole splittings of compounds **1-5** and **7** are in the range compatible with those expected for five-, *cis*-six- or seven-coordinate complexes, so that it is not possible to conclude which coordination number tin has got in the solid state from these experimental values. The value observed for QS of compound **6** are compatible with the structure proposed above.

Because di-*n*butyltin oxide is characterized by Q.S. = 2.60 and I.S. = 0.98 [4], Mössbauer spectroscopy obviously shows that all the di-*n*-butyltin oxide has reacted with the dicarboxylic acid.

Infra-red spectroscopy

The most important bands are given in table 5.

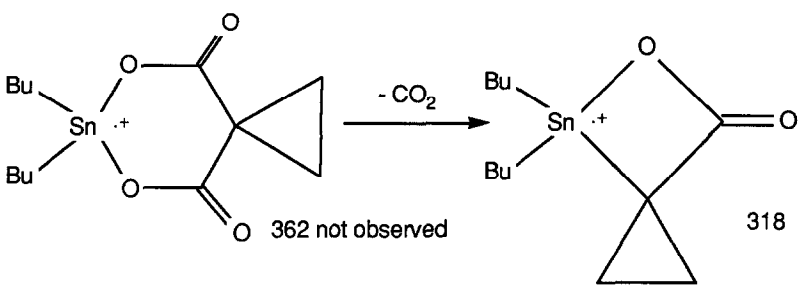
R	CH_3	C_2H_5	$-CH_2CH_2CH_2-$	CH_3	C_2H_5	C_6H_5	$-CH_2CH_2-$	Assignment
R'	CH_3	C_2H_5		H	H	H		
#	**2**	**4**	**1**	**3**	**5**	**6**	**7**	
	670	670	670	670	680	670	670	O-Sn-O
	1595	1585	1585	1580	1580	1595 1643	1550	C=O stretch
	2860	2870	2860	2850	2850	2850	2850	
	2930	2930	2930	2920	2920	2920	2920	} C-H stretch
	2960	2960	2950	2950	2950	2950	2950	

Table 5: Characteristic I.R. bands observed for compounds **1** to **7** (KBr)

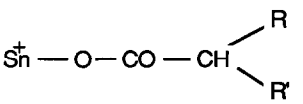
The I.R. spectrum of compound **6** can be related to a distannoxane carboxylate, like the ones depicted in figure 1. The I.R. spectrum of di-*n*-butyltin oxide is characterized by bands at about 2850 et 2920 cm^{-1}(CH stretching), 1410-1470 cm^{-1} (CH_2 bending) and a sharp band at 670 cm^{-1} (O-Sn-O bending). In all spectra, a band at about 670 cm^{-1} has been observed that corresponds to the O-Sn-O bending mode [8], [9].
The C=O stretching appears at lower frequencies as compared to the parent diacid. The CH stretchings are almost unaffected.

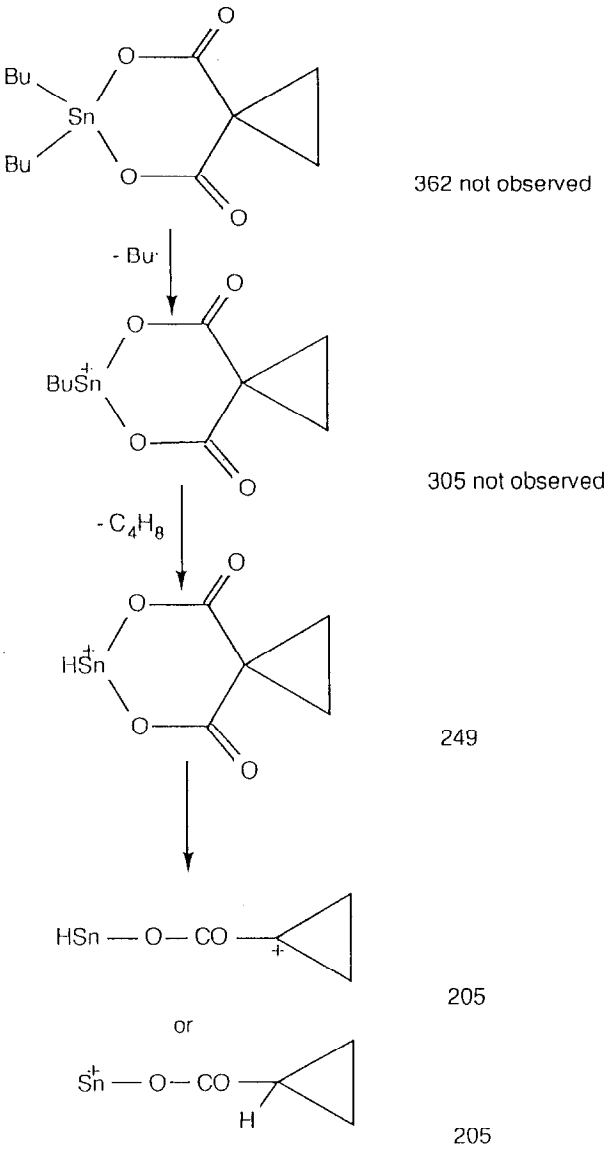
Mass spectrometry.

The 70 eV mass spectra recorded for compounds **1** to **7** are rather complex and very different from what one would expect from the fragmentation rules found in the literature for organotin compounds [5]: the molecular ion is never observed, which is unexpected owing to the presence of a tin-containing cycle in these compounds. The loss of a butyl radical is neither observed. A reasonable fragmentation scheme is proposed below for compound **7** (see scheme 1)
A tin-containing fragment is also observed at m/z = 318 that corresponds to the loss of CO_2 from the molecular



For compound **2**, a tin-containing fragment-ion appears at m/z = 336, that corresponds to the loss of CO from the molecular ion.
The tin-containing fragment appearing at m/z = 205 for compound **7** might have one of the structures proposed in scheme 1.

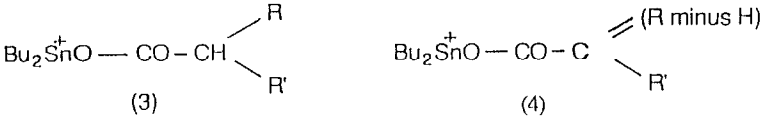
An equivalent fragment [fragment (1)]  (1)



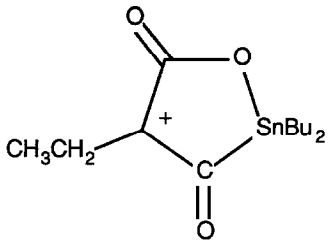
Scheme 1: Fragmentation scheme proposed for compound **7**.

is observed for compounds **2**, **3** , **5** and **7**.

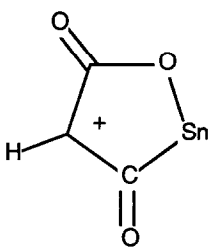
In some spectra, another tin-containing fragment with m/z = 203 [fragment (2)] is also observed for compounds **2** and **3**. To justify the tin-containing fragments appearing at a mass differing from the former ones with 114 units, fragments (3) and (4), a similar structure with two more butyl groups at tin seems reasonable.



They are observed for compounds **1**, **3**, **4** and **5**, and **4**, respectively.
Furthermore, ions corresponding to the loss of a OH. radical followed by the loss of ethylene [fragment (5)] are observed for compound **4**. An analogous ion without butyls on tin [fragment (6)] is observed for compound **5**.



(5)



(6)

Fragment-ions are always present at m/z = 120, 121, 137, 177, corresponding to Sn^+,SnH^+, $SnOH^+$, $SnBu^+$, respectively.

In vivo antitumour activity.

Compound **1** has been tested *in vivo* against L1210 leukemia in mice. The results are given in table 6.

Cpd	doses mg/kg/inj.	weigth change (day 5 - day 1)	median survival day	T/C x100 (%)
1	200	-	lethal before day 5	-
	100	- 3.10	6.0	73
	50	- 3.00	9.9	120
	25	+ 0.80	8.7	106
	12.5	+ 0.70	9.0	109
	6.3	+ 0.80	8.8	107
	3.1	+ 1.23	9.1	110
cis-platin	20	- 5.30	6.9	84
	10	- 1.70	21.0	256
	5.0	0.00	18.8	229
	2.5	+ 0.20	12.3	150
	1.3	+ 0.70	9.0	109
control	-	+ 1.40	8.2	100

Table 6: Results of the *in vivo* tests of compound **1** against L1210 leukemia in mice
Because T/C < 125, compound **1** is inactive.

Experimental part.

Synthesis of 1,1-cyclopropane dicarboxylic acid.
1,1-Cyclopropane dicarboxylic acid (m.p.: 134 - 136°C) has been prepared from 1,2-dibromoethane and diethylmalonate in basic triethylbenzylamine (TEBA) in 75% yield following the procedure decribed in ref. (7).

Synthesis of 7,7-dibutyl-5,9-dioxo-6,8-dioxa-7-stanna-spiro[3.5]nonane.
In a 2 l. flask, 5 g (0.0347 mole) diacid are dissolved at room temperature in a mixture of 150 ml ethanol and 750 ml toluene (6). 8.64 g (0,0347 mole) of dibutyltin oxide are added, a Dean-Stark funnel is adapted on the flask and the heterogeneous mixture is warmed up till reflux. After a few minutes at the boiling point, the reaction mixture becomes homogeneous. No ternary azeotrope is observed. When 50% of the solvent is distilled off, the reaction mixture is left to cool down. The precipitate formed is filtered and recrystallized from toluene. 11.52g (88%) of compound 1 is obtained in a pure form. Nice monocrystals are obtained when compound 1 is recrystallized from diethyl ether.

Synthesis of dibutyltin(IV) derivatives of mono- or disubstituted malonic acids.
The preparation is analogous with that of the spiro compound 1. The amount of diacid, solvent and dibutyltin oxide used are summarized in table 7.

#	R	R'	Amount of diacid (g)	Amount of Bu_2SnO (g)	Solvent (ml) EtOH	Solvent (ml) $C_6H_5CH_3$	Amount of organotin(g)	Yield (%)
2	CH_3	CH_3	7.26 (0.055 mole)	13.83 (0.055 mole)	220	1200	15	75
4	C_2H_5	C_2H_5	8.81 (0.055 mole)	13,69 (0.055 mole)	220	1200	19.78	92
3	CH_3	H	5.00 (0.042 mole)	10.54 (0.042 mole)	220	923	14.33	97
5	C_2H_5	H	5,00 (0.038 mole)	9.42 (0.038 mole)	160	750	13.60	99
6	C_6H_5	H	9.91 (0.055 mole)	13.83 (0.055 mole)	280	1200	22.00	93
7	$-CH_2CH_2-$		2.00 (0.0154 mole)	3.83 (0.0154 mole)	65	350	6.82	95

Table 7: Amounts of diacid, solvent and dibutyltin oxide used, and yields of the syntheses of dibutyltin(IV) derivatives of mono- or disubstituted malonic acids

Compound **7** is insoluble in ethanol/toluene so that, in that case, the reaction mixture does not become homogeneous. After 24 h reflux, the precipitate is filtered. Mössbauer spectroscopy is compatible with the structure of compound **7**.
Compounds **3** and **5** did not crystallize after leaving the reaction mixture to cool till room temperature. In these cases, the solvents were evaporated under reduced pressure and the compounds did become solids when the last traces of solvent were removed. Because the NMR of the crude products gave satisfactory NMR spectra, they were not recrystallized.

In vivo antitumour tests.
The tests have been performed as follows: male CDF_1 mice have received 10^5 L1210 cells by intraperitoneal injection on day 0. A suspension of the drug in a saline containing 0.1% tween 80 is then administered intraperitoneally on days 1, 5 and 9. The survival of mice was recorded daily and the T/C was computed. The T/C represent the ratio of the median survival time of treated mice (T) on the median survival time of the control (C). A $T/C \geq 125$ is requested to demonstrate an antitumour activity.

Instruments.
The NMR spectra have been recorded on a Bruker WM 250 instrument ($CDCl_3$ solutions, chemical shifts versusTMS as internal standard). The Mössbauer spectra have been recorded in the constant acceleration mode on an Elscint MVT4 instrument of Promeda ($Ca^{119}SnO_3$ source from Amersham, sample temperature: 90-100 K). The IR spectra have been recorded on a Perkin-Elmer 298 instrument (KBr). The mass spectra have been recorded on a VG M Micromass 7070F instrument (source temperature: 180-200 ºC, pressure: $\sim 10^{-5}$-10^{-7} mb).

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