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Organotin Gibberellates: Synthesis, Spectroscopic Characterization and Antitumour Activity

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The synthesis and characterization of di-n-butyl-, tri-n-butyl- and triphenyltin gibberellates are reported. Their antitumour activities *in vitro* against a panel of seven human tumour cell lines are given and compared with those of drugs used clinically. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Many di- and tri-organotin carboxylates, some of which are derived from natural bio-organic derivatives, exhibit interesting antitumour properties *in vitro*.[1–11] In the same line of investigation, the present paper reports the synthesis and characterization of three organotin gibberellates prepared for the same purpose from gibberellic acid,[12] and the results of their antitumour screening *in vitro* against a panel of tumour cell lines of human origin.

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RESULTS AND DISCUSSION

Di-n-butyltin digibberellate (**1**, Fig. 1) was synthesized by condensing[13–15] di-n-butyltin oxide with gibberellic acid in a molar ratio of 1:2. Attempts to obtain the corresponding dimeric distannoxane from a molar ratio of 1:1 invariably resulted in the generation of the same compound **1**.

The Mössbauer parameters of **1** are I.S. (relative to $CaSnO_3$) = 1.42; Q.S. = 3.34; $\Gamma_1 = 0.94$; $\Gamma_2 = 0.96$ mm s^{-1}. The quadrupole splitting observed is characteristic for a six-coordinate distorted trapezoidal-bipyramidal geometry[16–18] with two long =O→Sn coordination bonds and two shorter -O-Sn ones.[19,20]

The tri-n-butyl- and triphenyl-tin analogues **2** and **3** (Fig. 2) were synthesized similarly from tri-n-butyltin acetate[21,22] or triphenyltin hydroxide.[23,24]

The Mössbauer parameters of **2** (I.S. = 1.29; Q.S. = 2.77; $\Gamma_1 = 1.27$; $\Gamma_2 = 1.02$ mm s^{-1}) are characteristic of a monomeric structure with or without a C=O→Sn coordination bond whereas those of **3** (I.S. = 1.43; Q.S. = 3.60; $\Gamma_1 = 0.94$, $\Gamma_2 = 0.79$ mm s^{-1}) are typical for a polymeric structure with intermolecular C=O→Sn coordination bonds.[16–20,25]

The three compounds were characterized in $CDCl_3$ solution by 1D ^1H, ^{13}C and ^{117}Sn NMR spectroscopy, and heteronuclear ^1H–^{13}C HMQC (heteronuclear multiple-quantum correlation) and HMBC (heteronuclear multiple-bond correlation) spectroscopy.

The ^1H NMR data of **1, 2** and **3** are described in Table 1. The proton NMR spectrum of **1** has been assigned from the multiplicity and integration of the resonances and from the two-dimensional HMQC and HMBC ^1H–^{13}C correlations. The resonances of the α and β protons of **1** overlap and display complex patterns in the one-dimensional spectrum;

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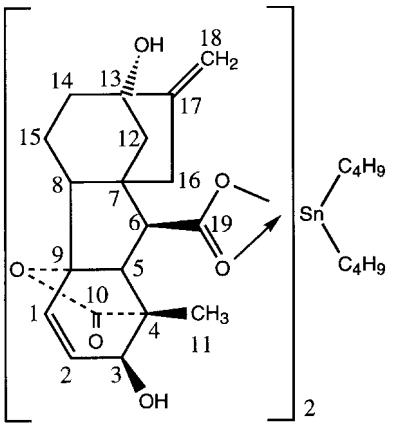

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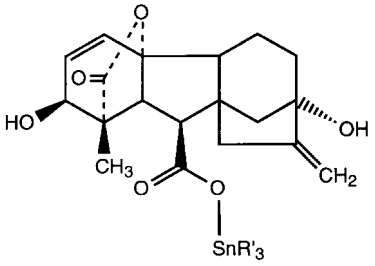
Figure 1 Di-n-butyltin digibberellate (**1**).

a single triplet is observed for the terminal δ-methyl group of **1**, in agreement with a monomeric six-coordinate distorted trapezoidal-bipyramidal geometry proposed for analogous derivatives[26–29] and evidenced in the solid state by Mössbauer spectroscopy. The H_8, H_{14} and H_{15} resonances of **1–3**, appearing as complex overlapping patterns in the one-dimensional NMR spectrum, are assigned by the chemical shift of the middle of the ^1H–^{13}C HMQC cross-peaks. The same holds for H_α of **1** and H_β of **1** and **3**.

The ^{13}C NMR data of **1, 2** and **3** are given in Table 2.

The $^1J(^{119/117}Sn–^{13}C)$ coupling constants ($CDCl_3$) of **1** confirm the distorted trapezoidal-bipyramidal structure proposed above, which is characteristic for diorganotin dicarboxylates.[17,26,29,30] Estimation of the C-Sn-C bond angles from the ^{13}C NMR parameters of n-butyltin compounds, using the empirical relationship



2 R' = C_6H_5

3 R' = n-C_4H_9

Figure 2 Tri-n-butyltin gibberellate (**2**) and triphenyltin gibberellate (**3**).

Table 1 ^1H data[a] for **1**

	1	**2**	**3**
H_1	d: 6.29 [9]	d: 6.24 [9]	d: 6.28 [9]
H_2	dd: 5.87 [9,4]	dd: 5.83 [9,4]	dd: 5.86 [9,4]
H_3	d: 4.12 [4]	ud: 4.05	ud: 4.10
H_5	d: 3.14 [11]	d: 3.13 [11]	d: 3.11 [11]
H_6	d: 2.82 [11]	d: 2.85 [11]	d: 2.76 [11]
H_8	1.90	1.92	1.90
H_{11}	s: 1.25	s: 1.07	s: 1.25
H_{12}	d: 1.79 [1]	d: 1.75 [1]	d: 1.75 [1]
	d: 1.99 [1]	d: 2.02 [1]	d: 2.01 [1]
H_{14}	1.78, 2.02	1.68, 2.02	1.78, 2.02
H_{15}	1.65, 1.90	1.60, 1.90	1.65, 1.90
H_{16}	d: 2.36 [2]	d: 2.34 [2]	d: 2.32 [2]
	d: 2.20 [2]	d: 2.21 [2]	d: 2.16 [2]
H_{18}	s: 5.26	s: 5.13	s: 5.24
	s: 4.94	s: 4.67	s: 4.91
H_α	1.62		t: 1.24 [7]
H_β	1.63 [7, 7]		1.63 [7, 7]
H_γ	tq: 1.33 [7, 7]		tq: 1.34 [7, 7]
H_δ	t: 0.88 [7]		t: 0.90 [7]
H_o		m: 7.68–7.71 {**58**}	
$H_{m/p}$		m: 7.42–7.47	

[a] Chemical shifts in ppm, $^nJ(^1H–^1H)$ coupling constants between brackets and $^nJ(^1H, ^{119}Sn)$ in bold, in Hz. Abbreviations: d, doublet; s, singlet; t, triplet; q, quartet; u, unresolved.

$|^1J(^{119}Sn–^{13}C)| = 9.99\ \theta - 746$,[31] yields a value of 132° for **1**, in the standard value range found for such distorted bipyramidal-trapezoidal structures.[32,33]

The ^{13}C resonances of the Bu_3Sn and Ph_3Sn moieties have been assigned from the $^nJ(^{119/117}Sn–^{13}C)$ ($n \leq 3$) coupling constants.

The ^{117}Sn NMR chemical shifts $\delta(^{117}Sn)$ of **1, 2** and **3** are respectively −132.8, −103.1 and 119.4 ppm. For compounds **1** and **2** they are as expected for the structures proposed from Mössbauer data. By contrast, **3** has a ^{117}Sn chemical shift characteristic for tetrahedral four coordination in solution as opposed to the five coordination suggested by the Mössbauer data different coordinations in solution and the solid state are not uncommon.[4,5]

The compounds were screened against seven human cancer cell lines, two mammary cancers, (MCF-7, EVSA-T), a colon carcinoma (WiDr), an ovarian cancer (IGROV), a melanoma (M19 MEL), a renal cancer (A 498) and a non-small-cell lung cancer (H226). The ID_{50} values observed are given in Table 3 and compared with those of some reference compounds used clinically[34,35]: doxorubicin (DOX), cisplatin (CPT), 5-fluorouracil (5-FU), methotrexate (MTX) and etoposide (ETO).

Surprisingly, except for H226 (against which it was totally inactive), **2** exhibited a much stronger

Table 2 ^{13}C data[a] for **1, 2** and **3**

Compound	**1**	**2**	**3**
C_1	132.7	132.7	132.9
C_2	132.6	132.7	132.6
C_3	69.8	69.9	70.0
C_4	53.6	53.6	53.6
C_5	53.4	53.5	53.4
C_6	50.6	51.0	51.1
C_7	50.5	50.5	50.1
C_8	50.9	51.2	51.6
C_9	90.7	90.5	90.5
C_{10}	178.7	178.8	178.8
C_{11}	14.7	14.3	14.5
C_{12}	45.0	45.0	45.0
C_{13}	78.1	78.2	78.2
C_{14}	38.5	38.3	38.5
C_{15}	17.1	17.1	17.1
C_{16}	43.1	43.0	43.1
C_{17}	156.7	157.2	157.6
C_{18}	107.5	107.0	107.0
C_{19}	181.4	178.3	177.0
$C_\alpha/C_i/C_\alpha$	25.8	137.8	16.7
	[576/557]	**[648/620]**	**[355/338]**
$C_\beta/C_o/C_\beta$	26.8 **[31]**	136.8 **[48]**	27.8 **[21]**
$C_\gamma/C_m/C_\gamma$	26.6 **[103]**	129.0 **[63]**	27.0 **[64]**
$C_\delta/C_p/C_\delta$	13.5	130.4	13.6

[a] (Chemical shifts in ppm, $^nJ(^{13}C, ^{119/117}Sn)$ coupling constants between brackets in bold, in Hz.

activity than the other two compounds, especially against the two mammary tumour and the colon carcinoma cell lines. Its activity was comparable with that of methotrexate and was even better than that of doxorubicin, two very active drugs in clinical use.

EXPERIMENTAL

Syntheses

Di-n-butyltin gibberellate (1)

Di-n-butyltin oxide (0.36 g, 1.45 mmol) and an excess (4.0 ml) of propanol were refluxed in 100 ml of dry benzene for 3 h. The ternary azeotrope benzene–water–propanol was removed using a Dean–Stark apparatus. The reaction mixture was cooled to room temperature; a solution of gibberellic acid (1.00 g, 2.90 mmol) in 15 ml of chloroform was added dropwise and the mixture was stirred for one night at room temperature. The solvent was removed under reduced pressure. The solid residue obtained was recrystallized from acetone–hexane, (yield 82%; m.p. 145–147 °C).

Triphenyltin and tributlytin gibberellates (2 and 3)

Reaction of stoichiometric amounts of gibberellic acid (1.00 g, 2.9 mmol) and triphenyltin hydroxide (1.06 g, 2.9 mmol) or tributyltin acetate (1.01 g, 2.9 mmol) was effected as above. Compounds **2** and **3** were recrystallized from dichloromethane–hexane (**2**: m.p. 109–111 °C, yield 85%; **3**: m.p. 65–66 °C; yield 78%).

NMR measurements

All two-dimensional NMR spectra and some of the one-dimensional spectra were recorded on a Bruker AMX500 spectrometer interfaced with an X32 computer and operating at 500.13, 125.77, and 186.50 MHz for the ^1H, ^{13}C, and ^{119}Sn nuclei, respectively. Other spectra were acquired on a Bruker AC250 instrument equipped with a Quattro probe tuned to 250.13, 62.93 and 89.15 MHz for ^1H, ^{13}C and ^{117}Sn nuclei, respectively. ^1H and ^{13}C chemical shifts were referenced to the standard Me_4Si (TMS) scale from the residual ^1H– and ^{13}C–^2H solvent resonances of chloroform ($CHCl_3$ 7.23 and $CDCl_3$ 77.0 ppm for ^1H and ^{13}C nuclei, respectively). The ^{119}Sn and ^{117}Sn reference frequencies were calculated from the absolute references $\Xi(^{119}Sn) = 37.290\,665$ MHz and $\Xi(^{117}Sn) = 35.632\,295$ MHz.[36,37] ^{13}C and ^{119}Sn BB proton-decoupled one-dimensional spectra, ^{13}C DEPT spectra, as well as the proton-detected

Table 3 Inhibition doses ID_{50} *in vitro* (ng ml^{-1}) of compounds **1, 2** and **3** and five drugs used clinically as references

	MCF-7	EVSA-T	WiDr	IGROV	M19 MEL	A 498	H 226
1	262	244	401	245	247	327	306
2	<3	<3	<3	7	5	20	>60 000
3	102	53	74	116	111	170	146
DOX	10	8	11	60	16	90	199
CPT	699	422	967	169	558	2253	3269
5-FU	750	475	225	297	442	143	340
MTX	18	5	<3	7	23	37	2287
ETO	2594	317	150	580	505	1314	3934

one- and two-dimensional $^{1}H–^{13}C$ HMQC[38] and HMBC[39,40] correlation spectra, were acquired using the pulse sequences of the Bruker program library, the latter being adapted to include gradient pulses,[41–44] as described recently.[45]

Mössbauer spectroscopy

The Mössbauer spectra were recorded as described previously.[15]

Antitumour screening

The protocol followed for the antitumour screenings has been reported previously.[34,35]

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REFERENCES

1. R. Willem, H. Dalil, P. Broekaert, M. Biesemans, L. Ghys, K. Nooter, D. de Vos, F. Ribot and M. Gielen, *Main Group Met. Chem.* **20**, 535 (1997).
2. M. Gielen, H. Ma, A. Bouhdid, H. Dalil, M. Biesemans and R. Willem, *Metal-Based Drugs* **4**, 193 (1997).
3. E. R. T. Tiekink, M. Gielen, A. Bouhdid, R. Willem, V. I. Bregadze, L. V. Ermanson and S. A. Glazun, *Metal-Based Drugs* **4**, 75 (1997).
4. R. Willem, A. Bouhdid, B. Mahieu, L. Ghys, M. Biesemans, E. R. T. Tiekink, D. de Vos and M. Gielen, *J. Organometal. Chem.* **531**, 151 (1997).
5. R. Willem, A. Bouhdid, M. Biesemans, J. C. Martins, D. de Vos, E. R. T. Tiekink and M. Gielen, *J. Organometal. Chem.* **514**, 203 (1996).
6. M. Gielen, E. R. T. Tiekink, A. Bouhdid, D. de Vos, M. Biesemans, I. Verbruggen and R. Willem, *Appl. Organometal. Chem.* **9**, 639 (1995).
7. M. Gielen, F. Kayser, O. B. Zhidkova, V. T. Kampel, V. I. Bregadze, D. de Vos, M. Biesemans, B. Mahieu and R. Willem, *Metal-Based Drugs* **2**, 37 (1995).
8. M. Gielen, A. Bouhdid, F. Kayser, M. Biesemans, D. de Vos, B. Mahieu and R. Willem, *Appl. Organometal. Chem.* **9**, 251 (1995).
9. M. Gielen, *Coord. Chem. Rev.* **151**, 41 (1996).
10. M. Gielen, R. Willem, A. Bouhdid and D. de Vos, European Patent 677 528 A1, Appl. 94/201 012.5, 12 April 1994; *Chem. Abstr.* **124**, 87376c (1996).
11. M. Gielen, R. Willem, A. Bouhdid and D. de Vos, *European Patent* 700 921, Appl. 94/202 612.1, 9 September 1994; *Chem. Abstr.* **124**, 343674z (1996).
12. R. L. Danheiser, in: *Strategies and Tactics in Organic Synthesis* Lindberg, T. (ed.), Academic Press, London, 1984, Chapter 2, pp.21–70.
13. M. Gielen, A. El Khloufi, M. Biesemans, B. Mahieu and R. Willem, *Bull. Soc. Chim. Belg.* **101**, 243 (1992).
14. M. Boualam, R. Willem, M. Biesemans and M. Gielen, *Appl. Organometal. Chem.* **5**, 497 (1991).
15. M. Boualam, R. Willem, M. Biesemans, B. Mahieu, J. Meunier-Piret and M. Gielen, *Main Group Met. Chem.* **14**, 41 (1991).
16. J. J. Zuckerman, in: *Chemical Mössbauer Spectroscopy*, Herber, R. H. (ed.), Plenum Press, New York, 1984, p. 167.
17. M. Gielen, J. Meunier-Piret, M. Biesemans, A. El Khloufi and R. Willem, *Polyhedron* **11**, 1961 (1992).
18. A. Meriem, R. Willem, J. Meunier-Piret, M. Biesemans, B. Mahieu and M. Gielen, *Main Group Met. Chem.* **13**, 167 (1990).
19. S. W. Ng, V. G. Kumar Das, W. H. Yip, R. G. Wang and T. C. W. Mak, *J. Organometal. Chem.* **393**, 201 (1990).
20. N. Donoghue and E. R. T. Tiekink, *J. Organometal. Chem.* **420**, 176 (1991).
21. M. Gielen, A. El Khloufi, M. Biesemans, F. Kayser, R. Willem, B. Mahieu, D. Maes, J. N. Lisgarten, L. Wyns, A. Moreira, T. K. Chattopadhay and R. Palmer, *Organometallics* **13**, 2849 (1994).
22. A. G. Davies and P. J. Smith, in: *Comprehensive Organometallic Chemistry*, Vol. 2, Wilkinson, G. (ed.), Pergamon Press, Oxford, 1982, p. 564.
23. K. C. Molloy, S. J. Blunden and R. Hill, *J. Chem. Soc., Dalton Trans.* 1259 (1988).
24. K. C. Molloy, K. Quill, S. J. Blunden and R. Hill, *Polyhedron* **5**, 959 (1986).
25. E. R. T. Tiekink, *Appl. Organometal. Chem.* **5**, 1 (1991).
26. A. Meriem, M. Gielen and R. Willem, *J. Organometal. Chem.* **365**, 91 (1989).
27. M. Gielen, A. El Khloufi, M. Biesemans, B. Mahieu and R. Willem, *Bull. Soc. Chim. Belg.* **101**, 243 (1992).
28. M. Boualam, R. Willem, M. Biesemans, B. Mahieu, J. Meunier-Piret and M. Gielen, *Main Group Met. Chem.* **14**, 41 (1991).
29. J. Holecek, M. Nadvornik, K. Handlir and A. Lycka, *J. Organometal Chem.* **315**, 299 (1986).
30. M. Gielen, M. Biesemans, A. El Khloufi, J. Meunier-Piret, F. Kayser and R. Willem, *J. Fluorine. Chem.* **64**, 279 (1993).
31. J. Holecek, K. Handlir, M. Nadvornik and A. Lycka, *J. Organometal Chem.* **258**, 147 (1983).
32. M. Gielen, A. Bouhdid and E. R. T. Tiekink, *Main Group Met. Chem.* **18**, 199 (1995).
33. M. Gielen, M. Boualam, B. Mahieu and E. R. T. Tiekink, *Appl. Organometal Chem.* **8**, 19 (1994).
34. V. T. DeVita Jr, S. Hellman and S. A. Rosenberg (eds), *Cancer: Principles and Practice of Oncology*, 5th edn, Lippincott–Raven, Philadelphia, 1967.
35. Y. P. Kepers, G. J. Peters, J. Van Ark-Otte, B. Winograd and H. M. Pinedo, *Eur. J. Cancer* **27**, 897 (1991).

36. J. Mason, *Multinuclear NMR*, Plenum Press, New York, 1987, pp. 625–629.
37. A. G. Davies, P. G. Harrison, J. D. Kennedy, R. J. Puddephatt, T. N. Mitchell and W. J. McFarlane, *J. Chem. Soc. A* 1136 (1969).
38. A. Bax, R. H. Griffey and B. H. Hawkins, *J. Magn. Reson.* **55**, 301 (1983).
39. A. Bax and M. F. Summers, *J. Am. Chem. Soc.* **108**, 2093 (1986).
40. A. Bax and M. F. Summers, *J. Magn. Reson.* **67**, 565 (1986).
41. J. Keeler, R. T. Clowes, A. L. Davis and E. D. Laue, *Meth. Enzymol.* **239**, 145 (1994).
42. J. -M. Tyburn, I. M. Bereron and D. M. Doddrell, *J. Magn. Reson.* **97**, 305 (1992).
43. J. Ruiz-Cabello, G. W. Vuister, C. T. W. Moonen, P. Van Gelderen, J. S. Cohen and P. C. M. Van Zijl, *J. Magn. Reson.* **100**, 282 (1992).
44. G. W. Vuister, R. Boelens, R. Kaptein, R. E. Hurd, B. K. John and P. C. M. Van Zijl, *J. Am. Chem. Soc.* **113**, 9688 (1991).
45. R. Willem, A. Bouhdid, F. Kayser, A. Delmotte, M. Gielen, J. C. Martins, M. Biesemans, B. Mahieu and E. R. T. Tiekink, *Organometallics* **15**, 1920 (1996).

