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Organometallic Complexes with Biological Molecules: VII. Dialkyl- and Trialkyl-tin (IV)[*meso*-tetra(4-carboxyphenyl)porphinate] Derivatives: Solid-state, Solution-phase Structural Aspects and *In Vivo* Effects

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The synthesis, the structural features and the *in vivo* biological activity of diorganotin(IV) and triorganotin(IV) derivatives of [*meso*-tetra(4-carboxyphenyl)porphine] (H_4TPPC) are reported. Derivatives with general formula $(R_2Sn)_2$TPPC and $(R_3Sn)_4$TPPC (R = Me, Bu, and Ph) were obtained, and the main information extracted from the infrared and Mössbauer spectral data, in the solid state, was in favor of the occurrence of five-coordinated tin(IV) atoms, in a polymeric trigonal-bipyramidal configuration, attained through two differently coordinated, ester-type and chelating respectively, carboxylate anions in $[R_2Sn]_2$TPPC, while in $[Alk_3Sn]_4$TPPC five-coordination of the tin(IV) atom is reached through bridging carboxylate groups.

^{1}H and ^{13}C NMR spectra, in DMSO-d_6 or $CDCl_3$ suggested that the soluble derivatives, at room temperature or at 342 K, were present in solution as simple monomers.

The interactions of (trimethyltin)$_4$[*meso*-tetra(4-carboxyphenyl)porphinate] (TMTPPC) and (tributyltin)$_4$[*meso*-tetra(4-carboxyphenyl)porphinate] (TBTPPC) with Bluescript KS(+) plasmid and cultured 3T3 fibroblasts were studied.

Both compounds have a clear inhibitory effect on the growth of cultured mouse embryonal fibroblasts (NIH-3T3), TBTPPC being much more active. No evidence was found, however, for DNA cleavage by the compounds at molar ratios as high as 1:10 (TMTPPC, TBTPPC/DNA base pairs).

According to our observations, the cytotoxicity of TBTPPC and TMTPPC does not seem to be based on direct interaction with DNA. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

Porphyrins and metal porphyrin complexes have been, in the recent past, the object of extensive investigations, both in solid and solution phases, because of their biological and technological applications.[1–3] Such research dealt, mainly, with metal ions inserted as the central atom. Recently, several papers have been published in which metallic or organometallic moieties were coordinated to side-chain carboxylate groups.[4–6] In particular bis[diorganotin(IV)chloro]protoporphyrin IX complexes have been investigated in solid and in solution phases,[4] and chromosome damage has been shown in early-developing embryos of *Anilocra physodes* L. (Crustacea, isopoda) following exposure to bis(dimethyltin(IV)chloro)protoporphyrin IX.[5]

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Furthermore, 15 platinum(II)porphyrin complexes were synthesized and chemically characterized, and their antitumor activity *in vivo* towards MDA-MB 231 mammary carcinoma cell line was tested.[6]

MATERIALS AND METHODS

Chemical methods

The organotin(IV) - [*meso* - tetra(4 - carboxyphenyl)porphinate] complexes were synthesized by refluxing, in dry methanol, R_2SnO or R_3SnOH, freshly prepared by hydrolysis of the parent R_2SnCl_2 and R_3SnCl respectively (gifts from Witco GmbH, Bergkamen, Germany), and [*meso* - tetra (4 - carboxyphenyl) porphine (H_4TPPC), Fig. 1, at high purity, from Porphyrin Products (Logan, UT, USA) in the molar ratio 2:1 and 4:1, respectively, for $[R_2Sn]_2$TPPC and $[R_3Sn]_4$TPPC derivatives [R = Me, Bu, Ph].

The microcrystalline solids were recovered by filtration and when possible recrystallized from a dry methanol–ether mixture. All the complexes obtained were analyzed for their C, H and N content at the Laboratorio di Chimica Organica, University of Milano (Table 1).

The tin content in the samples was determined in our laboratory, gravimetrically, as SnO_2 according to Neumann's method.[7]

Infrared spectra of the complexes were recorded as Nujol and hexachlorobutadiene mulls, in the 4000–180 cm^{-1} region, with a Perkin-Elmer model 983G spectrometer, computer-aided, using CsI windows (Table 2).

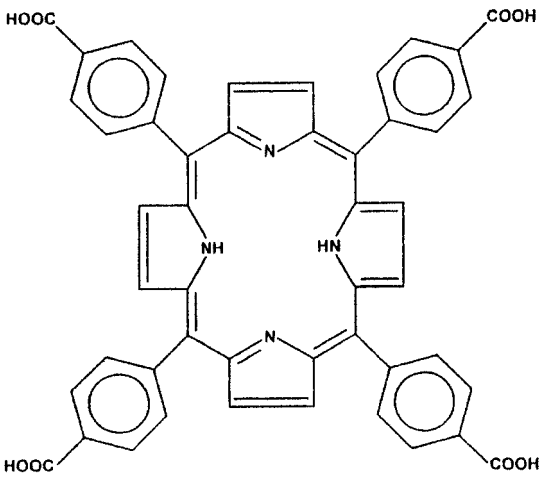

Figure 1 [*meso*-Tetra(4-carboxyphenyl)porphine] (H_4TPPC).

Table 1 Analytical data (calculated % values in parentheses) of $(R_2Sn)_2$TPPC and $(R_3Sn)_4$TPPC (R = Me, Bu, Ph; $TPPC^{4-}$ = *meso*-tetra(4-carboxyphenyl)porphinate]

Compound	C	H	N	Sn
$(Me_2Sn)_2$TPPC	57.17	3.58	4.98	21.90
	(57.60)	(3.53)	(5.16)	(21.89)
$(Bu_2Sn)_2$TPPC	61.46	5.10	4.24	18.96
	(61.36)	(4.98)	(4.47)	(18.95)
$(Ph_2Sn)_2$TPPC	64.02	3.47	4.24	17.70
	(64.89)	(4.12)	(4.20)	(17.81)
$(Me_3Sn)_4$TPPC	49.12	4.26	3.82	32.20
	(49.97)	(4.33)	(3.88)	(32.92)
$(Bu_3Sn)_4$TPPC	59.27	7.46	3.04	24.38
	(59.22)	(6.93)	(2.87)	(24.41)
$(Ph_3Sn)_4$TPPC	65.39	3.82	3.01	20.90
	(65.90)	(3.96)	(2.56)	(21.71)

^{119}Sn Mössbauer spectra were obtained with a 10 mCi $Ca^{119}SnO_3$ (Radiochemical Centre, Amersham, UK) source at room temperature. The absorber samples were powdered and pressed between aluminum foils in a copper sample holder, and maintained at liquid-nitrogen temperature in an Oxford Instruments model DN 700 (Oxford, UK) cryostat. The 77.3 ± 0.1 K temperature was controlled through an Oxford Instruments model ITC-2 temperature controller. The ^{119}Sn concentration was ~ 0.5 mg cm^{-2}.

The source motion was effected by the following Wissenschaftliche Elektronik GMBH apparatus (Germany): a velocity transducer (range 0 to ± 10 mm s^{-1}), a FG-2 function generator and a MR 250 driving unit. Velocity calibration was carried out with an enriched iron foil spectrum (^{57}Fe = 99.99%, thickness 0.06 mm, Dupont, MA, USA) at room temperature using a 10 mCi ^{57}Co source (Dupont) in a palladium matrix, while the zero point of the Döppler velocity was determined, at room temperature, through an absorption spectrum of natural $CaSnO_3$ containing 0.5 mg cm^{-2} of ^{119}Sn. Finally, a model 269 multichannel analyzer (Takes, Ponteranica, Bergamo, Italy) was used and *ca* 5×10^5 counts were collected for each velocity point. The obtained data were refined with appropriate software to obtain the Mössbauer parameter isomer shift, δ mm s^{-1}, nuclear quadrupole splitting, ΔE mm s^{-1}, and the width at half-height of the resonant peaks, Γ mm s^{-1}, reported in Table 3.

Table 2 Assignment of more relevant absorption bands of *meso*-tetra(4-carboxyphenyl)porphine (H_4TPPC), $[R_2Sn]_2$TPPC and $[R_3Sn]_4$TPPC (R = Me, Bu, Ph) derivatives in the 4000–250 cm^{-1} region[a]

Compound[b]	ν(NH)	ν_{as} (COO^-)	ν (COOH)	ν_{as}(COO^-)	ν_{sym} (COO^-)	ν_{sym} (COO^-)	ν_{as} (SnC_2)	ν_{sym} (SnC_2)	Y mode	$\Delta\nu_1$ (cm^{-1})	$\Delta\nu_2$ (cm^{-1})
H_4TPPC	3318w		1686s								
$(Me_2Sn)_2$TPPC	3317m	1706s		1630m	1470s	1402s	572s	486s		306	160
$(Bu_2Sn)_2$TPPC	3319m	1685s		1629s	1462s	1402s	572m	480s		283	167
$(Ph_2Sn)_2$TPPC	3313m	1686s			1479m	1403m			450s	283	150
$(Me_3Sn)_4$TPPC	3323m			1640s	1472s		550s	490m		168	
$(Bu_3Sn)_4$TPPC	3316m			1630s	1462s		550w	490s		168	
$(Ph_3Sn)_4$TPPC	3312m			1640s	1479s				450s	161	

[a] Nujol and hexachlorobutadiene mulls; s = strong; m = medium; w = weak.
[b] $TPPC^{4-}$ = [*meso*-tetra(4-carboxyphenyl)porphinate].

^{1}H and ^{13}C NMR spectra of all the organotin(IV) complexes were registered with a Bruker AC 250E instrument, operating at 250 and 63 MHz, respectively, using tetramethylsilane (TMS) as an internal standard and DMSO-d_6 and $CDCl_3$ as solvents (Tables 5 and 6).

Biological methods

Cell cultures and effects on cell growth

Cultured mouse embryonal fibroblasts (NIH-3T3) were harvested and detached from a sub confluent culture by successive rinsing with PBS^{o} (Ca^{2+}/Mg^{2+}-free phosphate buffered saline), 1×10^{-3} mol dm^{-3} EDTA in PBS^{o} and 1×10^{-3} mol dm^{-3} EDTA in PBS^{o} containing 5 mg cm^{-3} trypsin. Cells were counted and plated at a concentration of $(1.0–2.0)\times10^{5}$ cells/25 cm^{2} flask in DME (Dulbecco's modified Eagle's medium, Sigma, MO, USA), supplemented with antibiotics (40 mg penicillin, 8 mg ampicillin, 90 mg streptomycin per liter, Sigma) and 10% fetal calf serum (GIBCO, Italy). Cells were cultured at 37 °C in a 5% CO_2 humid atmosphere.

After 6–12 h (the optimal time for the cells to adhere to the flask surface) filter-sterilized TBTPPC or TMTPPC ethanolic solution was added to the medium at different concentrations (ranging from 0.5×10^{-7} to 0.5×10^{-5} mol dm^{-3}, respectively). After incubation (ranging from 1 to 24 h), the medium was changed and cells were grown for an additional 48 h. Untreated cells, and cells treated only with the solvent (ethanol) or with the parent chemical compounds (i.e. [*meso*-tetra (4-carboxyphenyl)porphine] or Me_3SnCl or Bu_3SnCl) were used as controls.

To measure cell toxicity of the compounds, cells, treated as described above, were harvested by trypsinization, pelleted and dissolved in 0.1 mol dm^{-3} NaOH at 50 °C for 90 min, and the absorbance of the solution was then measured at 260 nm, using a DU-65 spectrophotometer (Beckman, CA, USA), essentially as described by Di Liegro *et al*.[8]

Electrophoretic analysis of DNA/TBTPPC, TMTPPC mixtures

TMTPPC (or TBTPPC) was mixed with Bluescript plasmid DNA (Stratagene, CA, USA), at different molar ratios (from 1:10 to 1:100 TMTPPC/TBTPPC:DNA base pairs); after at least 30 min of preincubation at room temperature, the mixtures were analyzed by gel electrophoresis on 1% agarose in 1×10^{-2} mol dm^{-3} Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid], pH 7.4.

After running at 10 mA for 6 h, the gel was stained in ethidium bromide (2 mg cm^{-3} in distilled water) for 20 min, rinsed with water and photographed under ultraviolet illumination through a red filter.

RESULTS AND DISCUSSION

The analytical data reported in Table 1 strongly support the hypothesis of organotin(IV) moieties

Table 3 Experimental Mössbauer parameters,[a] isomer shift δ, and nuclear quadrupole splittings $|\Delta E|_{exp}$, measured at liquid-nitrogen temperature, and nuclear quadrupole splittings according to the point charge formalism applied to the idealized trigonal-bipyramidal structures of Fig. 2(a, b)

Compound[b]	δ (mm s^{-1})	$\lvert\Delta E\rvert_{exp}$ (mm s^{-1})	Γ_1 (mm s^{-1})	Γ_2 (mm s^{-1})	$\Delta E_{calcd.}$ (mm s^{-1})	Figure
$(Me_2Sn)_2TPPC$	1.24	3.20	1.06	1.02	3.24	2(a)
$(Bu_2Sn)_2TPPC$	1.31	3.32	0.95	0.92	3.24	2(a)
$(Ph_2Sn)_2TPPC$	1.21	2.96	1.01	1.00	2.84	2(a)
$(Me_3Sn)_4TPPC$	1.33	3.32	1.09	1.10	−3.69	2(b)
$(Bu_3Sn)_4TPPC$	1.41	3.45	0.85	0.83	−3.69	2(b)
$(Ph_3Sn)_4TPPC$	1.21	2.88	1.00	0.89	−3.24	2(b)

[a] Sample thickness ranged between 0.50 and 0.60 mg ^{119}Sn cm^{-2}; isomer shift, δ ±0.03 (mm s^{-1}) was measured with respect to room-temp. $BaSnO_3$; Γ_1 and Γ_2 values are the full width at half-height of the resonant peaks, respectively at greater and lower velocity with respect to the centroid of the Mössbauer spectra; nuclear quadrupole splittings, $|\Delta E|$ ± 0.02, (mm s^{-1}).

[b] $TPPC^{4-}$ = [*meso*-tetra(4-carboxyphenyl)porphinate]$^{4-}$.

reacting with H_4TPPC ligand (H_4TPPC = [*meso*-tetra(4-carboxyphenyl)porphine]) in the stoichiometric diorganotin(IV)/ligand, and triorganotin(IV)/ligand ratios 2:1 and 4:1, respectively. In all cases, however, the coordinating mode of the [*meso*-tetra(4-carboxyphenyl) porphinate]$^{4-}$ ion towards the organotin(IV) moieties can be deduced from comparison of the infrared spectra of the free and coordinated ligand (Table 2) for the solid phase and by ^{1}H and ^{13}C (Tables 5 and 6) for solutions.

Infrared spectra

$(R_2Sn)_2$[*meso*-tetra(4-carboxyphenyl) porphinate] complexes (R = Me, Bu and Ph)

The more relevant differences between the infrared spectra of free and coordinated [*meso*-tetra(4-carboxyphenyl)porphine] acid in the diorganotin(IV) derivatives are the following: the band at 1686 cm^{-1}, which in free [*meso*-tetra(4-carboxyphenyl)porphine] acid is attributed to COOH stretching, is shifted towards higher wavenumbers in the $(Me_2Sn)_2$[*meso*-tetra(4 - carboxyphenyl)porphinate] complex [$\nu_{as}(COO^-) > 1706$ cm^{-1}], while it is practically unchanged in $(Bu_2Sn)_2$- and $(Ph_2Sn)_2$-[*meso*-tetra(4-carboxyphenyl)porphinate]s.

In all of the three diorganotin complexes, a new band around 1400 cm^{-1} appears attributable to $\nu_S(COO^-)$, with $\Delta\nu$ [$= \nu_{as}(COO^-) - \nu_S(COO^-)$] ranging from 283 cm^{-1} (for $(Bu_2Sn)_2$- and $(Ph_2Sn)_2$-[*meso*-tetra(4-carboxyphenyl)porphinate]s to 306 cm^{-1} (for $(Me_2Sn)_2$[*meso*-tetra(4 - carboxyphenyl)porphinate]), which is characteristic of ester-type carboxylate groups (Table 2).[9–12]

Furthermore, in the infrared spectra of all the $(R_2Sn)_2$[*meso*-tetra(4-carboxyphenyl)porphinate]s, two more bands are present with $\Delta\nu$ lower than 180 cm^{-1}, suggesting the occurrence of a second carboxylate group, chelating or bridging (Table 2).[9–12] As a consequence, in the diorganotin - [*meso* - tetra(4 - carboxyphenyl)porphinate] complexes, the tin(IV) atom would reach at least five-coordination in an R_2SnO_3 configuration (Fig. 2a). In the region below 600 cm^{-1}, bands attributable both to $\nu_{as}(SnC_2)$ and $\nu_s(SnC_2)$ are present in $(Me_2Sn)_2$- and $(Bu_2Sn)_2$ - [*meso* - tetra(4 - carboxyphenyl)porphinate]s (Table 2).

$(R_3Sn)_4$[*meso*-tetra(4-carboxyphenyl) porphinate] complexes (R = Me, Bu and Ph)

In the triorganotin(IV)[*meso*-tetra(4-carboxyphenyl)porphinate] complexes, only two bands characteristic of asymmetric and symmetric COO^- stretchings are present in the IR spectra at around 1550 and 1400 cm^{-1}, respectively, for $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$. In all the triorganotin(IV)[*meso* - tetra(4 - carboxyphenyl)porphinate] complexes, the $\Delta\nu$ values are lower than 180 cm^{-1}, indicating a bridging or chelating carboxylate group. Also in these complexes, the tin atom would reach at least five-coordination in a trigonal-bipyramidal R_3SnO_2 configuration, with equatorial R_3 and axial bridging carboxylate oxygen atoms, (Fig. 2b) in a polymeric structure. Finally, both in $(Ph_2Sn)_2$- and $(Ph_3Sn)_4$-[*meso*-tetra(4-carboxyphenyl)porphinate] complexes the so-called Y-mode vibration (in the Wiffen notation) is present.[13]

Table 4 Experimental isomer shift δ (mm s^{-1}) and calculated partial atomic charge on tin atom, Q_{Sn} (CHELEQ),[17–19] calculated for a homologous series of pentacoordinated organotin(IV) derivatives

Compound[a]	δ[b] (mm s^{-1})	Q_{Sn}[b]	Point no.[c]
Alk_3SnCl	1.51	0.097	1
Alk_3SnCN	1.36	0.134	2
Alk_3SnNCO	1.43	0.160	3
Ph_3SnCl	1.35	0.167	4
Ph_3SnNCS	1.40	0.198	5
Ph_3SnNCO	1.20	0.229	6
Ph_3SnF	1.22	0.252	7
$Alk_2SnClpenG$	1.28	0.270	8
$Ph_2SnClpenG$	1.21	0.317	9
$Alk_3SnClpenGNa$	1.40	0.128	10
$Ph_3SnClpenGNa$	1.30	0.208	11
$(Alk_2Sn)_2TPPC$	1.20	0.338	12
$(Ph_2Sn)_2TPPC$	1.21	0.388	13
$(Alk_3Sn)_4TPPC$	1.37	0.184	14
$(Ph_3Sn)_4TPPC$	1.21	0.257	15

[a] penG, penicillin G; $TPPC^{4-}$, [*meso*-tetra(4-carboxyphenyl)porphinate]$^{4-}$.
[b] Average of the isomer shifts, δ, and of the partial atomic charge on tin atom, Q_{Sn}(CHELEQ), calculated or reported in Refs 6, 20–24.
[c] Identification numbers of the points on Fig. 3.

^{119}Sn Mössbauer spectra

The experimental Mössbauer parameters, isomer shift δ (mm s^{-1}) and nuclear quadrupole splitting ΔE (mm s^{-1}), of all the investigated complexes summarized in Table 3 are characteristic of organotin(IV) derivatives.[14–16]

In particular, the isomer shifts δ of the

Table 5 ^{1}H NMR of $[R_2Sn(IV)]_2$ and $[R_3Sn(IV)]_4$ TPPC [R = Me, Bu, Ph; $TPPC^{4-}$ = *meso*-tetra(4-carboxyphenyl)porphinate; TMS = internal reference]

Assignment	H_4TPPC^a 297 K	$[Me_2Sn]_2TPPC^a$ 297 K	$[Bu_2Sn]_2TPPC^a$ 297 K	$[Bu_2Sn]_2TPPC^a$ 342 K	$[Ph_2Sn]_2TPPC^a$ 297 K	$[Me_3Sn]_4TPPC^a$ 297 K	$[Bu_3Sn]_4TPPC^b$ 297 K	$[Ph_3Sn]_4TPPC^a$ 297 K
N—H	− 2.90 s	− 2.88 s	− 2.87 s	− 2.76 s	− 2.88 s	− 2.88 s	− 2.78 s	− 2.95 s
m − H	8.29d	7.42 s, bd	7.43 s, bd	8.36 d	8.42 s	8.28 d	8.27 d	8.24 d
	J_{HH} 7.8			J_{HH} 8.2		J_{HH} 7.6	J_{HH} 7.9	J_{HH} 7.4
o-H	8.39 d	8.41 s, bd	8.43 s, bd	8.42 d	8.42 s	8.36 d	8.45 d	8.28 d
	J_{HH} 8.2			J_{HH} 8.2		J_{HH} 7.2	J_{HH} 7.9	J_{HH} 7.5
β-Pyrrolic H	8.84 s	8.93 s	8.93 s	8.88 s	8.92 s	8.90 s	8.85 s	8.83 s
COOH	13.36 bd							
R		0.57	1.30, 1.98, 2.80, 2.96		7.42–8.18 m	0.67	0.98–1.03 m δ-CH_3 1.40–1.62 m, β,γ-CH_2 1.74–1.87 m α-CH_2	7.40–8.06 m
$^2J(^1H^{119}Sn)$		74.6				68.9		
C–Sn–C angle[c]		124°				118°		

[a] In DMSO-d_6.
[b] In $CDCl_3$.
[c] Calculated according to Ref. 45.

Table 6 ^{13}C NMR of $[R_2Sn(IV)]_2$ and $[R_3Sn(IV)]_4TPPC$ (R = Me, ^{n}Bu, Ph; $TPPC^{4-}$ = *meso*-tetra(4-carboxyphenyl)porphinate] at 297 K and/or at 342 K (TMS = internal reference)

	H_4TPPC		$[^nBu_2Sn]_2TPPC$[a]	$[Me_3Sn]_4TPPC$[a]		$[^nBu_3Sn]_4TPPC$[b]	$[Ph_3Sn]_4TPPC$[a]
Assignment	297 K	342 K	342 K	297 K	342 K	297 K	342 K
COO	167.66	167.24	169.25	169.6	169.48	171.71	168.10
α–C	146.01	145.72 bd	145.62	n.o.	145.84 bd	145.76	145.69
4′–C	145.57	145.30	145.21	143.95	143.70	n.o.	143.12
2′–C	134.61	134.19	134.15	134.66	134.54	134.39	133.94
β–C	131.70 bd	131.19	131.19	131.74	133.79	131.69	131.15
1′–C	130.68	130.59	130.61	130.21	131.16	n.o.	135.89
3′–C	128.06	127.30	127.66	128.0	127.64	128.45	127.63
meso–C	119.46	119.18	119.16	119.93	119.62	119.68	119.32
R			12.54, 23.82	0.63	0.24	13.91 δ-CH_3, 16.75 α-CH_2, 27.15 β-CH_2, 27.97 γ-CH_2	136.56 C(i) 136.19 C(*o*) 128.69 C(*m*) 128.11 C(*p*)
$^1J(^{13}C^{119}Sn)$				529.2	498.3	356.6	
$^2J(^{13}C^{119}Sn)$						20.1	
$^3J(^{13}C^{119}Sn)$						66.0	
CSnC angle[c]				123°	121°	110°	

[a] In DMSO-d_6.
[b] in $CDCl_3$. The low solubility of $[Me_2Sn]_2TPPC$ and $[Ph_2Sn]_2TPPC$ at both 297 and at 342 K and of $[Bu_2Sn]_2TPPC$ at 297 K, in both DMSO-d_6 and $CDCl_3$, did not allow ^{13}C NMR spectra to be recorded.
[c] Calculated according to Ref. 45.

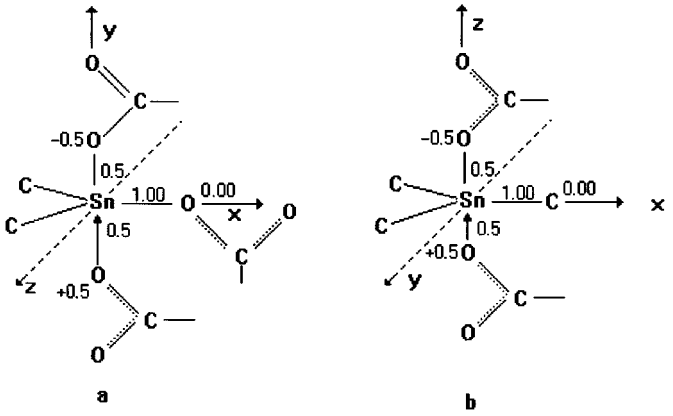

Figure 2 Regular tbp structures of tin assumed to estimate both the nuclear quadrupole splittings according to the point charge model (Table 3) and partial atomic charge on the tin atom, Q_{Sn} (see text, Table 4), for $(R_2Sn)_2TPPC$ (a) and $(R_3Sn)_4TPPC$ (b) derivatives ($TPPC^{4-}$ = [*meso*-tetra(4-carboxyphenyl)porphinate]$^{4-}$. *x*, *y* and *z* are the directions of the principal components of the electric field gradient, efg ($|\boldsymbol{V}_{zz}| \geqslant |\boldsymbol{V}_{yy}| \geqslant \boldsymbol{V}_{xx}|$); off-diagonal components of efg are diagonalized. The partial quadrupole splittings used in the calculations are: $\{Alk\}^{tbe} = -1.13$; $\{Ph\}^{tbe} = -0.98$; $\{COO\}^{unid}_{ax} = -0.10$; $\{COO\}^{bridg}_{ax} = 0.075$; $\{COO\}^{bridg}_{eq} = 0.293$ (see Refs 14, 26).

The reported bond orders and formal charges are assumed as input in the calculation of the partial atomic charge on the tin atom, Q_{Sn}.

diorganotin(IV) porphinate derivatives increase on going from $[Ph_2Sn]_2$- to $[Bu_2Sn]_2$-[*meso*-tetra(4-carboxyphenyl)porphinate], and from $[Ph_3Sn]_4$- to $[Bu_3Sn]_4$-[*meso*-tetra(4-carboxyphenyl)porphinate], reflecting the increase of 5s electron density on the tin(IV) atoms (Table 3).[14–16] The differences cannot be interpreted, however, in terms which are chemically meaningful. Nevertheless, very simple structural information may be extracted from the comparison of congener and isostructural compounds. As a consequence, partial atomic charges on tin atoms, Q_{Sn} (Table 4) have been calculated by using an orbital electronegativity equalization procedure[17–19] applied to the idealized trigonal-bipyramidal configuration, with the bond orders and formal charges reported in Fig. 2(a, b). In Table 4 are also reported isomer shifts δ and partial atomic charges Q_{Sn} of several organotin(IV) derivatives whose trigonal-bipyramidal configuration has been proposed in previous papers.[20–24] The linearity of the function $\delta(Q_{Sn})$ (Fig. 3) is in agreement with the results obtained over a number of homologous organotin(IV) compounds.[20–24]

In order to understand the dependence on the nature of the tin-ligand bond, it is important to realize that the major variations in the quadrupole splitting arise from structural rather than from bonding differences. Hence the ΔE values of the diorganotin(IV) and of the triorganotin(IV)-[*meso*-tetra(4-carboxyphenyl)porphinate]s have been rationalized according to the point charge model formalism[14, 25–29] applied to the idealized trigonal-bipyramidal structures of Fig. 2(a, b). The ΔE values so calculated (Table 3) are within the tolerance limit of the method (± 0.4 mm s^{-1}), suggesting the correctness of the hypothesized trigonal-bipyramidal configurations.

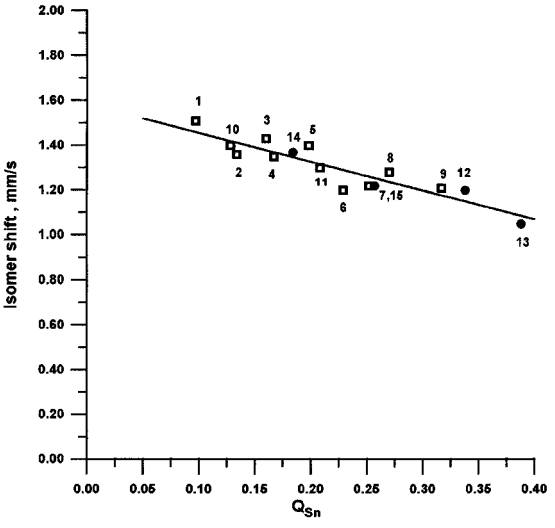

Figure 3 Isomer shifts the δ versus atomic charge Q_{Sn}, for $(R_2Sn)_2$TPPC, $(R_3Sn)_4$ TPPC derivatives ($TPPC^{4-}$ = [*meso*-tetra(4-carboxyphenyl)porphinate] (●, point nos 12–15, Table 4), and structurally correlated complexes (□, point nos1–11, Table 4). The full line is the least-squares fit of data points 1–11 (□, Table 4).

Solution studies of organotin(IV) derivatives

^{1}H and ^{13}C NMR spectra for the diorganotin(IV) and triorganotin(IV) complexes and for the free ligand are reported in Tables 5 and 6, in DMSO-d_6 or $CDCl_3$, at 297 and/or 342 K, depending from the solubility of the compounds.

^{1}H and ^{13}C NMR spectra of diorganotin(IV)- and triorganotin(IV) - [*meso*-tetra(4 -carboxyphenyl)porphinate] complexes

Caution must be exercised in interpreting NMR spectra of both *meso*-tetra(4-carboxyphenyl)porphine (H_4TPPC) and the closely related tetra(4-*N*-methylpyridylporphine (TMPyP) and *meso*-tetra(4-sulfonatophenyl)porphine (TTPS) and their metalloporphine derivatives, since they are reported to be dependent on experimental conditions such as concentration, temperature and solvent.[30–33]

Additionally, these studies demonstrated the existence of both homo- and hetero-dimers (i.e. dimers of two unequal molecules, mostly oppositely charged) in solution. While it is well known that in solution mixtures of the possible atropisomers exist, only recently[33] has the chromatographic separation of rotational isomers of Zn^{2+}, Cu^{2+}, and Ni^{2+} complexes of tetra(*N* - methyl - 3 - pyridiniumyl)porphine been accomplished, have the rotamers been isolated as stable solids and has a crystal structure of the Cu^{2+} derivative as the $\alpha,\alpha,\alpha,\beta$ isomer been obtained. The atropisomers could be identified by their ^{1}H NMR spectra, which are diagnostic of the rotational disposition of the neighboring *N*-methylpyridiniumyl groups.

In the context of a study on the monomer–dimer equilibrium in aqueous solutions of TPPS and its Zn^{2+} and Cu^{2+} complexes,[31] it was shown that in DMSO-d_6, even for concentrations as high as 0.044 mol dm^{-3}, the extent of aggrega-

tion of monomers is minimal.

Hence the spectra of the title complexes are run in this solvent at a concentration of 7×10^{-3} mol dm^{-3} for the ligand H_4TPPC and the triorganotin(IV) complexes, except for the Bu_3Sn compound, while due to their limited solubility nearly saturated solutions were used for the diorganotin(IV) derivatives reported.

$(R_2Sn)_2$ and $(R_3Sn)_4$[*meso*-tetra(4-carboxyphenyl)porphinate] complexes

^{1}H NMR spectra of the complexes clearly indicate that the only sites of the multifunctional ligand involved in coordination are the carboxylate groups Endocyclic NH groups are preserved and only minor shifts are observed relative to the free [*meso*-tetra(4-carboxyphenyl)porphine]. At 297 K the signals are shifted at most by 0.12 ppm downfield, except for the case of the triphenyltin derivative, where the signal is shifted by just 0.05 ppm in the opposite direction. A larger downfield shift is observed with increasing temperature, as in the case of the dibutyltin derivative. Pyrrolic β-hydrogens [which in dimethoxo(tetra-*p*-tolylporphyrinato) tin(IV), and closely related compounds,[34] show coupling with the central ^{119}Sn and ^{117}Sn nuclei] in all the complexes appear not to be affected by tin coupling. Only the hydrogens connected with the carboxylate groups, which appear as a broad signal at *ca* 13 ppm, disappear; this suggests metalation.

Noticeably, the spectrum of the free porphyrin shows, in the 7–9 ppm range, a pattern of two doublets arising from the coupling between *ortho* and *meta* protons of the 4-carboxyphenyl groups. Along with the β-pyrrole proton, their resonances, for solutions of H_4TPPC and Zn(TPPC) in mixed solvents (D_2O and CD_3OD), exhibit chemical shifts due to ring-current effects which depend on the formation of homo- and hetero-aggregates. Hofstra and co-workers[30, 31] were able to evaluate the dimerization constant for H_4TPPC (homodimerization), which was found to be 9.0×10^6 dm^3 mol^{-1}. Following the formation of homoaggregates, high-field shifts are observed for β-pyrrole and *ortho* and *meta* phenyl protons in 10^{-3} mol dm^{-3} concentrations, the β-pyrrolic signals being the ones which are shifted the most. Only minor shifts are observed for the triorganotin(IV) derivatives reported in this work, which may indicate that no major electron-withdrawing effect is exerted by coordination to the organometallic moiety. The diorganotin(IV) complexes under investigation show, for nearly saturated DMSO-d_6 solutions, only marginal shifts relative to the free H_4TPPC resonances of the β-pyrrolic and *ortho* proton signals.

A distinct pattern is observed for the *meta* hydrogen signals in dimethyltin(IV) and dibutyltin(IV) derivatives; at 297 K the signal appears to be shifted upfield (*ca* 0.9 ppm) relative to the free ligand. On increasing the temperature to 342 K, in the case of the dibutyltin(IV) complex the signal appears to return to the 'normal' chemical shift and resolution (Table 5). This might be taken as evidence of aggregation of the complex at the lower temperature, albeit different from the vertical interaction between monomers which are aligned with their planes approximately parallel as proposed by several authors[31, 32, 35–40] but rather in a rotated nonparallel conformation, whereby *meta* hydrogens of the exterior carboxyphenyl group are subjected to a larger ring current than β-pyrrolic and *ortho* hydrogens due to a shorter intermolecular contact.

Moreover, the carboxyphenyl group is not coplanar with the porphyrin ring, since in closely related aryl porphyrins it appears to be rotated with respect to the porphyrin plane by *ca* 63°, as observed in molecular crystals and in calculations related to their structure in solution.[31, 32, 40, 41]

^{13}C NMR spectra of the complexes are reported in Table 6.

Assignments for H_4TPPC and for the $TPPC^{4-}$ moiety in the complexes were made according to those reported for *meso*-tetra-arylporphines and their metal derivatives.[34, 42, 43]

The limited solubility, even in coordinating solvents, of some diorganotin(IV) complexes renders them unsuitable for ^{13}C NMR investigation.

It is worth noting that in spite of the possibility of atropisomerism, which arises from restricted rotation about the phenyl–porphyrin bond, only one set of resonances for the phenyl group has been observed.

In the ^{13}C spectrum of the free H_4TPPC in DMSO-d_6, at 297 K the α-pyrrole resonances appear as a sharp signal of weak intensity at 146.01 ppm while the β-pyrrole carbons display a broad resonance at 131.70 ppm. On increasing the temperature to 342 K, the appearance of the two signals is just the opposite, namely a broad signal is observed for the α-pyrrole carbons and

a sharp one for the β-pyrrole carbons. The chemical shifts of both the resonances, however, appear to be only marginally affected by the increased temperature, both in the free ligand and in the complexes (see Table 6). Broadening of these signals was shown to depend on the rate of NH tautomerism in the porphyrin by Abraham,[35–38] who observed at low temperature (213 K) a large chemical shift difference between α- and β-pyrrole carbons on 'pyrrole' or 'pyrrolenine' rings in TPPC-d_2 free base, while at 300 K linewidths for the α-pyrrole carbons of $TPPC^{4-}$ of *ca* 25 Hz and for β-pyrrole carbons of $TPPC^{4-}$-d_2 of *ca* 50 Hz were observed. It is reasonable to assume that in our experiments with H_4TPPC, both at 297 and at 342 K, α- and β-pyrrole ^{13}C resonances being an average of the two forms, a similar tautomeric equilibrium exists, and this exerts a greater influence on the chemical shifts of the porphyrin ring resonances than coordination of the organometallic moieties to the outer carboxylate group. Hence negligible shifts relative to the free base are observed. The organometallic moieties show coupling constants $^1J(^{13}C-^{119}Sn)$ and $^2J(^1H-^{119}Sn)$ which may be related to the coordination number of the tin atom and to the C–Sn–C angles of the organometallic moiety. Caution must be exercised when extracting this information from spectra obtained in complexing solvents such as dimethyl sulfoxide, as the coupling constants increase relative to those obtained in non-coordinating solvents, corresponding to the formation of weak or strong complexes.[44] The equations derived by Lockhart and Holecek[45–48] relating $^1J(^{13}C-^{119}Sn)$ and the geometry of the organometallic moiety were therefore used to evaluate the C–Sn–C angle in $(Bu_3Sn)_4$TPPC (the only complex which was soluble in $CDCl_3$), which was found to be 108–110°. This is consistent with four-coordinated pseudotetrahedral geometry around the central tin atom.

In a strongly coordinating solvent (DMSO-d_6), not unexpectedly, $^1J(^{13}C-^{119}Sn)$ and $^2J(^1H-^{119}Sn)$ values afforded a higher value of the C–Sn–C angle in $(Me_3Sn)_4$TPPC and $(Me_2Sn)_2$TPPC derivatives (*ca* 121°). This should not be considered as an indication of a *trans*-O_2 trigonal-bipyramidal geometry at tin in $(Me_3Sn)_4$TPPC or *cis*-Me_2 in $(Me_2Sn)_2$TPPC, but rather as due to the coordinating ability of the solvent.

The chemical shifts $\delta[^{13}C(1)–C(4)]$ of the butyltin(IV) derivatives have been assigned also in relation to the diagnostic values of ^nJ coupling constants. As previously observed,[47] a downfield shift is seen for resonances, relative to those obtained for $CDCl_3$ solutions. As indicated by Holecek,[47, 48] $^2J(^1H-^{119}Sn)$ values vary very little with a change in coordination, being the smallest among ^nJs, while $^3J(^{13}C-^{119}Sn)$ coupling constants lie in the range 58.1–71.6 Hz for a tetrahedral geometry whereas they are in the range 73.2–80.6 Hz for a trigonal bipyramidal one.

In a parallel fashion, for the $(Ph_3Sn)_4$TPPC complex at 342 K resonances were assigned on the basis of literature reports[49, 50] and of some $^nJ(^{13}C-^{119}Sn)$ resonances which could be identified, ^2J being 42.3 Hz and ^3J=66.5 Hz.

Unfortunately, the coupling constant that is most significant from a diagnostic point of view for the coordination geometry of tin (namely ^1J) could not be unambiguously assigned.

In conclusion, in relation to the solid-state investigations, the weak donor–acceptor (C=O---Sn bonds that are present in the solid break in coordinating dimethyl sulfoxide, and even in chloroform solutions, and the complexes exist essentially in the form of simple molecules with pseudotetrahedral configuration, while the TPPC moiety seems to adopt a nonstacked configuration.

In vivo effects

Cell cultures and effects of TBTPPC/TMTPPC on cell growth

3T3 cells were treated as described in the Materials and Methods section. Figure 4(a) shows the results of mock experiments run with pure ethanol or with *meso*-tetra(4-carboxyphenyl)porphine. These compounds do not have significant effects on cell growth, even for a 24 h incubation time and at the high concentrations reported in Fig. 4(a). The time-dependent effects of incubation of 3T3 cells with either TBTPPC or TMTPPC, at the maximal concentrations allowed by compound solubility, are shown in Fig. 4(b). TBTPPC is much more active than TMTPPC: a significant growth inhibition could be obtained only after 6 h of TMTPPC treatment, whereas TBTPPC already had a strong effect after 1 h. For this reason, in further analyses of the dose-dependence of TMTPPC or TBTPPC effect we incubated the cells for 24 h or for 1 h respectively.

The dose-dependent effects of 24 h of incubation of 3T3 cells with TMTPPC are shown in Fig. 4(c); 50% inhibition of cell growth was obtained at 1.0×10^{-6} mol dm^{-3} [TMTPPC]. The dose-dependent effects of 1 h of incubation of 3T3 cells with TBTPPC are shown in Fig. 4(d), as expected, 50% inhibition of cell growth was already obtained at about 1.5×10^{-7} mol dm^{-3} [TBTPPC]. The Figure shows also the effect of Bu_3SnCl. Interestingly, tributyltin(IV) chloride was less active than its tributyltin(IV)porphinate derivative (TBTPPC) but more active than the trimethyltin(IV)porphinate (TMTPPC).

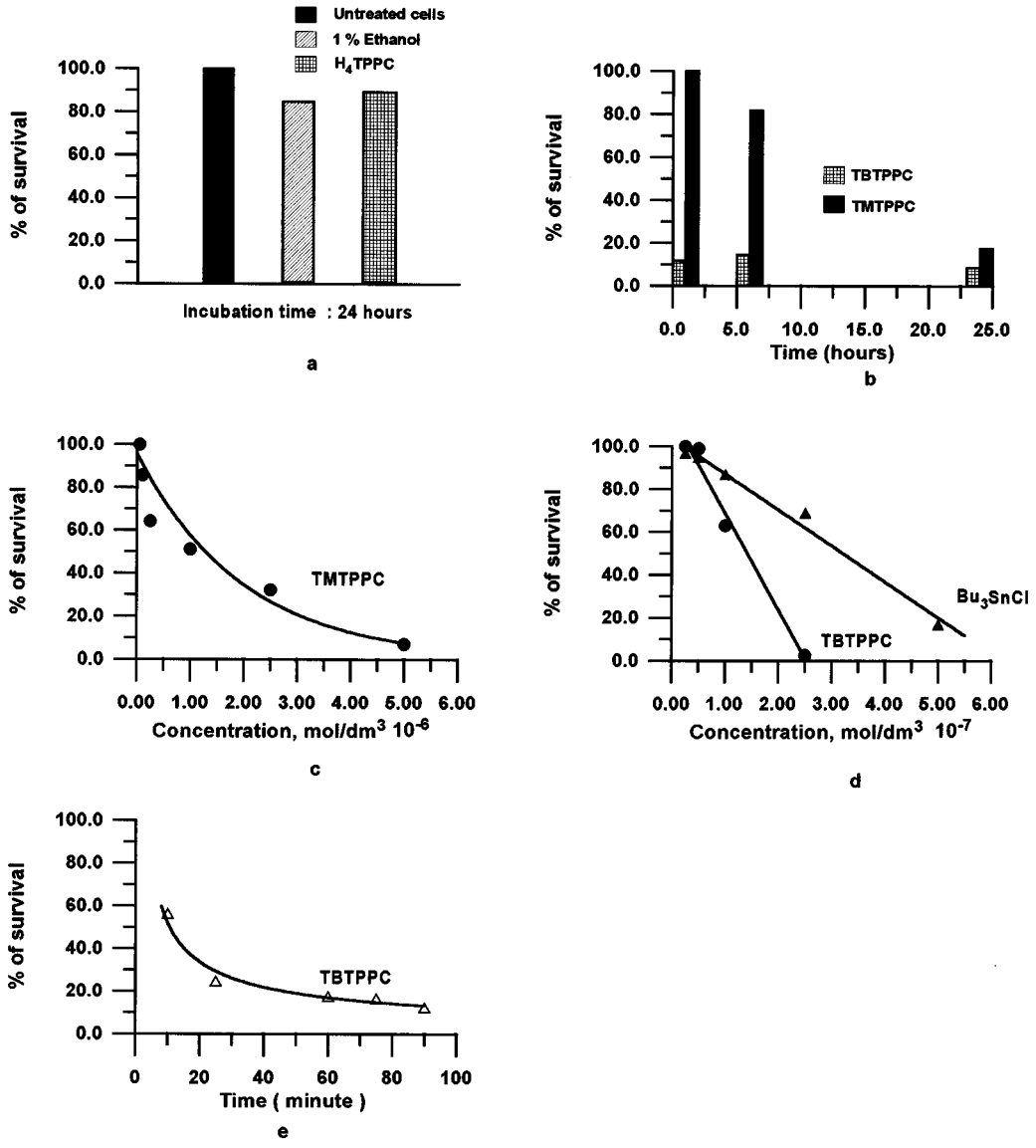

Figure 4 (a) Effects of 24 h incubation of 3T3 untreated cells, of 3T3 cells treated with 1% ethanol, or with 0.5×10^{-5} mol dm^{-3} H_4TPPC solution [H_4TPPC = *meso*-tetra(4-carboxyphenyl)porphine]. (b) time dependence of the effects towards the 3T3 cell growth of 0.5×10^{-5} mol dm^{-3} solutions of TMTPPC and TBTPPC. (c) Dose dependence of the effects on the growth of 3T3 cells treated for 24 h with TMTPPC solutions. (d) Dose dependence of 3T3 cell growth after incubation for 75 min with TBTPPC or Bu_3SnCl solutions. (e) Detailed time dependence of the effects of a 2.5×10^{-7} mol dm^{-3} solution of TBTPPC.

Given the high toxicity of TBTPPC, we further analyzed in more detail the time-dependent effects of this compound. TBTPPC (at a concentration of 2.5×10^{-7} mol dm^{-3}) was already able to induce 50% inhibition of cell growth after 15 min (Fig. 4e)

Electrophoretic analysis of DNA/TMTPPC, TBTPPC mixtures

TMTPPC (or TBTPPC) was mixed with plasmid DNA at different molar ratios; after at least 30 min of incubation, the mixtures were analyzed by agarose gel electrophoresis. Figure 5 shows one of these gels. No evidence was found for DNA cleavage by the compounds at molar ratios as high as 1:10 (TMTPPC, TBTPPC/DNA base pairs).

In conclusion, the data presented in this paper demonstrate the cytotoxicity of both TBTPPC and TMTPPC; as already suggested by other authors, both TBTPPC and Bu_3SnCl are more active than TMTPPC and Me_3SnCl; moreover, TBTPPC is much more active than the parent Bu_3SnCl.

Finally, according to our data, the cytotoxicity of these compounds does not seem to depend on DNA cleavage.

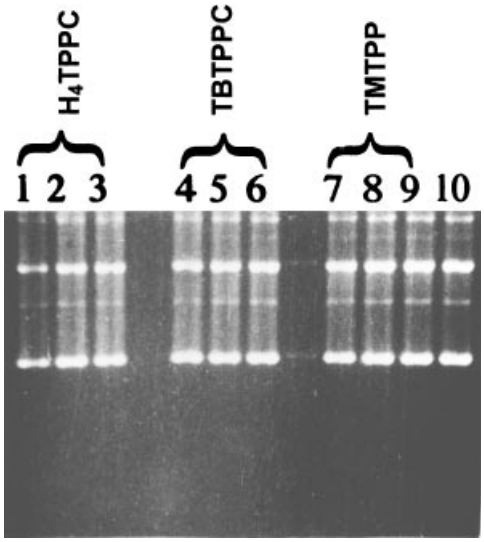

Figure 5 Electrophoretic analysis of Bluescript plasmid mixed with different amounts of H_4TPPC [*meso*-tetra(4-carboxyphenyl)porphine] (lanes 1–3), TBTPPC (lanes 4–6) or TMTPPC (lanes 7–9) at molar ratios (base pairs/compound) of 10:1 (lanes 1, 4 and 7), 50:1 (lanes 2, 5 and 8) and 100:1 (lanes 3, 6 and 9). In lane 10, untreated plasmid was electrophoresed as a control.

CONCLUSIONS

The diorganotin(IV)- and triorganotin(IV)-[*meso*-tetra(4-carboxyphenyl)porphinate]s (R = Me, Bu, Ph) are suggested to possess, on the basis of IR and Mössbauer investigations in the solid state, a polymeric trigonal-bipyramidal configuration, *cis*-R_2Sn and eq-R_3Sn, respectively. In solution in noncoordinating $CDCl_3$, they undergo dissociation into four-coordinated tetrahedral species, while pentacoordination is attained in coordinating solvents such as DMSO (via solvent interaction). Finally, these complexes show cytotoxicity increasing from methyl to butyl derivatives, and such cytotoxicity does not seem to depend on DNA cleavage.

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