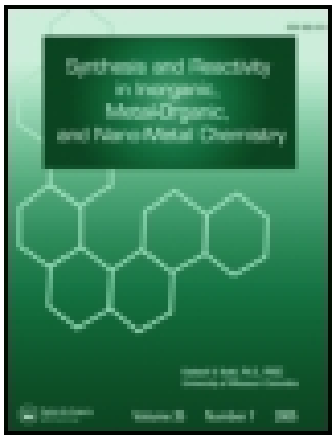

Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry

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Synthesis, Spectroscopy and in vitro Cytotoxicity of New Hydroxyanthraquinonato Triorganotin Compounds

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Synthesis, Spectroscopy and in vitro Cytotoxicity of New Hydroxyanthraquinonato Triorganotin Compounds

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We will present herein data on the synthesis, structural investigation and in vitro antitumor activity of new triorganotin compounds of the general type $(R_3Sn)_2Q$, where R = Bu, Ph, Bz, Q_1 = 1,4-dihydroxy-9,10-anthracenedione (quinizarin), Q_2 = 1,5-dihydroxy-9,10-anthracenedione (anthrarufin), Q_3 = 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione (leucoquinizarin) and R_3SnQ_4 where Q_4 = 1,2-dihydroxy-9,10-anthracenedione (alizarin). The compounds were synthesized by refluxing the organotin hydroxide with the parent quinone and were characterized by IR, ^{1}H- NMR and thermal measurements. The spectroscopic analysis of the new triorganotins, provides evidence on the formation of a monodentate Sn-O bond for quinizarin, anthrarufin and leucoquinizarin, which are coordinated to Sn(IV) central atom via the phenolic oxygen donor atoms, with the R-substituents of the organotin moiety completing the tetrahedral coordination environment. On the contrary, organotin alizarinates form a six-membered chelate ring, which stabilizes the Sn central atom in a five-coordinated environment exhibiting distorted trigonal bipyramidal geometry. The ligand is coordinated to Sn (IV) via the quinoidal oxygen and its neighbouring phenolic one. The new compounds were tested for their cytotoxicity against human tumor and normal cell lines and the results are reported.

Keywords hydroxyanthraquinones, triorganotins, SRB assay

INTRODUCTION

The last two decades' organotin compounds have found a solid application field within pharmaceuticals since most of them show potent antitumor activity. In general, triorganotins exhibit higher biological activity than their di-and mono-analogues,[1] probably due to their ability to bind proteins[2] and although the exact mechanisms of action remain unknown, researchers tend to attribute their biological applications to interactions of the organotin moieties with cellular membranes.[3,4] Organotins are known to interact in-vitro with the activity of several enzymes, e.g., human aromatase[5] and acetyltransferase,[6] while there is evidence that they enhance human CG secretion.[7]

During the last 30 years a respectful number of organotin complexes has been synthesized and tested for their antitumor profile[8,9] but both the structure and anti-cancer activity varies according to the ligand- metal interactions. Subsequently, we find reports on carboxylates[10,11] and carboxylate-derivatives,[12–14] peptides and amino acids,[15] diketones and di-ketone-derivatives,[16–22] steroids,[23] NSAIDs,[24] other drugs[25,26] as well as on a wide range of N, S, O donor ligands.[27] In each case, the binding mode to organotin cations depends on the ligand's properties chelation encountered may be monodentate, bridging or is some cases polymeric.

Despite the thorough tin-chemistry study, only a very limited amount of work has been done to investigate the potential triorganotin quinonoids. Brown et al.[28] have studied some organotin (IV)–orthoquinone systems concluding on the reaction conditions and coordination modes of benzoquinone derivatives with diphenyl- and triphenyltin but no further work leading to safe conclusions on the coordination mode of organotin quinonates has been conducted.

Among quinonoids, anthraquinones with hydroxy groups directly attached to the quinonic ring constitute an intriguing class of compounds with unique biological activity.[29] For example, hydroxyquinones constitute the functional core of anti-tumor agents daunorubicin and adriamycin (also known as doxorubicin), which are considered highly active agents in metastatic breast cancer (MBC).[30] The deriving semiquinone radicals have been implicated in their anti-tumor action and their associated toxic effects.[31] Recently, it has been shown

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that the chelated forms of these drugs achieve intercalation with DNA, and, more importantly, in the free radical chemistry of the drug.[32]

Our group has previously contributed to the study of hydroxyanthraquinone chelation with reports on the mononuclear and homobinuclear chelates of 1,5-dihydroxy-9,10-anthracenedione, 1,8-dihydroxy-anthracenedione, 1-amino-4-hydroxy-9,10-anthracenedione and 1,2-dihydroxy-9,10-anthracenedione.[33–35] Herein, we continue our research on quinone chelates by presenting the profile of new triorganotins: anthrarufin, alizarin and leucoquinizarin, typical examples of hydroxyanthraquinones with established biological activity (Figure 1). The first three have been tested for genotoxicity with the hepatocyte/ DNA repair test and for effects on oxidative phosphorylation in isolated rat liver mitochondria. Among them, only alizarin has exerted an uncoupling and inhibitory effect on mitochondrial respiration.[36]

On the other hand, leucoquinizarin's peptides have been recently reported to afford topoisomerase I inhibition.[37] The chelation process of these hydroxyanthraquinones usually evolves via their tautomeric anthraquinoid structures.[38] The formation of a wide variety of metal complexes has been reported for quinizarin and anthrarufin,[39] while alizarin exhibiting linkage isomerism, acts as a chelate via the 1,2- or the 1,9-oxygen atoms and forms complexes with Ru, Cu, Ni, Hg, and a variety of transition metals.[40,41]

EXPERIMENTAL

Materials

Triorganotin chlorides were purchased from Sigma-Aldrich and hydroxy-anthraquinones from FLUKA. All reagents were used without further purification. Tribenzyltin chloride was prepared by a standard method reported in the literature.[42] All the solvents used in the reactions were of AR grade and were purchased from Sigma Aldrich.

Measurements

Carbon and hydrogen elemental analysis was performed with a Perkin-Elmer 240 B analyzer. Attempts to define the melting points using a Buchi B-540 model (maximum

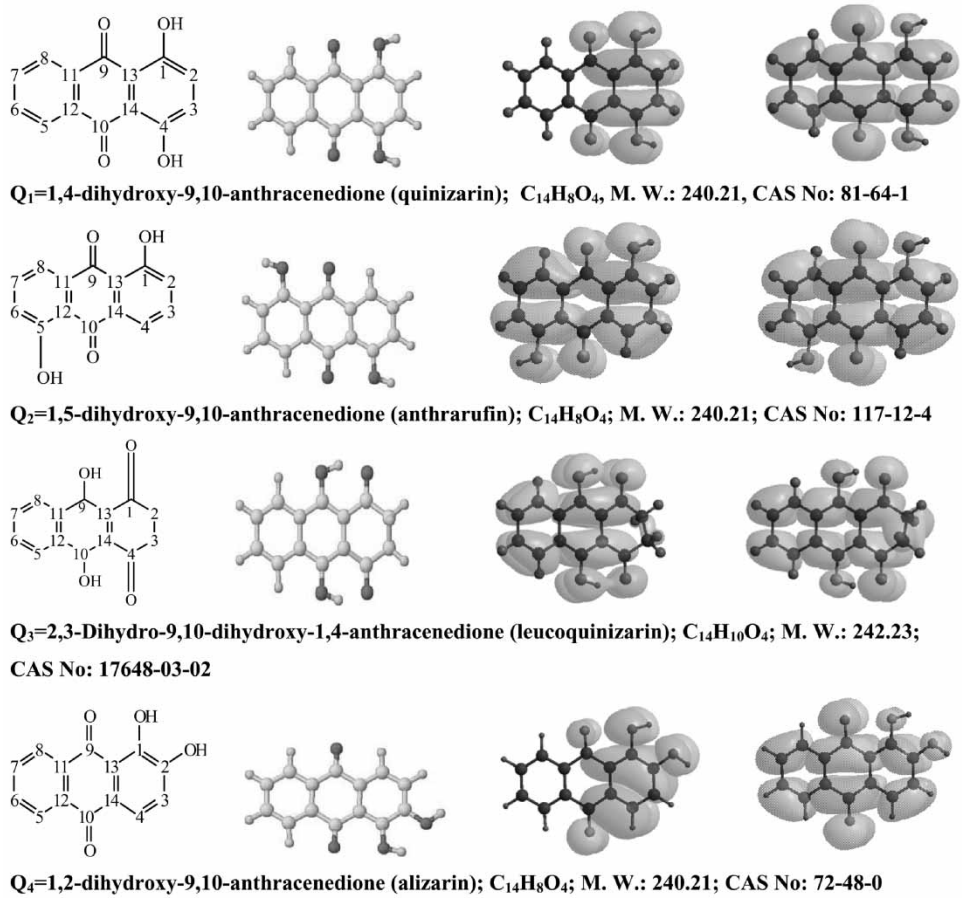

FIG. 1. (a) Structure and nomenclature of the hydroxyanthraquinones studied, (b) Computer-simulated structures of quinizarin, anthrarufin, leucoquinizarin and alizarin using B3LYP/6-31G(d) calculations for energy minimization; (c) HOMO & LUMO orbitals of the free ligands.

410°C), in most cases gave out no clear values. Infrared spectra in the range 4000–200 cm^{-1} were recorded as KBr discs (2:100 ratio) on a Perkin-Elmer Spectrum One spectrometer. ^{1}H-NMR spectra were recorded with a Bruker AM 300 (300 MHz) in ca. 5% solution of $CDCl_3$ using Me_4Si as internal standard, at room temperature. Simultaneous thermogravimetric (TG) and differential thermal analysis (DTA) were carried out on samples weighing 10 mg, with a Setavam SetSys-12 Model. Spectra recording and analysis was conducted with the SetSys 2000 software. A heating rate of 10°C/min from ambient temperature to a maximum of 650°C was applied, under nitrogen atmosphere.

Computational Details (Figure 1)

To depict Figure 1 standard ab initio molecular orbital theory and DFT was used on the GAUSSIAN-98 program suite on a Intel CELERON 3,2 GHz PC. DFT calculations were performed on hydroxy-anthraquinones by the hybrid B3LYP method, using the RB3LYP/6-31G(d) basis set. The HOMO and LUMO orbitals were visualized with ChemOffice 2002 (PC version).

Cell Lines

Five human tumor cell lines and one human non-tumour cell line were used: K-562 (Chronic myelogenous leukemia), MCF-7 (Breast adenocarcinoma, estrogen receptor positive, ER+), HeLaS3 (Epitheloid carcinoma of cervix), PC-3 (Prostate cancer), Hs 294T (Melanoma, metastatic to lymph node) and MRC-5 (Lung foetal fibroblasts). The cells were grown in RPMI 1640 (K562 and Hs294T cells) or Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose (MCF-7, PC3, HeLaS3, and MRC5 cells). Media were supplemented with 10% of fetal calf serum (FCS, NIVNS) and antibiotics: 100 IU/ml of penicillin and 100 μg/ml of streptomycin (ICN Galenika). All cell lines were cultured in flasks (Costar, 25 cm^2) at 37°C in the 100% humidity atmosphere and 5% of CO_2. Only viable cells were used in the assays. Viability was determined using Trypan blue in a due exclusion assay.

SRB Assay

Cytotoxicity was evaluated by the colorimetric SRB assay reported from Skehan et al.[43] Single cell suspension was plated into 96-well microtitar plates (Costar, flat bottom): 1×10^4 of K562 and 5×10^3 of MCF-7, PC3, HeLa, Hs294T and MRC5 cells, per 180 ml of medium. Plates were preincubated 24 h at 37°C, 5% CO_2. Tested substances were added to all wells except the control wells and microplates were incubated for 48 h. After incubation period (48 h) SRB assay was carried out: 50 μl of 80% trichloroacetic acid (TCA), was added to all wells. An hour later, plates were washed with distillate water and 75 ml of 0.4% SRB was added to all wells. Half an hour later, plates were washed with citric acid (1%) and dried at room temperature. Finally, 200 μl of 10 mmol TRIS (pH = 10.5) basis was added to all wells. Optical density was measured on a microplate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells, containing compete medium only, acted as blank. Cytotoxicity was calculated according to the formula:

$$(1 - OD_{TEST}/OD_{CONTROL}) \times 100$$

and was expressed as a percent of cytotoxicity (CI%). Data presented herein, represent the mean of the quadruplicate wells obtained from two independent experiments. IC_{50} values define the dose of compound's dose that inhibits cell growth by 50%. The IC_{50} of compounds was determined by median effect analysis and were calculated using the CalcuSyn program.

Synthesis

Quinizarin and Anthrarufin Derivatives (Compounds 1–6)

2,2 mmol of tributyl (0.95 ml), triphenyl (0.84 g), and tribenzyl tin chloride (0.94 g) accordingly were dissolved in 40 ml propanol. The stoicheiometric quantity of NaOH was added and the resulting mixture was left stirring for about 15 minutes. Then, 1 mmol (0.24 g) of quinizarin (or anthrarufin) suspended in 30 ml propanol was added slowly and under continuous magnetic stirring to the resulting triorganotin hydroxides. The mixture was refluxed for 12 hours and was then condensed via evaporation. The resulting compound was filtered and the precipitate was washed several times with water, propanol, acetone and diethyl ether. The solid was recrystallized from propanol.

Leucoquinizarin Derivatives (Compounds 7–9)

The same method and quantities were applied as described previously.

Alizarin Derivatives (Compounds 10–12)

The same method was applied as described above, but the ratio of triorganotin hydroxide to hydroxy-anthraquinone was altered to 1.2:1 (therefore 0.52 ml tributyl, 0.46 g triphenyl, 0.51 g tribenzyl tin chloride and 0.24 g alizarin were used).

RESULTS AND DISCUSSION

The new triorganotin hydroxyanthraquinonates are mostly amorphous but vividly coloured. They exhibit stability to light, air and heat exposure. They are insoluble in water and most inorganic and organic solvents and only slightly soluble in $CHCl_3$ and DMSO, a property that has obstructed their thorough study. Analytical data of the new compounds are presented in Table 1. Their synthesis occurred via the interaction of the organotin hydroxide with the parent quinone. The triorganotin derivatives formulated as $R_3Sn(\mu\text{-}Q)SnR_3$ (R = Bu, Ph, Bz; Q = hydroxyanthraquinonato ligand) may be described as the corresponding hydroxyanthraquinonates, in

TABLE 1
Physical properties of the new triorganotin hydroxyanthraquinonates

Compound	Molecular type	M.W.	Yield (%)	Color	Elemental analysis theoretical (found) (%)
Quinizarin derivatives					
1. $(Bu_3Sn)_2Q_1$	$C_{38}H_{60}O_4Sn_2$	818	62	Dark purple red	55.75 (55.90) C 7.33 (7.52) H
2. $(Ph_3Sn)_2Q_1$	$C_{50}H_{36}O_4Sn_2$	938	74		63.96 (64.03) C 3.83 (3.70) H
3. $(Bz_3Sn)_2Q_1$	$C_{56}H_{48}O_4Sn_2$	1022	60		65.75 (65.24) C 4.69 (4.57) H
Anthrarufin derivatives					
4. $(Bu_3Sn)_2Q_2$	$C_{38}H_{60}O_4Sn_2$	818	54	Deep brown red	55.75 (55.20) C 7.33 (7.02) H
5. $(Ph_3Sn)_2Q_2$	$C_{50}H_{36}O_4Sn_2$	938	69		63.96 (63.50) C 3.83 (40.10) H
6. $(Bz_3Sn)_2Q_2$	$C_{56}H_{48}O_4Sn_2$	1022	57		65.75 (65.30) C 4.69 (4.42) H
Leucoquinizarin derivatives					
7. $(Bu_3Sn)_2Q_3$	$C_{38}H_{62}O_4Sn_2$	820	53	Dark brown	55.61 (55.13) C 7.56 (7.82) H
8. $(Ph_3Sn)_2Q_3$	$C_{50}H_{38}O_4Sn_2$	940	68		63.83 (63.35) C 4.04 (4.21) H
9. $(Bz_3Sn)_2Q_3$	$C_{56}H_{50}O_4Sn_2$	1024	57		65.63 (64.00) C 4.88 (4.54) H
Alizarin derivatives					
10. Bu_3SnQ_4	$C_{26}H_{34}O_4Sn$	529	68	Deep red	58.97 (58.54) C 6.42 (6.70) H
11. Ph_3SnQ_4	$C_{32}H_{22}O_4Sn$	589	76		65.19 (65.20) C 3.74 (3.81) H
12. Bz_3SnQ_4	$C_{35}H_{28}O_4Sn$	631	64		66.56 (66.22) C 4.44 (4.55) H

accordance with the reaction stoicheiometry and spectroscopic results. The general reaction scheme is outlined in Figure 2.

The anthraquinones used herein, may coordinate to metal ions by different modes. Our main goal has been to determine which one of the anthraquinoid tautomeric structures will prevail to the chelation with Sn and whether monodentate or bidentate Sn-O bonding will be preferred. Experimental observation indicates that quinizarin (Q_1) and anthrarufin (Q_2) react

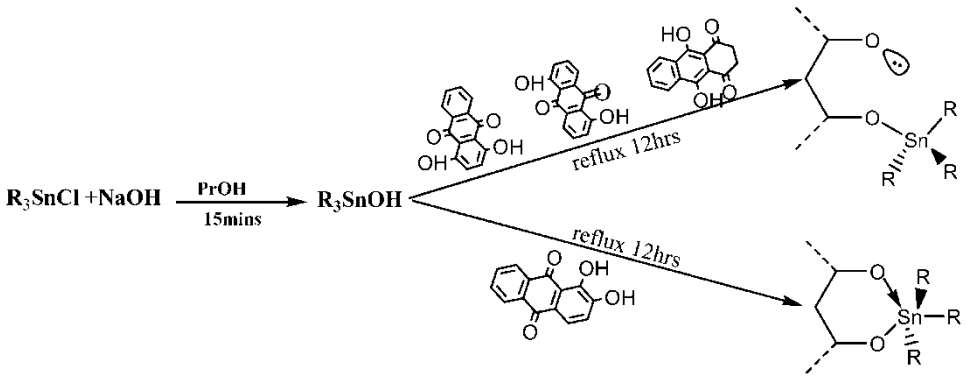

FIG. 2. General reaction scheme, applied to tributyl, triphenyl and tribenzyl-tin derivatives.

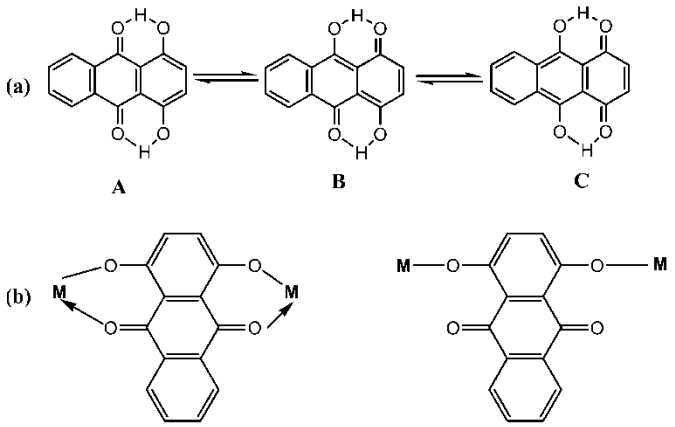

FIG. 3. (a) Tautomeric forms of quinizarin; (b) Potential coordination modes for quinizarin.

instantly while leucoquinizarin (Q_3) and alizarin (Q_4) derivatives precipitate gradually with texture and color alterations.

According to the literature,[44] quinizarin may exist in solution as an equilibrium mixture of 3 main tautomers depicted in Figure 3: 1,4-dihydroxy-9,10-anthraquinone (**A**), 4,9-dihydroxy-1,10-anthraquinone (**B**), and 9,10-dihydroxy-1,4-anthraquinone (**C**) (For numbering, see Figure 1). Formation of the majority of monometal complexes proceeds through the 1,10-anthraquinoid structure, while the 9,10-anthraquinoid structure is typical of bimetal complexes. Similar studies for anthrarufin[45] show that the ligand may occur in seven excited states that differ not only in the ionization degree but also in the contribution of the tautomeric 9,10-, 1,10-, and 1,5-anthraquinoid structures while affording complexation.

On the other hand, alizarin as a 1,2-chelate displays ligand-based redox chemistry and its ionization follows the equilibria:

$$AQ(OH)_2 \rightleftharpoons AQ(OH)O^- \rightleftharpoons AQO^{2-}$$

where $AQ(OH)_2$ is alizarin, and $AQ(OH)O^-$ and AQO^{2-} are the corresponding mono- and dianions. The main tautomers of alizarin are depicted in Figure 4a. The 2,9-, 1,10-, and 1,2-tautomers are more probable to participate in chelation processes than the 9,10-anthraquinoid structure. Furthermore,[46] complexation energies of these tautomers reveal that chelation has a negligible influence on the structure of the anthraquinone backbone and in most cases the ligand tends to keep a planar conformation. The metal–oxygen bonds involve p or d metal orbitals depending on whether the d shell is full or empty. Bimetallic alizarinates with 9,10-anthraquinoid structures are not formed because of the adjacent negatively charged oxygen atoms; therefore the complexation of red metal alizarinates is expected to occur at the peri- or ortho-hydroxycarbonyl group (Figure 4b).

Combining the above with the spectroscopic analysis of our newly synthesized triorganotins, we have concluded on the formation of monodentate Sn-O bonding for quinizarin, anthrarufin and leucoquinizarin, which are coordinated to Sn(IV) central atom via the phenolic oxygen donor atoms, with the R-substituents of the organotin moiety completing the tetrahedral coordination environment. On the contrary, organotin

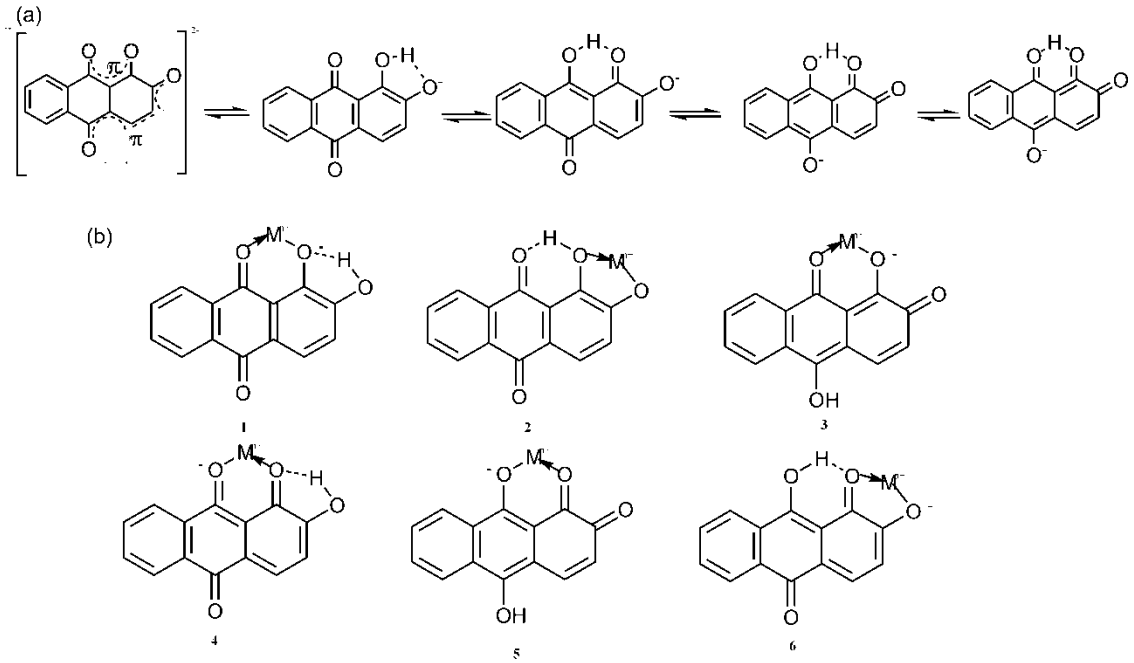

FIG. 4. (a) Tautomeric forms of alizarin; (b) Potential coordination modes for alizarin.

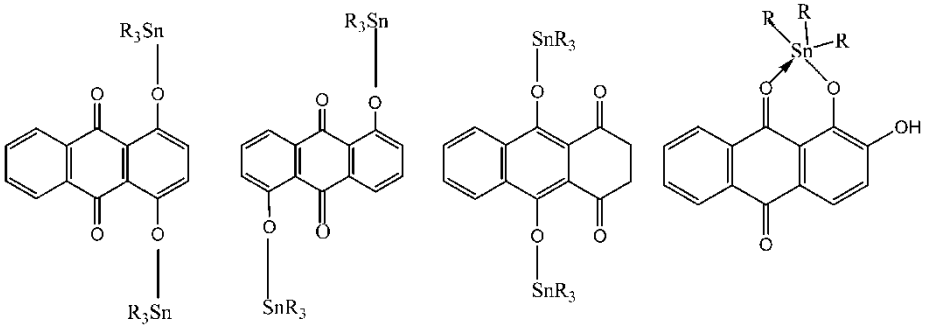

FIG. 5. Proposed structures of the new triorganotin hydroxyanthraquinonates, where R=Bu, Ph and Bz.

alizarinates form a six-membered chelate ring, which stabilizes the Sn central atom in a five-coordinated environment exhibiting distorted trigonal bipyramidal geometry. The ligand is coordinated to Sn (IV) via the quinonic oxygen of C_9 and its neighbouring phenolic oxygen of C_1. The R-substituents of the tin cation complete the trigonal bipyramidal coordination environment (Figure 5).

IR Studies

Selected IR data for the free ligands and their complexes are reported in Table 2. Assignments of the infrared bands were made by comparing the spectra of the complexes with the ones of the free ligands. The sharp bands in the region $\sim$3400 cm^{-1} in the spectra of the free hydroxyanthraquinones are assigned to the ν(—OH) stretching vibrations and disappear in the triorganotin derivatives of quinizarin, anthrarufin and leucoquinizarin, providing evidence that the hydroxyl groups participate to the coordination. The ν(=C—H) stretching vibrations of the free ligands appear in the region 3015–3080 cm^{-1}.

Upon complex formation no major spectral changes are noticed for the tributyltin derivatives, but in the case of the triphenyl and tribenzyltins both the range and tension of these bands increase (3015–3190 cm^{-1}), probably due to the incorporation of the Ph and Bz substituents. The same applies to the medium ν(-C-H) stretching vibrations of leucoquinizarin' s saturated ring present (2855–2930 cm^{-1}).

The carbonyl ν(C=O) stretching vibration is a sharp band (only one because of the ligands' symmetric nature) at 1629 cm^{-1} for quinizarin, 1634 cm^{-1} for anthrarufin and 1633 cm^{-1} for leucoquinizarin. Upon complexation, the band is not influenced, providing strong evidence that the carbonyl moiety does not interact with tin. The non-involvement of the carbonyls is further substantiated by the fact that no major changes occur in the region 1570–1590 cm^{-1}, where the ν(C=C) skeletal in-plane vibrations of the conjugated aromatic ring appear.

In the free ligands, the ν(C—O) band appears in 1225 cm^{-1} for the quinizarinato moiety, in 1214 cm^{-1} for anthrarufin and 1197 cm^{-1} for leucoquinizarin. Upon derivatization, a minor shift in higher frequencies by 10–60 cm^{-1} is noticed, which is consistent and indicative of the coordination of the phenolic oxygens with tin. We should notice that free quinizarin has a strong broad band $\sim$800 cm^{-1}, which is shifted to $\sim$850 cm^{-1} in all its triorganotin derivatives.

In free alizarin, the ν(—OH) stretching vibrations appear in the area 3385–3480 cm^{-1}. In the corresponding complexes, a slight shift to higher frequencies by 15–30 cm^{-1} in the form of broader bands may be attributed to the breaking down of extensive hydrogen bonding of the free ligand. These bands are related to the stretching vibrations of the phenolic hydroxyls and their maintenance strengthens the notion that at least one of the two phenolic hydroxyls (C_2-O) does not participate to coordination. The carbonyl ν(C_9=O) stretching vibration of alizarin is found at 1664 cm^{-1}.

In the corresponding complexes, a bathichromical shift to lower frequencies by 15–35 cm^{-1}, depending on the derivative (see Table 2), highlights the participation of the C_9=O group to coordination. Respectively, the ν(C_1-O) band, at 1198 cm^{-1} for alizarin, is shifted in higher frequencies, supporting the bidentate coordination of alizarin, which forms a six-membered ring, energetically favored when compared to the five-membered that would form with the participation of the two phenolic oxygens.

In the spectra of tributyltin complexes, we assign the pattern of medium bands in the area $\sim$2850–2990 cm^{-1} at the stretching vibrations of the n-Bu skeleton. Some strong to medium intensity bands also appear in the tributyltin complexes spectra in the region 1180–1220 cm^{-1} and are assigned to ν(Sn–CH_3) stretching vibrations. The strong band at 510–585 cm^{-1} is also due to the Sn—C stretching vibration and has been previously reported by Singh[47] to be characteristic of tributyltins. The triphenyltin hydroxyanthraquinonates show the expected symmetric and asymmetric stretching vibrations of the $SnPh_3$ moiety at ca. 460, 270 and 240 cm^{-1}.

The usual benzene ring absorptions appear in the complexes spectra together with the typical band of the phenyltin group at $\sim$1070 cm^{-1}. The ν(OH) band of Ph_3SnOH ($\sim$3610 cm^{-1}) is not found in the spectra of the products, indicating complex formation of the triphenyltin moiety. The incorporation of

TABLE 2
Characteristic IR frequencies (cm^{-1}) of the free ligands and their triorganotin derivatives

	-OH	C-Har	C-Haliph	C═O	C═C-C-O	C-O	Sn-C	Sn-O
Quinizarin derivatives								
Free ligand	3436br	3020–3070 m	—	1629vs	1590s	1225 s	—	—
1. $(SnBu_3)_2Q_1$	—	3020–3070 m	2890–2955 m	1630vs	1586s	1274 s	510 m; 580 m	460–530 m
2. $(SnPh_3)_2Q_1$	—	3020–3090 m	—	1628vs	1585s	1256 s	460 m; 270 m; 240 m	
3. $(SnBz_3)_2Q_1$	—	3020–3110 m	2910–2970 m	1629 vs	1590 s	1257 s	240 m; 460 m	
Anthrarufin derivatives								
Free ligand	3417br	3015–3080 m	—	1634vs	1571s	1214s	—	—
4. $(SnBu_3)_2Q_2$	—	3015–3080 m	2890–2945 m	1636vs	1570s	1270s	515 m; 570 w	470–510 m
5. $(SnPh_3)_2Q_2$	—	3015–3140 m	—	1635vs	1570s	1241s	455 m; 260 m; 240 m	
6. $(SnBz_3)_2Q_2$	—	3015–3090 m	2930–2985 m	1629vs	1577s	1261s	555 m; 240 w	
Leucoquinizarin derivatives								
Free ligand	3426br	3025–3060 m	2855–2930 m	1633vs	1581s	1197s	—	—
7. $(SnBu_3)_2Q_3$	—	3025–3060 m	2860–2975 m	1630vs	1590s	1226s	530 m; 570 m	490–545 m
8. $(SnPh_3)_2Q_3$	—	3020–3190 m	2850–2930 m	1637vs	1589s	1209s	470 m; 280 m	
9. $(SnBz_3)_2Q_3$	—	3030–3075 m	2915–2980 m	1630vs	1585s	1215s	540 m; 260 m	
Alizarin derivatives								
	-OH	C-Har	C-Haliph	C_9═O	C═C-C-O	C_1-O	Sn-C	Sn-O
Free ligand	3385–3480br	3020–3075 m	—	1664vs	1588s	1198s	—	—
10. $SnBu_3Q_4$	3390–3500br	3020–3075 m	2910–2990 m	1645vs	1526s	1217s	570 m; 545 w	490–520 m
11. $SnPh_3Q_4$	3400–3490br	3015–3120 m	—	1629vs	1479s	1226s	460 m; 260 m	
12. $SnBz_3Q_4$	3395–3505br	3020–3085 m	2940–2990 m	1648vs	1531s	1219s	540 m; 260 m	

vs = very strong; s = strong; m = medium; w = weak; br = broad.

the tribenzyl- group is indicated by the typical pattern present in the region 2920–3080 cm^{-1}, accounting for ν(=C-H) and ν(C-C-H) stretching vibrations. The occurrence of new vibrational modes in the region 545–460 cm^{-1}, which is absent in both the spectra of the ligands and parent organotins, may be assigned to the ν(Sn—O) stretching vibrations and support the linkage between the oxygen atoms and tin. The bands observed at ~280 and 240 cm^{-1} in the far-IR spectra of the complexes have been tentatively assigned to v_{asym} and v_{sym} (Sn—C) modes.

^{1}H-NMR Studies

The ^{1}H-NMR spectra of the new complexes show the expected integration and peak multiplicities with aromatic signals undergoing a more complex pattern upon chelation. Selected ^{1}H-NMR data are presented in Table 3. A typical singlet at 1.5δ, when observed, has been assigned to the water of $CHCl_3$. The chemical shifts of the signals for the R-substituents appear at the same position as in the free organotins and have been assigned in the spectra of the complexes.

The tributyltin (IV) derivatives show the typical butyl skeleton pattern between 0.70–2.0δ. In the spectra of the triphenyltin (IV) complexes it is not possible to distinguish between signals due to aromatic protons of the ligands and those linked to tin, but integration has taken their presence into account. A complicated set of multiplets due to the ligand aromatic and phenyl ring protons of the Sn-Ph_3, moiety is observed in the range 6.90–8.50δ for all the complexes. The compounds' poor solubility has prevented observation of any tin satellites.

In the Q_1, Q_2, Q_3 triorganotins, the signal due to the phenolic protons of the free ligands disappears suggesting coordination with tin. The downfield shifts of the aromatic and quinoid ring protons are attributed to the complexation, which generates a delocalization of electron density on the anthraquinone ring.

In alizarin complexes, only the H_1 signal disappears, substantiating our thoughts for its non-participation to the coordination with tin. The other phenolic proton (H_2) resonance is still present in a form of a weaker signal. An experiment with D_2O exchange has verified the existence of a phenolic proton in alizarin derivatives (obviously H_2). The ^{13}C NMR spectra that we managed to acquire show a significant downfield shift of all carbon resonances compared with the free hydroxy-anthraquinones due to electron density transfer from the ligand to the acceptor.

Thermal Analysis

While trying to define the melting points of the new triorganotins we have observed a complicated, multi-step process of color and texture alterations, which in most cases ends with an apparent decomposition above 350°C. This behavior prompted us to a more detailed thermal study, which revealed that up to 400°C several phenomena occur in association with respective weight loss. The most striking one is the abrupt transition from a highly endothermic to a considerable exothermic phenomenon in the region 250–350°C, where the greatest mass loss occurs. This phenomenon may be correlated to the ligands' coordination mode. Temperature accounts for the tautomerisation process of hydroxyanthraquinones, which is respectively associated with the way the ligand approaches the metal. At least some endothermic phenomena seen herein may be attributed to such an alteration in the coordination scheme especially if one takes into consideration the possibility

TABLE 3
Selected ^{1}H-NMR data of the new triorganotin hydroxyanthraquinonates

1. $(SnBu_3)_2Q_1$: 0,7–1,8 δ(m): 54H, Sn-Bu; 6,9–7,2 δ(m): 2H, H_2,H_3; 7,4–7,5 δ(m): 2H, H_6,H_7; 7,8–8,32 δ(m): 2H, H_5,H_8
2. $(SnPh_3)_2Q_1$: 1,5 δ(s) H_2O in $CHCl_3$; 6,8–7 δ(m): 2H, H_2,H_3; 7–8,2 δ(m): 34H (ligand and Sn-Ph protons, undistinguishable)
3. $(SnBz_3)_2Q_1$: 3,25 δ(s): 12H, -CH_2 of the benzyl group; 6,9–7 δ(m):2H, H_2,H_3; 7–8 δ(m): 34H (ligand and Sn-Ph protons, undistinguishable)
4. $(SnBu_3)_2Q_2$: 0,8–1,6 δ(m): 54H, Sn-Bu; 6,9–7,1 δ(m): 2H, H_2, H_6; 7,5–7,7 δ(m): 2H, H_3, H_7; 7,8–8 δ(m): 2H, H_4, H_8
5. $(SnPh_3)_2Q_2$: 1,5 δ(s) H_2O in $CHCl_3$; 6,9–7 δ(s): 2H, H_2, H_7; 7–8,1 δ(m): 34H, (ligand and Sn-Ph protons, undistinguishable)
6. $(SnBz_3)_2Q_2$: 3,5 δ(s): 12H, -CH_2 of the benzyl group; 6,8–6,9 δ(s): 2H, H_2, H_6; 7–8,2 δ(m): 34H, (ligand and Sn-Ph protons undistinguishable)
7. $(SnBu_3)_2Q_3$: 0,9–1,5 δ(m): 54H, Sn-Bu; 3,1 δ(s): 4H, H_2, H_3; 7,4–7,8 δ(m): 2H, H_6, H_7; 8,3 δ(s): 2H, H_5, H_8
8. $(SnPh_3)_2Q_3$: 3,1 δ(s): 4H, H_2, H_3; 7–8,4 δ(m): 34H, (ligand and Sn-Ph protons, undistinguishable
9. $(SnBz_3)_2Q_3$: 1,5 δ(s) H_2O in $CHCl_3$; 3,3 δ(s): 4H, H_2,H_3; 3,5 δ(s): 12H, -CH_2 of the benzyl group; 6,9–7,8 δ(m): 32H, H_6,H_7 and Sn-Ph protons; 8,3 δ(s): 2H, H_5, H_8
10. $SnBu_3Q_4$: 0,8–1,6 δ(m): 27H, Sn-Bu; 6,9–8,0 δ(m): 6H,H_3, H_4, H_5, H_6, H_7, H_8; 10,4 δ(s):1H, H_2
11. $SnPh_3Q_4$: 1,5 δ(s) H_2O in $CHCl_3$; 6,9–8,6 δ(m):21H, (ligand and Sn-Ph protons, undistinguishable; 10,6 δ(s): 1H, H_2
12. $SnBz_3Q_4$: 2,9–3,5 δ(m): 12H, -CH_2 of the benzyl group; 6,8–7,0 δ(s): 1H, H_3; 7–8,2 δ(m):14H, (ligand and Sn-Ph protons, undistinguishable); 10,2 δ(s):1H, H_2

of forming a five-coordinate tin center by means of the α-N pair lone coordination.

The triphenyl derivatives of Q_1, Q_2 and Q_3 were studied in a range up to 650°C. Up to this temperature, the total mass loss does not exceed the 60% of the initial mass. This implies either that breakdown is not complete or that tin oxides may co-exist with residue. Given the high percentage of residues, we have acquired their IR spectra, where it is evident that part of the hydroxyanthraquinonic ring is still present at 600°C. The thermogravimetric (TG) data show that decomposition is temperature- related and differentiates according to the R- substituent of the organotin moiety. The weight losses observed due to thermal decomposition are in respect with the calculated values and within the margins of experimental mistakes ($\pm$3%.). The degradation patterns of quinizarin (Q_1) and anthrarufin (Q_2) derivatives are remarkably similar and therefore we only present data for the triphenyl and tributyl quinizarinate.

Compound 2 (triphenyltin quinizarinate), shows a slight weight loss of $\sim$ 10% up to 230°C. Two endotherms are observed at 100°C and 125°C, followed by an exotherm at 200°C and a weak endotherm at $\sim$300°C. None of these phenomena may be attributed to the compounds' melting and have been assigned to phase alteration processes of organotins. From the temperature of 250°C and up, a rapid weight loss begins. At 325°C, an exotherm is noticed, corresponding to approximately 35% weight loss, which may be accounting for the SnPh moiety. A gradual weight loss continues up to 600°C without any evidence of quinizarin degradation. A last weak exotherm is present at $\sim$ 425°C.

An equivalent degradation pattern -although a bit more complicated- accounts for the triphenyltin derivative of leucoquinizarin (compound 8). A slight weight loss, not more than 5% occurs up to 230°C. Up to this temperature, we notice two endotherms at 100°C and $\sim$ 170°C, followed by an exotherm at 200°C and finally another endotherm at $\sim$230°C. Within the range of 200°C and 230°C, there is a 15% weight loss, which corresponds to phenyl groups of the triorganotin moiety. At 350°C, the rapid weight loss stabilizes, after approximately 37% of the initial mass has been lost. A strong exotherm is noticed in this area.

The degradation patterns of the tributyltin derivatives show a higher degree of mass loss exceeding 60% of the initial mass for compound 1 and 65% for compound 7. In both cases, the rapid weight loss begins at $\sim$250°C and ends at $\sim$350°C. Compound 1 shows three endotherms at $\sim$180°C, 200°C and 350°C, while compound 7 displays only two endotherms one at 200°C and another at $\sim$340°C.

Cytotoxicity Studies

We have measured the cytotoxicity of some of the new triorganotin hydroxyanthraquinonates against five human tumour K562, MCF-7, HeLaS3, PC3, Hs 294T and one non-tumor human cell line MRC5, using the colorimetric SRB assay.

TABLE 4
IC_{50} values of some of the new triorganotin hydroxyanthraquinonates

	K562	MCF 7	HeLa	Hs 294T	MRC 5
Doxorubicin	0.82	1.19	5.08	29.85	0.32
$(Ph_3Sn)_2Q_1$	0.83	0.08	0.21	0.4	0.03
$(Bu_3Sn)_2Q_1$	>100	3.02	2.06	2.24	12.29
$(Ph_3Sn)_2Q_2$	5.66	0.02	1.42	0.45	0.039
$(Ph_3Sn)_2Q_3$	0.45	0.17	0.36	0.44	0.098
$(Bu_3Sn)_2Q_3$	18.77	1.09	1.41	0.29	1.96
$Ph_3Sn\ Q_4$	0.82	0.29	0.92	0.62	0.02

The results are summarized in Table 4. Most of the compounds were found to be toxic in both tumor and normal cells and were continuously present in the culture for 48 h in the range of concentrations from 10^{-8} to 10^{-4} M.

Regarding the cytotoxicity profile, dose-dependent response was found for all cell lines except PC3 (prostate carcinoma cell line). By a second, independent experimentation, we have verified this as a true response, which has obstructed us from calculating the IC_{50} values for all compounds except tributyl quinizarinate, for which the IC_{50} value was found to be 0.55 μM. Most of the compounds at concentrations of 10^{-5} and 10^{-4}M gave cytotoxicity above 80% regardless of the cell type, while in most cases no tissue specific cell response was observed. All tested compounds are more active against HeLa and Hs294T than against K562 and MCF7. The melanoma cells (Hs 294T) are known to be non-sensitive to doxorubicin but in our case, a remarkable sensitivity is expressed towards these new compounds. The triphenyltin derivatives of quinizarin and alizarin were found as active as doxorubicin, while the tributyl leucoquinizarinate is remarkably less active. This same compound shows an analogous response to doxorubicin when tested to MCF7 cells. The undesirable effect of high cytotoxicity against MRC5 cells is depicted in almost all compounds.

The justification of this highly toxic profile lies predominantly on the limited solubility, which allows no margin for cellular penetration or interactions with proteins. Although it has been repeatedly stated that triorganotins bearing tetrahedral geometry possess a better biological activity,[8–10] in our case this is not confirmed. Actually, we have associated the toxicity to the non-involvement of the lone pair on the oxygen atom of carbonyl group in coordination. This has been reported to play a key role in the escalation of toxicity.[2,3] Moreover, toxicity is affected by the R substituents of tin (IV) and the function of the ligands bound to it. In the R_3SnQ unit, the function of Q is to transport the active organotin (IV) moiety to the action site and release it there on hydrolysis. We therefore assume that the complicated structure of hydroxyanthraquinones may not be desirable for this process.

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