Spectroscopic Investigation of Organotin(IV) Derivatives of 7-Epiclusianone: a preliminary *in vitro* Antitumor Evaluation of the HN-5 Human Carcinoma Cell

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ABSTRACT

A series of organotin(IV) compounds have been prepared by reaction with 7-epiclusianone (*Epi*), a natural product extracted from fruits of *Rheedia gardneriana*. This compound has an interesting motif with several coordinating sites for metal-ligand bond formation, and in solution, it shows a keto-enol tautomerism. The ^{1}H NMR of the organotin(IV) derivatives has revealed intramolecular hydrogen bonding, indicating that the keto-enol tautomerism of 7-epiclusianone is not involved upon coordination of those, but the absence of this bonding type in the case of the $SnCl_4$ derivative suggests a strong interaction. The Mössbauer spectroscopy has revealed five- and six-fold coordination for the organotin(IV) and $SnCl_4$ derivatives in the solid state. However, in solution all tin complexes have six-fold coordination, as shown by ^{119}Sn NMR. The overall data point out that the organotin(IV) precursors $SnCl_xPh_{4-x}$ (x = 1, 2) are weakly bonded to the 7-epiclusianone, except the $SnCl_4$. Bioassay *in vitro* of the substance test [$SnClPh_3$(*Epi*)] (**1**) has been investigated using two epithelial cells: normal MDCK from canine kidney, and HN-5 from a human carcinoma of the tongue. The results clearly demonstrate that the time for cellular reproduction has been reduced in the presence of the substance test.

Keywords: 7-epiclusianone, organotin, carcinoma, spectroscopy, coordination chemistry

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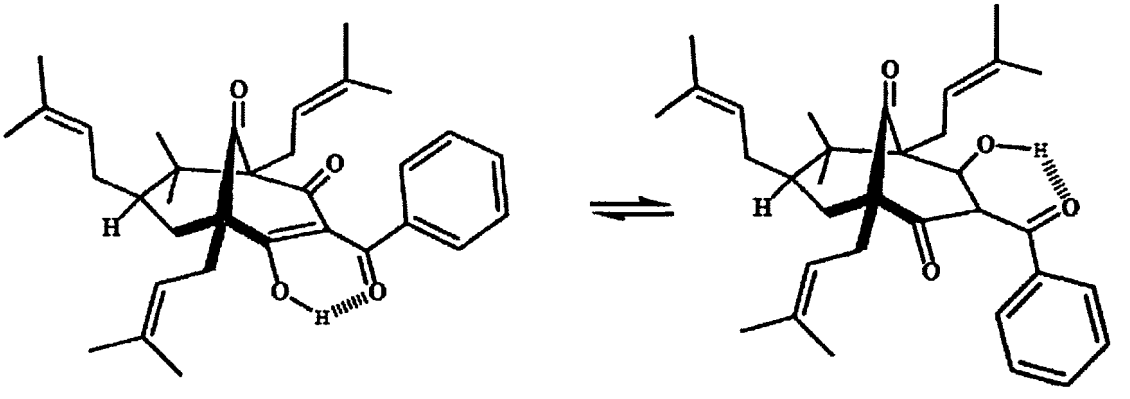
INTRODUCTION

Organotin compounds have been of interest to inorganic chemists due to their versatile chemistry, among other aspects. For instance, they have presented appealing pharmacological properties as antitumor and antifungal drugs. Triorganotin derivatives have been acknowledged as the most active compounds against several cancer cell lineages /1/. Varieties of organic compounds in combination with organotin precursors have revealed antitumor activity. However, correlations between the biological activity and the motif of the active compound have not been clearly established. The 7-epiclusianone (3-benzoyl-4-hydroxi-6-6-dimethyl-1,5,7-tri(3-methyl-2- butenyl)bicycle-[3.3.1]non-3-eno-2,9-dione) is a natural substance extracted from fruits of *Rheedia gardneriana*. This compound in solution has a keto-enol tautomerism as shown in Scheme 1 /2/. Crystallography studies have shown 7-epiclusianone as a new isomeric form of clusianone /3/. The former is biologically active against *Clavibacter michiganense*, *Listeria monocytogenes* and *Staphylococcus aureus* /4/. In addition, it is active *in vitro* but inactive *in vivo* against *Trypanossoma cruzi*, an agent of a well-known disease in Brazil named "Doença de Chagas". The biological activity against organisms such as *Artemia salina*, the fungus *Cladosporium shaerospermum* and the snail *Biomphalaria glabrata* have also been reported /4/. To inorganic chemists, 7-epiclusianone is a polydentate ligand accounting for the number of available nonbonding electrons on the oxygen atoms. In view of that, new potential bioactive compounds are conceivable by coordination of metals to those coordinating sites. This chemical feature has been the motivation for studying the coordination chemistry of 7-epiclusianone towards organotin(IV) precursors and SnCl4.



Scheme 1: Keto-enol tautomerism of 7-epiclusianone.

In this work, a series of organotin derivatives have been prepared by reacting the $SnCl_xPh_{4-x}$ (x = 1, 2, 4) precursors with 7-epiclusianone. As far as our knowledge has reached, this is the first attempt to study the coordination chemistry of 7-epiclusianone. Several spectroscopic methods such as ^{1}H and ^{119}Sn NMR, infrared and Mössbauer spectroscopy were the techniques employed to characterise the reaction products. Two epithelial cell lineages have been used for the *in vitro* antitumor bioassay of the substance test (1): HN-5 from human carcinoma of the tongue /5-7/ as well as standard epithelial MDCK from canine kidney /8/.

MATERIALS AND METHODS

Two pieces of equipment were used to obtain the ^{1}H and ^{119}Sn NMR spectra: a Varian Mercury 300 MHz and Bruker DPX-400 spectrometers. The latter was equipped with an 89 mm wide-bore magnet. Both have tetramethylsilane ($SiMe_4$) and tetramethyltin ($SnMe_4$) as internal standard ($\delta = 0$), respectively. The ^{119}Sn Mössbauer spectroscopy data was collected at 30 K in constant acceleration equipment moving a CaSnO3 source at room temperature. All spectra were computerfitted assuming Lorentzian single lines. The infrared spectra were recorded on a Perkin Elmer Spectrum 1000 grating spectrometer using Nujol suspension between CsI windows, scanning from 4000 to 200 cm^{-1}. A Perkin Elmer 2400 CHN analyzer was used for the chemical analysis. The 7-epiclusianone was extracted from fruits of *Rheedia gardneriana* during the summer season as outlined in the literature /2/. Vacuum techniques, nitrogen atmosphere, and Schlenk glassware was used throughout the experiments to prepare the organotin(IV) and $SnCl_4$ derivatives. Solvents and reagents have been purchased from Aldrich and Sigma companies.

2.1. Preparation of the organotin compounds

All 7-epiclusianone derivatives were prepared by an equimolar reaction with the $SnCl_xPh_{4-x}$ (x = 1, 2, 4) precursors under inert atmosphere of nitrogen. Ethanol, a mixture of hexane/ethanol and dichloromethane were the solvents for the preparation of complex **1**, **2** and **3**, respectively. The metal reagent was dissolved in 20 mL of the appropriate solvent followed by addition of solid 7- epiclusianone into the metal solution. Complex **1** and **2** have been prepared under heating (50÷C) and stirring for 3 h. For the SnCl4 derivative, the reaction has occurred at room temperature under stirring for the same interval of time. Removal of half the solvent under reduced pressure led to pale-yellow or yellow solids in complexes **1** and **2**, and orange in complex **3**. The solids were filtered off in air, washed with hexane under reduce pressure and kept in desiccators. Several attempts of reacting tributyltin(IV) chloride with 7-epiclusianone failed, irrespective of the reaction conditions.

[$SnClPh_3$(*Epi*)] (**1**). Yield: 0.71g (80 %). Mp (oC): 74 -75. *Anal.* Calc. for $C_{51}H_{57}O_4ClSn$: C, 68.97; H, 6.46. Found: C, 68.57; H, 6.30. IR (CsI pellets, cm-1): 3435 (OH), 3052, 3068 (CH, Ph, alkene), 2963, 2856 (CH_2, CH_3), 1727 (C=O, non-conjugated), 1673 (C=O, conjugated), 518 (Sn-O), 332 (Sn-Cl), 275 (Sn-C). ^{1}H NMR (CDCl3, 300 MHz, δ, ppm; d, doublet; m, multiplet): 17.90, 17.82 (d, hydrogen bonding), 7.79 - 7.35 (m, Ph), 5.24 - 4.81(m, CH, alkene), 2.79 - 0.99 (m, CH, CH_2, CH_3). ^{119}Sn NMR (CH3OH, 149.2 MHz, δ, ppm; s, singlet): -549.0 (s). Mössbauer: IS = 1.34(5) mm/s, QS = 2.53(5) mm/s. SnClPh3: IS = 1.33(1) mm/s, QS = 2.54(1) mm/s /9/.

[$SnCl_2Ph_2$(*Epi*)] (**2**). Yield: 0.43g (51 %). Mp (oC): 103 -104. *Anal.* Calc. for $C_{45}H_{52}O_4Cl_2Sn$: C, 63.85; H, 6.19. Found: C, 63.02; H, 6.63. IR (Nujol/CsI, cm-1): 3441 (OH), 1729 (C=O, nonconjugated), 1671 (C=O, conjugated), 539 (Sn-O), 304 (Sn-Cl), 248 (Sn-C). ^{1}H NMR (CD3OD, 300 MHz, δ, ppm; d, doublet; m, multiplet): 17.76, 17.71 (d, hydrogen bonding), 7.56 - 7.36 (m, Ph), 5.27 - 4.65(m, CH,

alkene), 2.72 - 0.86 (m, CH, CH_2, CH_3). ^{119}Sn NMR (CH_3OH, 149.2 MHz, δ, ppm; s, singlet): -516.0 (s). Mössbauer: IS = 0.80(5) mm/s, QS = 1.57(5) mm/s. $SnCl_2Ph_2$: IS = 1.41(1) mm/s, QS = 2.83(1) mm/s /9/.

[$SnCl_3$(*Epi*)].9H_2O.CH_2Cl_2 (**3**). Yield: 0.69 g (93 %). Mp (oC): 112 d. *Anal.* Calc. for $C_{34}H_{61}O_{13}Cl_5Sn$: C, 41.94; H, 6.31. Found: C, 41.71; H, 6.75. IR (Nujol/CsI, cm-1): 3449 (H_2O), 1723 (C=O, non-conjugated), 1668 (C=O, conjugated), 602, 654 (Sn-O), 352, 334 (Sn-Cl). ^{1}H NMR (CD_3OD, 300 MHz, δ, ppm; d, doublet; m, multiplet): 7.35 - 7.26 (m, Ph), 3.76 - 3.42(m, CH, alkene), 1.82 - 0.75 (m, CH, CH_2, CH_3). ^{119}Sn NMR (CH_3OH, 149.2 MHz, δ, ppm; s, singlet): - 507.0 (s), -596.0 (s). Mössbauer: IS = 0.33(5) mm/s, QS = 0.43(5) mm/s. $SnCl_4$: IS = 0.82 mm/s, QS = 0.00 mm/s) /10, 11/.

2.2. Bioassay of organotin(IV) compounds

The antitumor activity was evaluated based on the procedure described in the literature /12/ with slight modifications. Two epithelial cell lineages, MDCK and HN-5, were used in order to investigate the activity of complex **1** as a potential antitumor drug.

Standard cell culture medium preparation:

The culture medium for the living cells was prepared by mixing 13.5 g Eagle medium, 3.7 g sodium bicarbonate, 100 U mL-1 of penicillin, 0.1 mg mL^{-1} of streptomycin and 0.25 μg mL^{-1} amphotericin B with 1.0 L of distilled water.

Trypsin solution:

This solution was prepared by combining 0.25 g trypsin, 0.71 g sodium phosphate, 0.90 g sodium chloride, 0.05 g EDTA with 100 mL deionised distilled water.

Phosphate buffer solution:

This solution was prepared by adding 4.0 g sodium chloride, 1.0 g potassium chloride, 1.0 g potassium dihydrogenphosphate, 7.7 g sodium phosphate monobasic to 5.0 L distilled water. The final pH of the solution was 7.6.

Cell culture and bioassay for testing 7-epiclusianone as an antitumor drug

The standard preparation for the antitumor test of those cell lineages was performed following the outlined methodology. MDCK and HN-5 cells /7, 8/ were kindly provided by professor David Garrod (Manchester University, UK) and Dr. Donna Davis (Medical Oncology Unit, Southampton University, UK). These cells were grown in DMEM supplemented with 10% of treated calf serum, 2 mM L-glutamine and 1 mM non-essential aminoacids. These cells were put in culture flasks (Nunclon) and allowed to grow in a humidified incubator at 37°C with 5% CO2. When the cells were sub-confluent, they were transferred at 2.5 x 10^4 cells to six-well plates (Nunclon). Two six-well plates were used for each cell line. After removing the growth medium and washing the plates with PBS, [$SnClPh_3$(*Epi*)] at a concentration of 28.25 x 10^{-12} g/mL in

growth medium was added in one plate of each cell lineage. As control, normal medium with 2.77 % of ethylic alcohol, used as solvent, was also added to one plate of each cell lineage. All the plates were kept in the incubator for 24 h, followed by trypsinization with 0.25 % trypsin (w/v) (Sigma) plus 1 mM EDTA (Sigma) in PBS. The detached cells were then recovered from the wells by adding DMEM with supplements, which interrupted the trypsinization. The number of recovered cells was obtained by counting them using a Neubauer hemocytometer and a trypan-blue assay for counting only viable cells. This procedure was performed selecting a well randomly from the six wells plates, from control and test plates, at 24 h intervals and the number of cells at each time point was plotted in graphs where the growth curves were drawn.

RESULTS AND DISCUSSION

The 7-epiclusianone is a natural product obtained from fruits of *Rheedia gardneriana*, which is harvested in the Brazilian summer season. This compound and its organotin(IV) derivatives are soluble in usual organic solvents such as chloroform and methanol, except in water. Several attempts of obtaining suitable crystals of complex **1** and **2** in various solvents or in solvent mixtures have failed. From the crystals available, the structure of 7-epiclusianone has been once more determined by X-ray crystallography techniques /3/.

3.1. Infrared spectroscopy

The spectroscopic characterisation of 7-epiclusianone has been reported before /2/. Absorptions in the region of 1500 cm-1 have been assigned to the C=C bond from the phenyl ring of 7- epiclusianone. The infrared spectrum of this compound has exhibited two stretching frequencies which are related to the infrared-active stretching modes of the conjugated and non-conjugated C=O moiety. Those bands did not shift appreciably in the infrared spectra of complex **1** and **2** upon coordination of 7-epiclusianone. In fact, an insignificant shift around 1 cm^{-1} has been observed. This suggests the absence of reaction between the organotin(IV) precursors and the 7-epiclusianone. Nevertheless, a decreased intensity of the non-conjugated C=O infrared band in both organotin(IV) derivatives is evident, suggesting a weak interaction between the ligand and the organotin moiety in the solid state.

Two possible effects for this bonding weakness are conceivable. Firstly, the steric hindrance involving both the phenyl groups of the metal precursors and the bulk moieties of 7-epiclusianone. This can be envisaged by the hybrid sp^2 orbital of the oxygen atom from the non-conjugated C=O group of 7-epiclusianone and the rotating 1,5,7-tri(3-methyl-2-butenyl) moiety. In this situation, the non-bonding electrons at the oxygen atom of the non-conjugated C=O group are pointing towards this rotating moiety. This makes the approaching of the metal difficult towards the conjugated or the non-conjugated C=O fragment. In addition, steric hindrance between the metal precursors and the 1,5,7-tri(3-methyl-2-butenyl) moiety is also conceivable, accounting for the geometrical disposition of the phenyl groups at the metal centre. Secondly, the electron density entails the carbon and oxygen atoms at the non-conjugated C=O fragment. The bonding angle intrinsic to the C-(C_{sp2}=O)-C group is less than 120°. This bonding angle

requires more *p*-bond character along the C-C_{sp2} bond, increasing subsequently the *s* character in the non-conjugated C_{sp2}=O bond. Thus, the electron density is retained between the carbon and oxygen atoms. Consequently, no obvious infrared shift from this moiety has been observed upon coordination. In the case of the $SnCl_4$, starting material of complex **3**, the infrared band relative to the conjugated C=O groups has shifted by approximately 5 cm^{-1} towards low frequency upon coordination. Although this is not a significant shift, it is evidence for coordination through the conjugated C=O moiety of 7-epiclusianone.

At the low infrared frequency, new weak bands in the range of 500 to 660 cm^{-1} have been assigned to the ν(Sn-O) stretching due to metal-ligand bond formation as well as from coordinating water /13, 14/. Geometrical isomerism involving the ν(Sn-Cl) stretching mode has been deduced by the number of absorptions at the low frequency region, except in the case of complex **1**. This compound has shown only one band, indicating that in the solid state the chloride is still attached to the tin(IV) nucleus. Only one ν(Sn-Cl) infrared band has been exhibited in complex **2**, which indicates that the chlorides are in *trans* position relative to the metal centre. Steric hindrance seems to be an important aspect of the product formation. This is corroborated by the fact that no reaction with 7-epiclusianone has occurred when triphenyltin(IV) chloride was replaced by tributyltin(IV) chloride. Two bands were distinctive in complex **3** at the low frequency, indicating *fac/mer* isomerism for the chloride groups /15, 16/. The infrared spectra of *fac* and *mer* isomers usually exhibit two and three absorptions of ν(Sn-Cl), respectively, in the range of 200 to 360 cm^{-1}. However, the number of infrared bands is not always unequivocal to decide between those isomeric conformations. For instance, both rhodium-*fac* and *mer* isomers, derivatives of diphenylphosphinobutane, exhibit two infrared bands at the low frequency /16/. The higher value of the ν(Sn-Cl) stretching in the infrared spectrum of complex **3** puts forward a chloride ion in *trans* position to an oxygen atom of the conjugated carbonyl group of 7-epiclusianone.

This shift towards higher wavelengths is most likely due to the difference in electronegativity between the chloride and the oxygen atom. In this context, a *mer* isomer is presumably the main product of the reaction between $SnCl_4$ and 7-epiclusianone. Possible coordination modes for the tin(IV) precursors can be envisaged in Figure 1.

3.2. NMR spectroscopy

NMR spectroscopy has revealed the existence of keto-enol tautomerism in the organotin derivatives of 7-epiclusianone. This tautomeric equilibrium for 7-epiclusianone is well-know and it has been established before (see Scheme 1) as a doublet signal at δ 17.77 and 17.69 by ^{1}H NMR /2/. The same pattern has been displayed in the spectra of complexes **1** and **2**, revealing signals in the same region. In the case of complex **3**, the doublet has disappeared, indicating that the metal is bonded through the conjugated carbonyl group of 7-epiclusianone. No chemical shift relative to the hydroxyl fragment has been identified by ^{1}H NMR within the spectrum of this compound, corroborating the loss of hydrogen chloride which is consistent with the microanalysis of **3**.

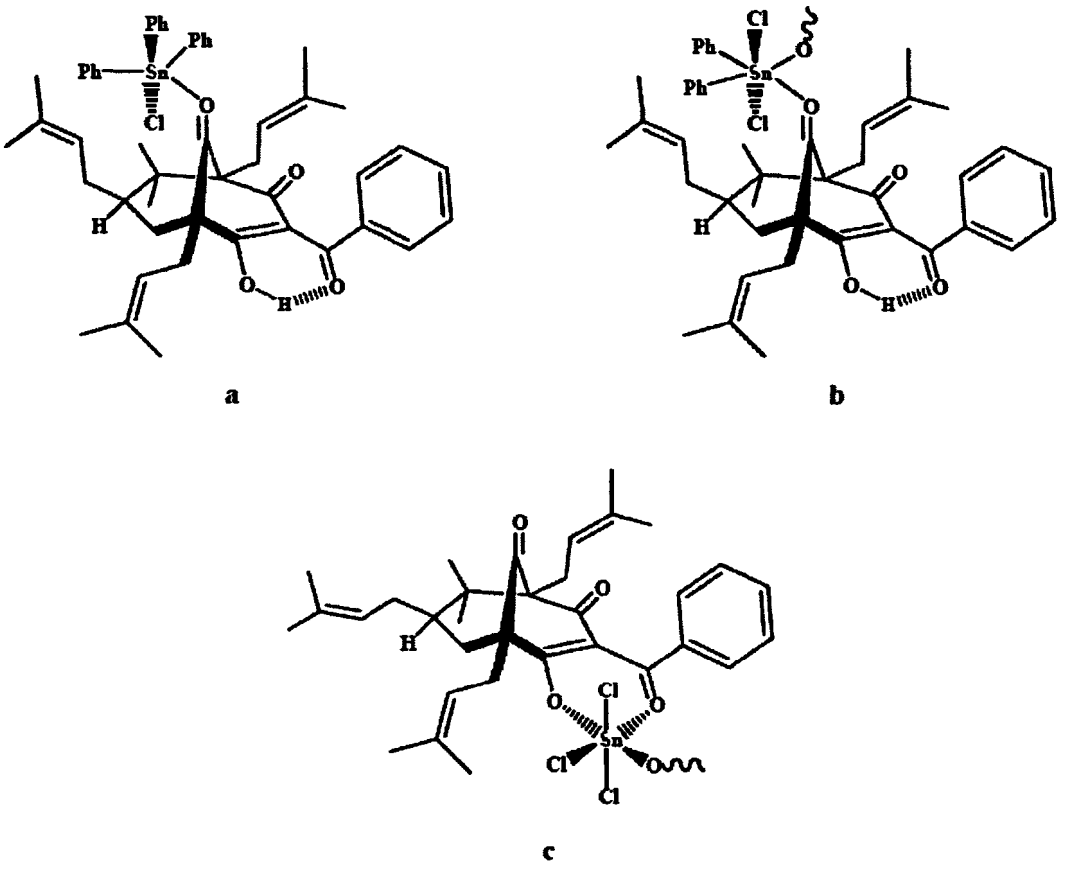


Fig. 1: Possible coordination modes of 7-epiclusianone towards the organotin(IV) precursors SnClPh3 (**a**), SnCl2Ph2 (**b**) and SnCl4 (**c**) in the solid state.

The ^{119}Sn NMR spectrum of **1** and **2** has revealed one signal for each complex, except in **3** which has exhibited two. To all those tin derivatives, the ^{119}Sn NMR has given a chemical shift commonly found in six-fold coordination species. To investigate whether this chemical shift is related to the 7- epiclusianone coordination, the NMR spectra of the $SnCl_xPh_{0-x}$ (x = 1, 2) precursors have been obtained in methanol. The NMR spectra exhibit chemical shifts at δ -176.0 (x = 1) and -235.6 (x = 2). These NMR chemical shifts are distant from those of complex **1** and **2**, revealing evidence for coordination of 7-epiclusianone. Subtracting those chemical shifts from the corresponding derivatives, a significant value of δ -324 and -265 has been found. This is a remarkable chemical shift which supports not only the coordination but also auto-association between the solvent and the ligand itself /17/. The auto-association of 7-epiclusianone may occur through both conjugated and non-conjugated carbonyl fragments. In view of that, dimers, tetramers, or polymeric species are probably the reaction products in which the metal is in the same magnetic chemical environment. This phenomenon might be related to the stereochemistry of 7-epiclusianone in solution. For instance, rotation along the C-C bond of the 1,5,7-tri(3-methyl-2-butenyl) moiety in this ligand may reduce the steric hindrance for the approaching of the metal precursor towards the non-conjugated as well as the conjugated carbonyl group. Two peaks have been revealed in the spectrum of complex **3** which have a difference in

chemical shift of δ -89. This value suggests a subtle magnetic variation surrounding the metal centre, due to the bulk of 7-epiclusianone, or they correspond to dissimilar tin sites as a consequence of auto-association in solution /18/.

3.3. Mössbauer spectroscopy

Mössbauer spectroscopy has been an important tool for structural elucidation of organotin compounds in the solid state. The parameters associated with this technique, isomer shift (IS) and quadrupole splitting (QS), provide respective information about the coordination number as well as the stereochemistry of the aryl or alkyl substituents at the metal centre. The IS datum of complex **1** has revealed a five-fold coordination species, and its QS points out the phenyl groups at the edge of a trigonal-bipyramidal arrangement /9/. The QS values associated with planar configuration for the phenyl groups of the SnPh3 moiety are commonly found in the range of 3.0 to 4.1 mm s^{-1} /19/. In this context, the QS datum of **1** cannot be correlated with a planar configuration for the phenyl groups, revealing instead that one of those is at the axial and two are at the equatorial position of a trigonal-bypiramidal arrangement /20/. Unusual values of IS and QS, however, are possible because of distortion in the geometrical configuration of the organotin compounds. Distorted trigonalbipyramidal arrangements of triorganotin(IV) derivatives have been disregarded in complex **1**, accounting for the values of IS and QS (1.28 mm/s and 3.35 mm/s) reported in the literature /21/. Every attempt at getting suitable crystals of **1** for crystallography studies has failed. Only the crystal structure of 7-epiclusianone has been determined from the crystalline material obtained by slow solvent evaporation technique at room temperature. This fact corroborates a weak bond between the triphenylorganotin(IV) and the 7-epiclusianone. The Mössbauer parameters of complex **2** are uncommon, but in agreement with an increase in the coordination number of the metal centre accounting for the low IS, whereas the QS parameter indicates the phenyl groups in *cis* configuration surrounding the metal at the centre of an octahedron. The QS data of *cis*diphenytin(IV) species are typically found around 3.14 mm/s or below. Above this value, the phenyl groups are associated to occupy the axial position of an octahedron. Nevertheless, distortions from the regular octahedral geometry as well as the electronegativity of the substituent may change the Mössbauer parameters /22/. Several octahedral organotin(IV) complexes have been reported with low values of IS and QS (IS = 0.67 – 0.78 mm/s and QS = 1.52 – 1.99 mm/s) as a result of these effects /23, 24/. On the other hand, other geometrical patterns are conceivable for the metal in complex **2**. This could be a distorted or a regular trigonal-bipyramidal arrangement. Those agree with the molecular formula established by microanalysis. However, the values of IS and QS from diorganotin(IV) derivatives on such a geometrical pattern are above the magnitude found in complex **2**, and for that reason, it has been disregarded /25-27/. Thus, the Mössbauer datum of **2** is in favour of a six-fold coordination in the solid state. This can be envisaged by looking at the 7- epiclusianone acting as a bridging bidentate ligand through both the conjugated and non-conjugated carbonyl moieties. In this context, it seems reasonable that both parameters of complex **2** do indicate that the metal is at the centre of a distorted octahedral arrangement. A monodentate as well as a bridging bidentate coordination of 7-epiclusianone in complex **1** and **2** corroborates with the auto-association revealed by ^{119}Sn NMR.

The datum of complex **3** has also exhibited parameters within the range associated to six-fold coordination species /23, 28/. The QS value of **3** reflects the small difference in electronegativity amongst the substituent attached to the metal centre.

3.4. Antitumor bioassay

The number of cells versus time plotted in the graphs given in Figure 2 demonstrates the cellular reproduction of both MDCK and HN-5 in ethanol as well as in the presence of the substance test **1**. The MDCK cells have a maximum in ethanol at 120 h, decaying from that onwards. Similar fact has occurred at 72 h in the case of HN-5, decaying subsequently until no cell being detected at 144 h. The MDCK cells seem to have a two-stage reproduction process in ethanol: one at 72 h and another at 120 h. By comparing the MDCK replication in ethanol as well as in the presence of the substance test **1**, it is clear that the two-stage process has shifted to less time in the presence of the latter: the first-stage at 24 h and the second at 96 h. The amount of MDCK cells has increased in 24 h when in comparison with the same process in ethanol. However, at the end of 144 h, the cell number in the presence of the substance test **1** was approximately the same as that at the beginning of the experiment. The amount of MDCK cells in ethanol, on the other hand, was higher at 144 h. This fact suggests that the MDCK cells have a better cellular reproduction in ethanol than in the presence of the substance test **1**. The carcinoma cells HN-5 have shown behavior as similar as the MDCK, shifting the maximum cellular replication from 72 h to an interval of 48 to 72 h, in the presence of the substance test **1**. Nevertheless, irrespective of the experimental conditions, at the end of 144 h there were no detectable HN-5 cells in ethanol as well as in the presence of the substance test **1**.

The effect on the cellular reproduction relative to the time for that occurs is comparable for both lineages. The death of the HN-5 is significant since it is in contrast with the MDCK at 144 h. However, the bioassay of the substance test on the carcinoma HN-5 has not provided conclusive evidence for the antitumor activity, in view of the fact that these cells died in the presence as well as in the absence of complex **1**. Nevertheless, the time for cellular replication seems to be altered by this organotin derivative of 7-epiclusianone.

CONCLUSION

The organotin(IV) precursors are weakly bonded to the 7-epiclusianone in the solid state, except the $SnCl_4$ derivative. In solution, beyond solvent coordination, 7-epiclusianone seems to follow a pattern of auto-association accounting for the ^{119}Sn NMR chemical shift. The bioassay for the antitumor activity is not conclusive owing to the death of HN-5 in ethanol as well as in the presence of the substance test **1**. Generally, the only observable effects were the reduction in the time for cellular reproduction.

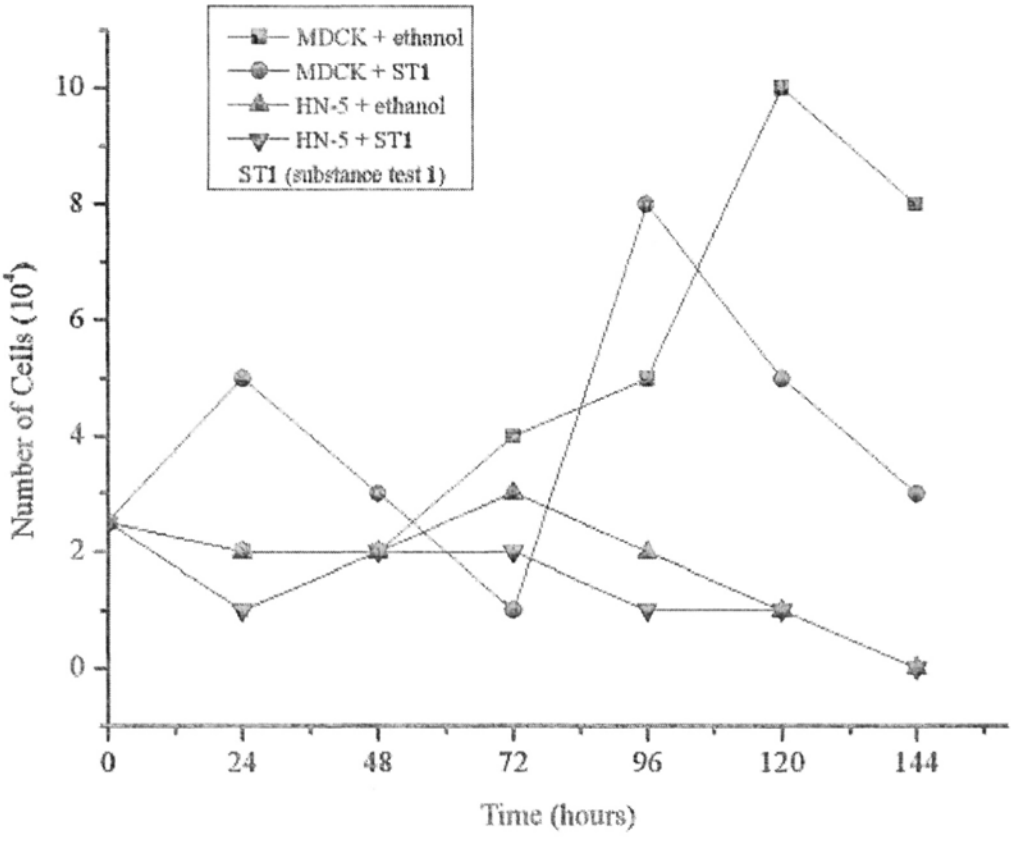


Fig.-2: Bioassay *in vitro* of MDCK and HN-5 cells in ethanol as well as in the presence of the substance test [SnClPh3(*Epi*)] (**1**).

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