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SÍNTESE E CARACTERIZAÇÃO DE TRÊS CARBOXILATOS DE ORGANOTINA (IV) DO ÁCIDO ENT-CAURENÓICO: ATIVIDADE ANTIFÚNGICA CONTRA OS *TRAMETES VERSICOLOR* (L .: FR) PILÀT

SYNTHESIS AND CHARACTERIZATION OF THREE ORGANOTIN(IV) CARBOXYLATE OF *ENT*-KAURENOIC ACID: ANTIFUNGAL ACTIVITY AGAINST *TRAMETES VERSICOLOR* (L.: FR) PILÀT

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RESUMO

A indústria de produtos florestais tem um papel muito importante no desenvolvimento de uma sociedade sustentável de base biológica. No entanto, muitos fungos que deterioram os principais componentes estruturais da madeira, como celulose, hemicelulose e lignina, são capazes de causar a decomposição em árvores em pé, levando a perdas significativas na produção florestal devido aos impactos na produção de biomassa. *Trametes versicolor* (L .: Fr) Pilát é um fungo capaz de atacar as árvores por estratégias de vida saprotróficas e parasíticas, causando podridão branca de espécies de madeira, um problema sério para a indústria que é focada na madeira e seus usos. Os objetivos do trabalho foram a síntese de carboxilato de organoestanho (IV) de um produto natural (*ácido ent-caurenóico*), caracterização das estruturas e avaliação antifúngica contra *T. versicolor*, como contribuição de novas alternativas para a preservação da madeira. A síntese de complexos organoestânicos (IV) derivados do ácido tetracíclico ent-caur-16-en-19-óico diterpênico (ácido ent-caurenoico, CA) deu três novos complexos potencialmente ativos contra *T. versicolor*. Os complexos foram caracterizados por espectroscopia de FTIR e RMN. A atividade biológica foi avaliada por meio de um método de diluição em gel com inoculação em placa superficial, utilizando concentrações de 60 e 120 µg / mL, para os complexos CA e três organoestânicos (IV). Uma bioatividade consideravelmente maior foi observada para a concentração de 120 µg / mL e para o complexo com maior peso molecular.

Palavras-chave: *Ácido ent-caurenóico, indústria de produtos florestais, complexos organoestânicos (IV), estudos espectroscópicos, Trametes versicolor.*

ABSTRACT

The forest products industry has a very important role in the development toward a sustainable, biobased society. However, many fungi that deteriorate the main structural components of wood, such as cellulose, hemicellulose and lignin, are able to cause decay in standing trees, leading to significant losses in forest output due to impacts on biomass production. *Trametes versicolor* (L.: Fr) Pilát is a fungus able to attack the trees by saprotrophic and parasitic life strategies, causing white-rot of wood species, a serious problem for the industry that is focused on wood and its uses. The objectives of the work were the synthesis of organotin(IV) carboxylate of a natural product (*ent*-kaurenoic acid), characterization of the structures and anti-fungal evaluation against *T. versicolor*, as a contribution of new alternatives for the wood preservation. The synthesis of organotin(IV) complexes derived from the diterpene tetracyclic *ent*-kaur-16-en-19-oic acid (*ent*-kaurenoic acid, KA) gave three new complexes potentially active against *T. versicolor.* The complexes were characterized by FTIR and NMR spectroscopy. The biological activity was evaluated by a gel dilution method with superficial plate inoculation, using 60 and 120 µg/mL concentration, for the KA and the three organotin(IV) complexes. A

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considerably higher bioactivity was observed to the 120 µg/mL concentration, and for the complex with greater molecular weight.

Keywords: *ent*-kaurenoic acid, forest products industry, organotin(IV) complexes, spectroscopic studies, *Trametes versicolor*.

1. INTRODUCTION

Trametes versicolor (L.: Fr.) Pilát is a Basidiomycete causing of white-rot in the wood (Velásquez et al., 2006). This fungi together an others Agaromycetes, and a limited number of bacteria to cause decay in standing trees by parasitic and saprotrophic strategy (Bari et al., 2019). *T. versicolor* is able to attack wood of almost all heavily lignified plants, utilizing a full range of enzyme system for complete decomposition of the substrate, altering the physical-chemistry characteristic of de wood. Although that decay wood is a natural process, to forest products industry it is an economical lost, and in urban environmental, it represent a risk for the population due to deterioration structural constructions fabricated with the wood (Sikkema et al., 2016).

For many years the chemical preservatives have been used to protect wood, for example, copper chromium arsenic (CCA), alkaline copper quat and copper azole. Actually, others environmentally friendly methods (ultrasound, magnetic, microwave and biological methods) and materials (vitamins, sugars, plant extracts, biodegradable polymers and microorganisms) are used in the production of nanoparticles for impregnation of some wood species (Can et al., 2018).

Medicinal Inorganic Chemistry plays an important part in the exploration of metallic ion properties for new drug design (Muhammad and Saqib, 2016), since new compounds are obtained taking advantage of the coordination geometry and structural diversity (monomeric, dimeric, hexameric and oligomeric) offered by this study area (Win et al., 2012). In particular, for the organotin(IV) carboxylates of general formula R_2SnL_2 or R_3SnL (R= Me, Et, Ph, and L= carboxylate ligand, either mono or bidentate), the geometries could be tetrahedral, trigonal bipyramid or octahedral (Hadjikakou and Hadjiliadis, 2009; Iqbal et al., 2015). It is expected that this geometric characteristics of the complexes, play an important role in the biological action of the new compounds (Matela and Aman, 2012; Farooq et al., 2015).

In the literature, has been reports of organotin(IV) carboxylates that have anticancer activity (Muhammad and Saqib, 2016) and antimicrobial activity (Win et al., 2012; Kumar and Pankaj, 2014; Quintero-Rincón et al., 2016). The objective of the design and synthesis of these complexes is obtain new compounds with improved activity in comparation with the serie head (*ent*-kaurenoic acid) as an alternative to the resistance of the microorganisms to several chemical molecules. Also, the tendency is in the direction of the development of ecologically friendly technologies based on the Green Chemistry principles (Contreras, 2014), to be able to obtain compounds for wood preservation, trying the correct combination of metallic agents and natural products with bioactive properties, mainly extracted from plants, that have a synergic action as fungicides and antitermite properties (González-Laredo et al., 2015).

In this work is reported the synthesis and characterization of organotin(IV) complexes with *ent*-kaurenic acid (KA), a diterpene of *ent*-kaurane type (IUPAC name: *ent*-kaur-16-en-19-oic acid). The fungicidal activity of natural product and the organotin(IV) carboxylates was tested against *Trametes versicolor* (L.: Fr.) Pilát.

2. MATERIALS AND METHODS

2.1. Reagents and equipment

All the reagents and solvents were obtained from different companies (Aldrich, Merck, Riedel de Haën, Fisher Chemicals, Research Organic/Inorganic Chemical Corp. ($(CH_3)_2SnCl_2$), Alfa Division ($(C_2H_5)_2SnCl_2$) and Alfa División Ventron ($(C_6H_5)_3SnCl$ 95%) and were used directly. The melting points were measured on a Barnstead/Electrothermal, 9300 apparatus.

The FTIR were recorded on a Perkin Elmer 1725-X FTIR (KBr pellet, 4000- 450 cm^{-1}). The NMR of ^1H, ^{13}C, bidimensional were done on the following: (Bruker, 300 MHz, 300 MHz for ^1H and 75,49 MHz for ^{13}C; Bruker, 500 MHz, 500 MHz for ^1H and 125,74 MHz ^{13}C and Bruker, 600 MHz , 600 MHz for ^1H and 150,91 MHz for ^{13}C).

2.2. Extraction and purification of the ent-kaurenic acid [KA]

The aerial parts of *Espeletia semiglobulata* Cuatrec. (about of 9.0 Kg) were collected in Piedras Blancas paramo (3100 meters above sea level) in Mérida state. The extraction and purification of KA were carried by an acid-base extraction (Aparicio et al., 2013).

2.2.1. Preparation of the sodium salt of KA.

The sodium salt of *ent*-kaurenic acids were prepared by heating to reflux at 40 ºC in a hexane solution of each acid with an equimolar quantity of NaOH, diluted in a few drops of distilled water.

2.3. Antimicrobial activity

A gel dilution method with inoculation on a plate surface was used (Jansser et al., 1987). On each of Petri dishes (10 cm diameter) were added 20 mL of malt agar extract with each one of the compounds containing 60 and 120 µg/mL concentrations. Simultaneously, 95% ethanol was added as blank or control. The new growth medium with each one of the incorporated compounds for each trial, was allowed to solidify at room temperature for one hour and later on, was inoculated placing on top of it, in the center of the dish, a circular sample of the *Trametes versicolor* (L:Fr) Pilát (FP-133255-R) (10 mm) fungus. The incubation period was during six days at 26 ± 2 °C. The determinations were done in triplicate for each compound and concentration. The growth diameter of the fungus (mm) was measured at the end of each incubation period (6 days). The inhibition percentage was expressed as a total growth function of the control as discussed in the literature (Gopalakrishnan et al., 1997).

2.4. Statistical analysis

A descriptive statistical analysis was done using the SPSS program for Windows, version 19, giving frecuency and simple percentage distribution tables for the variables used in the research.

3. RESULTS AND DISCUSSION:

3.1. Spectroscopy

3.1.1. Ent-kaur-16-en-19-oic acid, [KA]

FT-IR, vmax (cm^{-1}), functional group: 3460, ν(OH); 1710, ν(C=O); 1650 ν(C=C)]. **NMR-^{1}H**, *M* ($CDCl_3$): ppm (group)]: δ: [$H_{1β}$, *dt*: 0,77], [$H_{3α}$, *d*: 2,13], [$1H_5$, *m*: 1,07], [$H_{9β}$, *m*: 1,05], [$H_{12α}$, *m*: 1,59], [H_{13}, *s*: 2,63], [$H_{14α,□}$, *d*: 1,97], [$H_{14β,}$ *dd*: 1,97], [$2H_{15α, β,}$ *m*: 2,04 (methylene)], [$2H_{17,}$ *s*: 4,74; 4,79], [$3H_{18,}$ *s*: 1,24], [$3H_{20,}$ *s*: 0,95]. [**RMN-^{13}C**, ($CDCl_3$): ppm]: δ: [C_1: 40,87], [C_2: 19,25], [C_3: 37,95], [C_4: 43,91], [C_5: 57,23], [C_6: 21,99], [C_7: 41,44], [C_8: 44,39], [C_9: 55,28], [C_{10}: 39,86], [C_{11}: 18,59], [C_{12}: 33,26], [C_{13}: 44,01], [C_{14}: 39,83], [C_{15}: 49,19], [C_{16}: 156,03], [C_{17}: 103,15], [C_{18}: 29,12], [C_{19}: 184,72], [C_{20}: 15,78]. White crystalline solid, m.p. 178-180°C.

3.1.2. [Bis (ent-kaur-16-en-19-oate)] dimethyltin(IV), $(CH_3)_2Sn[C_{20}H_{29}O_2]_2$ [1]

FTIR, vmax (cm^{-1}), functional group: 3432, υ(O-H), lattice water ; 1570, υ(COO as); 1390, υ(COO s); 1656, υ(C=C); 576, υ(O-Sn-O); 530, υ(Sn-C); Δν (Δν = $ν(COO)_{as}$ - $ν(COO)_s$) = 180). [**NMR-^{1}H**, ($CDCl_3$): ppm (functional group)]: δ: [$2H_2$, 1.8; 2.45 (methylene)], [$2H_3$, 1.92; 2.39 (methylene)], [$1H_5$, 1.63 (methyne)], [$2H_6$, 1.45; 1.85 (methylene)], [$2H_7$, 0.9; 2.12 (methylene)], [$1H_{11}$, 1.8; 2.48 (methylene)], [$2H_{12}$, 1.42; 1.93 (methylene)], [$1H_{13}$, 2.784 (methyne)], [$2H_{15}$, 2.4 (methylene)], [$2H_{17}$, 4.8923; 4.7745 (methylene)], [$3H_{18}$, 1.200 (methyl)], [$3H_{20}$, 0.988 (methyl)], [$3H_{a,a'}$ 0.93 (methyl)]. [**NMR-^{13}C**, ($CDCl_3$): ppm (functional group)]: δ: [C_2: 18.4 (methylene)], [C_3: 38.0 (methylene)], [C_4: 44.8 (quaternary)], [C_5: 46.2 (methyne)], [C_6: 20.0 (methylene)], [C_7: 38.5 (methylene)], [C_8: 46.5 (quaternary)], [C_{11}: 18.5 (methylene)], [C_{12}: 29.5 (methylene)], [C_{13}: 41.0 (methyne), [C_{15}: 50.0 (methylene)], [C_{16}: 158.8 (quaternary)], [C_{17}: 105.48 (methylene)], [C_{18}: 28.5 (methyl)], [C_{19}: Not observed (quaternary)], [C_{20}: 23.71 (methyl)], [$C_{a, a'}$: 16.00 (methyl)]. White solid, m.p. 126 °C.

3.1.3. [Bis (ent-kaur-16-en-19-oate)] diethyltin (IV), $(C_2H_5)_2Sn[C_{20}H_{29}O_2]_2$ [2]

FTIR, vmax (cm^{-1}), functional group: 3404, υ(O-H) lattice water; 1548, υ(COO as); 1405, υ(COO s); 1656, υ(C=C); 700, υ(O-Sn-O); 558, υ(Sn-C); Δν (Δν = $ν(COO)_{as}$ - $ν(COO)_s$) = 143). [**NMR-^{1}H**, ($CDCl_3$): ppm (functional group)]: δ: [$2H_1$, 1.82; 1.18; 1,409 (methylene)], [$2H_2$, 2.58; 1.78 (methylene)], [$2H_3$, 2.32; 1.92 (methylene)], [$1H_5$, 1.55 (methyne)], [$2H_6$, 1.4 (methylene)], [$2H_7$, 2.4; 2.0 (methylene)], [$1H_{11}$, 1.78 (methylene)], [$1H_{13}$, 2.74 (methyne)], [$2H_{14}$, 1.9 (methylene)], [$2H_{15}$, 2.6; 2.1 (methylene)], [$2H_{17}$, 4.885; 4.765) (methylene)], [$3H_{18}$, 1.08 (methyl], [$3H_{20}$, 0.85 (methyl)], [$2H_a$, 1.50 (methylene)], [$3H_b$, 1.25 (methyl)]. [**NMR-^{13}C**, ($CDCl_3$): ppm (functional group)]: δ: [C_1: 40.8 (methylene)], [C_2: 18.77 (methylene)], [C_3: 37.92 (methylene)], [C_4: 42.28 (quaternary)], [C_5: 46.36 (methyne)], [C_6: 20.69 (methylene)], [C_7: 37.93 (methylene)], [C_8: 45.46 (quaternary)], [C_{11}: 16.41 (methyne)], [C_{13}: 41.3 (methyne)], [C_{14}: 38.87 (methylene)], [C_{15}: 50.38 (methylene)], [C_{16}: 158.64 (quaternary)], [C_{17}: 105.395 (methylene)], [C_{18}: 28.85 (methyl)], [C_{19}: not observed (quaternary)], [C_{20}: 23.9 (methyl)], [C_a: 44.97 (methylene)], [C_b: 9.05 (methyl)]. White solid, m.p. 240 °C.

3.1.4.[Ent-kaur-16-en-19-oate] triphenyltin(IV), $(Ph)_3Sn[C_{20}H_{29}O_2]$ [3]

FTIR, vmax (cm^{-1}), functional group: 1961-1817 (overtones), υ(C-H); 1537, υ(COOas); 1414, υ(COOs); 1656, υ(C=C); 695, υ(O-Sn-O); 576, υ(Sn-C); Δν (Δν = ν(COOas) - ν(COOs) = 123). [**NMR-^{1}H**, ($CDCl_3$): ppm (functional groups)]: δ: [$2H_1$, 1.9; 1.22 (methylene)], [$2H_2$, 1.84; 1.48 (methylene)], [$2H_3$, 2.39; 1.98 (methylene)], [$1H_5$, 1.71 (methyne)], [$2H_7$, 1.6; 1.48 (methylene)], [$1H_{11}$, 2.45; 1.84 (methyne)], [$2H_{12}$, 1.98; 1.42 (methylene)], [$1H_{13}$, 2.747 (methyne)], [$2H_{14}$, 2.18; 1.02 (methylene)], [$2H_{15}$, 2.58; 2.16 (methylene)], [$2H_{17,17'}$, 4.891; 4.773) (methylene)], [$3H_{18}$, 1.236 (methyl)], [$3H_{20}$, 0.963 (methyl)], [H_o, 7.65 (aromatic)], [H_m, 7.448 (aromatic)], [H_p, 7.708 (aromatic)]. [**NMR-^{13}C**, ($CDCl_3$): ppm (functional group)]: δ: [C_1: 40.2 (methylene)], [C_2: 20.0 (methylene)], [C_3: 37.9 (methylene)], [C_4: 43.2 (quaternary)], [C_5: 46.8 (methyne)], [C_7: 45 (methylene)], [C_8: 44.4 (quaternary)], [C_{10}: 38.9 (quaternary)], [C_{11}: 18.4 (methyne)], [C_{12}: 29.4 (methylene)], [C_{13}: 41.9 (methyne)], [C_{14}: 38.5

(methylene)], [C_{15}: 50.0 (methylene)], [C_{16}: 158.0 (quaternary)], [C_{17}: 105.504 (methylene)], [C_{18}: 28.4 (methyl)], [C_{19}: not observed (quaternary)], [C_{20}: 24 (methyl)], [C_{ipso}, 140.0 (aromatic)], [C_o, not observed (aromatic)], [C_m, 131.09; 129.105 (aromatic, not equivalent)], [C_p, 134.145 (aromatic)]. White solid, m.p. 123 °C.

3.2. Spectroscopic analysis

In the FTIR spectra of complexes [1-3] was observed a decrease in the C=O stretching due to back-donation (Stuart, 2004), that causes a bathochromic displacement of the carbonyl signal in KA ligand, indicating that the metal coordination was through the oxygen atoms of the carboxylate group (Mahmood et al., 2004). The spectra show bands corresponding to the asymmetric [vCOOas] and symmetric [vCOOs] vibrations of the acetate group. Generally, the Δv = [vCOOas - vCOOs] values are used as a coordination mode indicator of the carboxylate anion with the tin atom (Win et al., 2010). In our case, the Δv = [vCOOas - vCOOs] values for the all complexes is < 200 cm^{-1} indicates a bidentate coordination. The unidimensional and bidimensional NMR spectra analysis for [1-3] complexes shows signals that are characteristic of the natural product KA. However, in the spectra of the complexes, are observed the signals associated with the alkyl chains bonding to tin. In the NMR-^{13}C spectra analysis it was not possible to assign the signal corresponding to C19 of the KA.

3.3. Antimicrobial analysis

The results presented in Table 1, show the micellar growth inhibition percentages of *T. versicolor.* As can be observed, all the compounds analyzed inhibit the fungus growth. When the percent inhibition is analyzed and compared, it is shown that it depends on the type and nature of the compound, as well as on the concentration used. The variance analysis (ANOVA) at two concentrations (60 y 120 µg/mL) show statistically significant differences of the bioactivity between the compounds evaluated ($p<0,05$).

The biological effectiveness of the compounds studied was done by Tukey test for multiple comparision (Table 2), shows that at a 60 µg/mL concentration, *ent*-kaurenic acid (KA) shows the minimum biological activity compared with its organotin(IV) derivatives ($p<0.05$). The absence of statistically significant differences between the compounds [*bis*- (*ent*-kaur-16-en-19-oate)] dimethyltin(IV) (1), and [*ent*-kaur-16-en-19-oate] triphenyltin(IV) (3) ($p>0,05$), show that these compounds have the same biological activity. On the other hand, [*bis*-(*ent*-kaur-16-en-19-oate)] diethyltin(IV) (2) and (3), show the greatest bioactivity against *T. versicolor*, with absence of statistical differences between them ($p=0.59$).

When the concentration is increased to 120 µg/mL (Table 1), the compounds KA, (1), (2), and (3), show greater bioactivity compared to the percent inhibition against the fungus using the 60 µg/mL concentration. The results in Table 3, show that most of the compounds indicated notable differences in the micellar growth control of *T. versicolor* (p<0.05). The bioactivity for compound (3) is superior to the other organotin(IV) compounds , and did not show important significant differences between them (p=0.99). These results demonstrate that the organotin(IV) carboxylate derived from the natural product *ent*-kaurenic acid present biological activity against the *Trametes versicolor* fungus. The percent micellar inhibition is superior to the KA and increases at a greater concentration of the compounds. Also, there is a greater activity when the molecular weight of the compound (3), increases. In general, the bioactivity of the compounds decreases in the following order: 2 > 3 > 1 > AK, at 60 µ/mL concentration, and 3 > 1 > 2 > AK, for 120 µ/mL concentration.

Wood is a renewable natural resource that is very important in the world economy, especially for house building and furniture construction. Its preservation must be done using technology based on sustainable principles and preserving the environment, using natural products derived from plants with fungicide and antitermite properties, and using the correct combination of these natural products with metals such as boron or copper (González-Laredo et al., 2015). Based on the results obtained, it is important to continue the studies of the organotin(IV) complexes derived from *ent*-kaurenoic acid as potentially active molecules against the *Trametes versicolor* fungus.

4. CONCLUSIONS:

Three new organotin(IV) carboxylate derived from the natural product *ent*-kaurenoic acid (KA), isolated from frailejon *Espeletia semiglobulata* CUATREC, were synthetized and characterized by FTIR and NMR spectroscopy. The carboxylate group is bidentate bonded in all compounds. The KA and the three organotin(IV) derivatives (1-3) are biologically active against *Trametes versicolor* fungus that causes the wood white rot. The bioactivity increases with the concentration of the compounds and the molecular weight of the complexes. These results incorporate the Medicinal Organometallic Chemistry as a study area for development of wood preserving molecules that have ecological importance.

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6. REFERENCES:

1. Aparicio, R., Villasmil, T., Peña, A., Rojas, J., Usubillaga, A. Estudio fitoquímico de las hojas de *Espeletia semiglobulata* CUATREC. *Rev. Fac. Farm.*, **2013**, 55: 2-5.
2. Bari, E., Daryaei, M. G., Karim, M., Bahmani, M., Schmidt, O., Woodward, S., Sistani, A. Ehsan, B., Mehrdad, G., Karimb, M., Mohsen, B., Schmidtd, O., Woodwarde, S., Tajick, M., Sistan, A. Decay of Carpinus betulus wood by Trametes versicolor-An anatomical and chemical study. *Inter. Biodeter. Biodegr.*, **2019**, *137*, 68-77.
3. Can, A., Sivrikaya, H., Hazer, B. Fungal inhibition and chemical characterization of wood treated with novel polystyrene-soybean oil copolymer containing silver nanoparticles. *Inter. Biodeter. Biodegr.*, **2018**, *133*, 210-215.
4. Contreras, R. Química verde: haciendo química amigable con el medioambiente. Mérida: Ediciones del CDCHTA-ULA, Venezuela, **2014**.
5. Farooq, A., Shaista, S., Kaneez, F., Saqib, A., Ishtiaq, Q., Corrado, R. Organotin(IV) based anti-HCV drug: synthesis, characterization and biochemical activity. *Dalton trans.*, **2015**, 1-38.
6. González-Laredo, R., Rosales-Castro, M., Rocha-Guzmán, N., Gallegos-Infante, J., Moreno-Jiménez, M., Karchesy, J. Wood preservation using natural products. *Mad. Bosq*., **2015**, 21: 63-76.
7. Gopalakrishnan, G., Banumathi, B., Suresh, G. Evaluation of the antifungal activity of natural xanthones from *Garcinia mangostana* and their synthetic derivatives. *J. Nat. Prod*. **1997**, 60: 519-524.
8. Hadjikakou, S., Hadjiliadis, N. Antiproliferative and anti-tumor activity of organotin compounds. *Coord. Chem. Rev*., **2009**, 253: 235-249.
9. Iqbal, H., Ali, S., Shahzadi, S. Antituberculosis study of organotin(IV) complexes: a review. *Cogent Chem*., **2015**, 1: 1-12.
10. Jansser, A., Senefief, J., Baerherm, A. Antimicrobial activity of essential oils: a 1976-1986 literature review. Aspects of the test methods. *Planta Med*., **1987**, 53: 395-398.
11. Kumar, P., Pankaj. M. Characterization and pesticidal studies of dibutyltin (IV) derivatives of diphenylamine-2-hydroxy-2`-carboxylic acid. *Res. J. Chem. Sci.*, **2014**, 4: 75-77.
12. Mahmood, S., Ali, S., Bhatti, M., Mazhar, M., Shahid, K., Khan, K., Maharvi, G. Synthesis, spectral characterization and biological applications of tri- and diorganotin(IV) derivatives of 2-[N-(2,6-dichloro-3-methylphenyl)amino]benzoic acid. *Turk. J. Chem*., **2004**, 28: 17-26.
13. Matela, G., Aman, R. Organotin(IV) complexes of carboxylic acid derivatives. *Cent. Eur. J. Chem*. **2012**, 10: 1-15.
14. Muhammad, S., Saqib, A. Organotin(IV) carboxylates as promising potential drug candidates in the field of cancer chemotherapy. *Curr. Pharm. Design*., **2016**, 22 (44): 6665-6681 (17).
15. Quintero-Rincón, P., Fontal, B., Fonseca, Y., Bellandi, F., Contreras, R., Vielma-Puente, J., Carrillo-Rodríguez, F., González-Romero A., Velásquez, J. In vitro antimicrobial activity of two dibutyltin(IV) complexes derivatives of kaurenic acids. *Emir. J. Food Agric*. **2016**, 28 (12): 865-871.
16. Sikkema, R., Caudullo, G., de Rigo, D. Carpinus betulus in Europe: distribution, habitat, usage and threats. *European Atlas of Forest Tree Species; Publications Office of the European Union: Luxembourg*, **2016**, 73-75.
17. Stuart, B. Infrared spectroscopy: fundamentals and applications. John Wiley & Sons, Ltd., **2004**.
18. Velásquez, J., Toro, M., Rojas, L., Encinas, O. Actividad antifúngica in vitro de los extractivos naturales de especies latifoliadas de la Guayana venezolana*. Mad. Bosq*., **2006**, 12: 51-61.
19. Win, Y., Teoh, S., Vikneswaran, M., Chan, M., Ha, S., Ibrahim, P. Synthesis and characterization o organotin(IV) complexes derived of 2-amino-5-nitrobenzoic acid: in vitro antibacterial screening activity. *Am. J. Appl. Sci*., **2010**, 7: 886-891.
20. Win Y., Teoh, S., Ha, S., tengku-Muhammad, T. Preliminary in vitro cytotoxic assay of human liver carcinoma cells (HepG2) of organotin (IV) complexes: synthesis and characterization of organotin (IV) complexes of 2,4-dinitrobenzoic and 3,5-dinitrobenzoic acids*. Afr. J. Biotechnol*., **2012**, 11: 13140-13146.

Table 1. *Biological Activity and ANOVA of ent-kaurenic acid (KA) and organotin(IV) complexes (1-3), against Trametes versicolor* (L.: Fr.) Pilát.

Concentration	Compounds				ANOVA					
	KA	1	2	3		Square Sum	gl	Quadratic Mean	F	Sig.
60 µg/mL	73.88 (0.95)	82.22 (0.02)	87.78 (0.03)	83.33 (0.26)	Inter-groups	673.44	4	168.36	25.14	0.00
					Intra-groups	167.43	25	6.69		
					Total	840.87	29			
120 µg/mL	87.78 (1.43)	96.10 (0.21)	93.33 (0.69)	99.98 (0.04)	Inter-groups	620.92	4	155.23	290.22	0.00
					Intra-groups	13.37	25	0.54		
					Total	634.29	29			

The mean value is indicated, and standard deviation in parenthesis

Table 2. *Comparation of multiple of Tukey for percent inhibition of Trametes versicolor* (L.: Fr.) Pilát *growth at 60 µg/mL concentration (95 % confidence).*

		Mean Difference (I-J)	Typical Error	Sig.	Confidence Interval at 95%	
(I) Comp1	(J) Comp1	Lower Limit	Upper Limit	Lower Limit	Upper Limit	Lower Limit
KA	1	-8.34(*)	1.49	0.00	-12.72	-3.95
	2	-13.89(*)	1.49	0.00	-18.28	-9.50
	3	-9.45(*)	1.49	0.00	-13.83	-5.06
1	KA	8.34(*)	1.49	0.00	3.95	12.72
	2	-5.56(*)	1.49	0.008	-9.94	-1.17
	3	-1.11	1.49	0.94	-5.49	3.28
2	KA	13.89(*)	1.49	0.00	9.50	18.28
	1	5.56(*)	1.49	0.008	1.17	9.94
	3	4.45(*)	1.49	0.046	0.06	8.83
3	KA	9.45(*)	1.49	0.00	5.06	13.83
	1	1.11	1.49	0.94	-3.28	5.49
	2	-4.45(*)	1.49	0.046	-8.83	-0.06

* The mean difference is significant at 0.05 level.

Table 3. *Comparation of multiple of Tukey for percent inhibition of Trametes versicolor* (L.: Fr.) Pilát *growth at 120 µg/mL concentration (95 % confidence).*

(I) Comp1	(J) Comp1	Mean Difference (I-J) Lower Limit	Typical Error Upper Limit	Sig. Lower Limit	Confidence Interval at 95% Upper Limit	Lower Limit
KA	1	-8.33(*)	0.42	0.00	-9.57	-7.08
	2	-5.55(*)	0.42	0.00	-6.79	-4.31
	3	-12.21(*)	0.42	0.00	-13.44	-10.96
1	KA	8.33(*)	0.42	0.00	7.08	9.57
	2	2.77(*)	0.42	0.00	1.53	4.01
	3	-3.88(*)	0.42	0.00	-5.12	-2.64
2	KA	5.55(*)	0.42	0.00	4.31	6.79
	1	-2.77(*)	0.42	0.00	-4.01	-1.53
	3	-6.65(*)	0.42	0.00	-7.89	-5.41
3	KA	12.21(*)	0.42	0.00	10.96	13.45
	1	3.88(*)	0.42	0.00	2.64	5.12
	2	6.65(*)	0.42	0.00	5.41	7.89

* The mean difference is significant at 0.05 level.

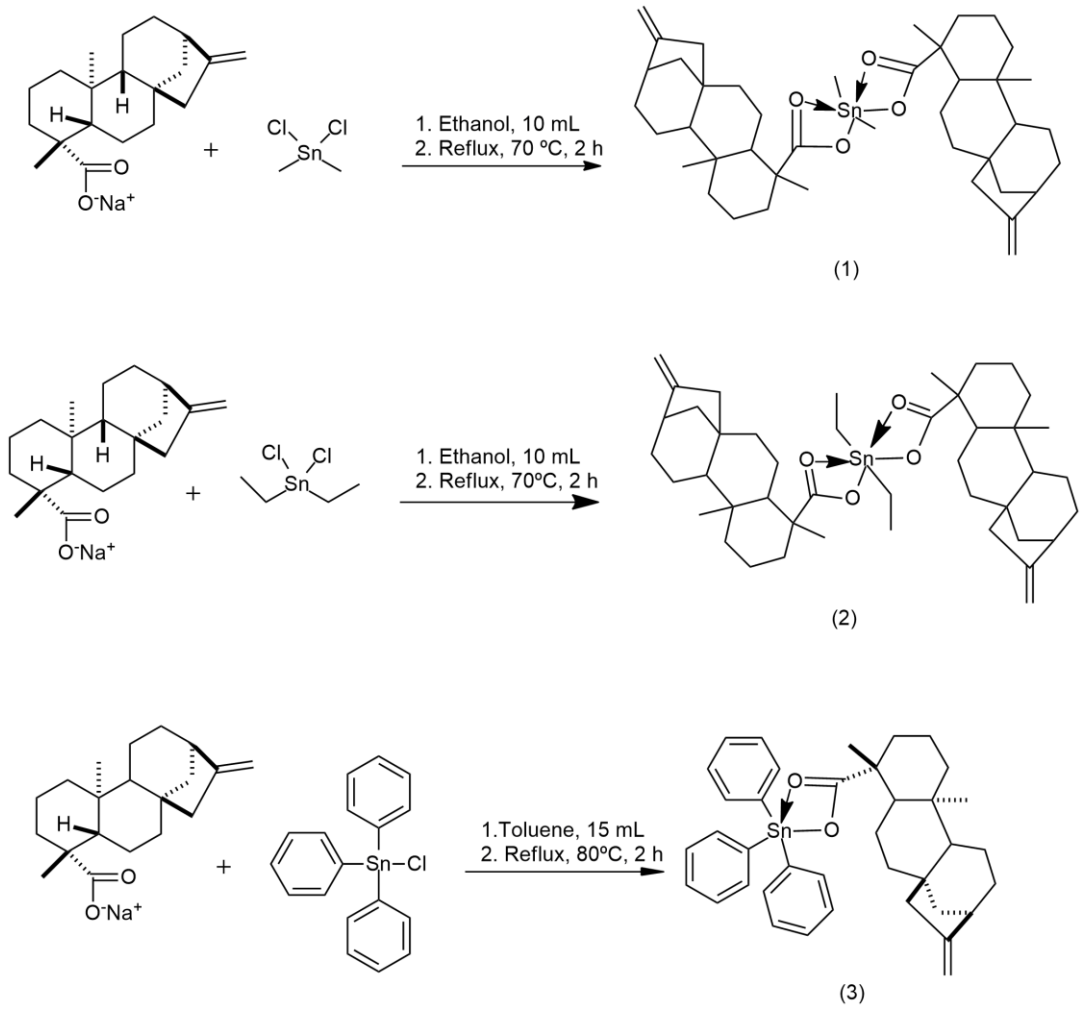

Figure 1*. Reactives, conditions, and chemical structures of organotin(IV) carboxylates [1-3].*