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REGULAR ARTICLE

In vitro antimicrobial activity of two dibutyltin(IV) complexes derivatives of kaurenic acids

Patricia Quintero-Rincón[1]*, Bernardo Fontal[2], Yuraima Fonseca[2], Fernando Bellandi[2], Ricardo Contreras[2], Joel E. Vielma-Puente[2], Freddy Carrillo-Rodriguez[3], Ana González-Romero[4], Jesús Velásquez[5]

[1]Postgrado Interdisciplinario en Química Aplicada, Facultad de Ciencias, Universidad de Los Andes. Mérida 5101, Venezuela, [2]Departamento de Química, Universidad de Los Andes, Facultad de Ciencias, Laboratorio de Organometálicos, Mérida 5101, Venezuela, [3]Departamento de Química, Universidad de Los Andes, Facultad de Ciencias, Laboratorio de Productos Naturales, Mérida 5101, Venezuela, [4]Departamento de Microbiología y Parasitología, Universidad de Los Andes, Facultad de Farmacia y Bioanálisis, Mérida 5101, Venezuela, [5]Laboratorio Biotecnológico de Productos Forestales, Centro Biotecnológico de Guayana CEBIOTEG-UNEG, Upata, Edo Bolívar, Venezuela

ABSTRACT

The antimicrobial activity *in vitro* of *ent*-kaurenic, KA [1] and grandiflorenic acid, GA [2], two natural products obtained from *Espeletia semiglobulata* Cuatrec., and two dibutyltin (IV) complexes [3] and [4] derived from natural products synthesis, *ent*-kaurenic acid and grandiflorenic acid, respectively, against strains of *Escherichia coli*, *Pseudomonas aeruginosa*, both pathogens for man, and *Trametes versicolor*, a fungus responsible of white wood rotting. For the human pathogens, an agar diffusion disk was used, with a 30 μg/mL concentration. There was an improved activity with the dibutyltin (IV) with grandiflorenic acid [4] against *E. coli*; while the anti-fungal activity against *T. versicolor* was done by a gel dilution method with surface plate inoculation getting an improved anti-fungal activity with 120 μg/mL concentration. The new compounds were characterized with FTIR spectroscopy, ^{1}H, ^{13}C unidimensional and bidimensional NMR experiments for the natural products [1], [2] and the organotin complexes [3] and [4].

Keywords: Dibutyltin (IV) complexes; Kaurenic acids; *Escherichia coli*; *Pseudomonas aeruginosa*; *Trametes versicolor*

INTRODUCTION

Ent-kaurenic acid (*ent*-kaur-16-en-19-oic acid) [1] and grandiflorenic acid (*ent*-kaur-9(11), 16-dien-19-oic) [2], are *ent*-kaurane diterpenes amply distributed in the vegetal kingdom (Peixoto et al., 2008; Boeck et al., 2005; Vieira et al., 2002; Otto and Simoneit, 2001). Both are natural products common in frailejones species (Espeletiinae, Asteraceae). These compounds show wide spectral biological activity, and have been used in traditional medicine, for example, *ent*-kaurenic acid has been used as anti-microbial, anti-inflammatory (Sosa-Sequera et al., 2010; Cavalcanti et al., 2006), anti-fungal (Boeck et al., 2005), anti-parasitical (Diamantino et al., 2008) and others. While grandiflorenic acid has been reported with diuretic effect (Somova et al., 2001), uterotonic properties (Villa-Ruano et al., 2013) and anti-trypanosomiasis (Batista et al., 2009). Occasionally, cattle consume different species of Espeletia and other paramo vegetation, although of poor quality (Molinillo and Monasterio, 2002). Both compounds have complicated structures, and have a tetracyclic skeleton and lipophilic properties (Hueso-Falcón et al., 2010), they differ in an additional endocyclic double bond presented by compound [2] in the 9-11 position. Different investigators have produced an important number of derivatives by hemi-synthesis, for instance, derivatization of the acid group on carbon-19 to obtain alcohols, esters and amides (Diamantino et al., 2008; Mthembu et al., 2010; Haraguchi et al., 2011). However, derivatives have also been obtained by funtionalization of the exocyclic and endocyclic double bonds in grandiflorenic acid (Peixoto et al., 2008; Hueso-Falcón et al., 2010; Hueso-Falcón et al., 2011).

Evaluation of anti-microbial activity has been reported and shows that *ent*-kaurenic acid is active against dermatophytes such as *Trichophyton rubrum*, *T. mentagrophytes* and *Epidermophyton floccosum* (Boeck et al., 2005). Ghisalberti et al., 1997 reported this acid against *Bacillus subtilis*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Saccharomyces cerevisiae*, *E. coli*, *Cladosporium herbarum* and *C. albicans*. The

***Corresponding author:**
Patricia Quintero-Rincón, Postgrado Interdisciplinario en Química Aplicada, Facultad de Ciencias, Universidad de Los Andes, Mérida 5101, Venezuela. E-mail: patriciaquintero@ula.ve

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activity of [1] against *S. aureus* and *E. coli* was confirmed by Velikova et al., 2000. The anti-fungal activity of *ent*-kaurenic acid has been evaluated against *Botrytis cinerea*, a fungus that produces gray rotting in various plant species (Cotoras et al., 2004). Lastly, the phytotoxicity of amide derivatives of compound [1] has been evaluated and could be used as herbicides (Diamantino et al., 2008).

Recently, the biological properties of organotin(IV) derivatives which have electron donating groups, such as oxygen, nitrogen and sulfur, have been evaluated (Win et al., 2010). It is noteworthy, the cell apoptosis induction by Sn metal (Shpakovsky et al., 2014); the anti-microbial activity is also known, and has been reported as a pesticide against the flour red scarab or *Tribolium casteneum* (Win et al., 2012; Kumar and Pankaj, 2014). However, of all the biological activities tried for organotin(IV) compounds, the most noteworthy is the anti-tumoral activity (Sedaghat et al., 2013; Nath et al., 2003).

The present investigation reports the anti-microbial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Trametes versicolor* strains by two complexes synthetized from dibutyltin(IV)dichloride and natural products [1] y [2], and compared with [1] and [2] alone. The synthesis of these two compounds [3] and [4] and the characterization by FTIR and unidimensional and bidimensional ^{1}H, ^{13}C NMR are reported.

EXPERIMENTAL

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 ^{1}H, ^{13}C, bidimensional were done on the following: (Bruker, 300 MHz, 300 MHz for ^{1}H and 75,49 MHz for ^{13}C; Bruker, 500 MHz, 500 MHz for ^{1}H and 125,74 MHz ^{13}C and Bruker, 600 MHz, 600 MHz for ^{1}H and 150,91 MHz for ^{13}C).

Extraction and purification of the kaurenic acids [1] and [2]

The vegetal material (about 9,0 Kg of the aerial parts of *Espeletia semiglobulata* Cuatrec.) was collected in Piedras Blancas paramo (3100 meters above sea level) in Mérida state. The extraction and purification of [1] and [2] were carried by and acid-base extraction following Aparicio et al., (2013) method (Fig. 1).

Ent-kaur-16-en-19-oic acid, [1]

FT-IR, νmax (cm^{-1}), functional group: 3460, ν(OH); 1710, ν(C=O); 1650 ν(C=C)]. NMR-^{1}H, *M* ($CDCl_3$): ppm (group)]: δ: [H1β, *dt*: 0,80], [H1α, *dt*: 00,89], [H3α, *m*: 1,40], [H3α, *d*: 2,17], [H3β, *d*: 1,0], [1H5β, *m*: 1,05], [H6α,β, *m*: 1,83], [H7α, *t*: 1,42], [H7 β, *t*: 1,79], [H9β, *m*: 1,05], [H11α,β, *m*: 1,50], [H12α, *m*: 1,59], [H12 β, *m*: 1,45], [H13, *s*: 2,63], [H14α, *d*: 1,99], [H14b, *d*: 1,12], [H15α,β, *m*: 2,05 (methylen)], [2H17, *s*: 4,74; 4,79], [3H18, *s*: 1,24], [3H20, *s*: 0,95]. [RMN-^{13}C, ($CDCl_3$): ppm]: δ: [C1: 40,87], [C2: 19,25], [C3: 37,95], [C4: 43,91], [C5: 57,23], [C6: 21,99], [C7: 41,44], [C8: 44,39], [C9: 55,28], [C10: 39,86], [C11: 18,59], [C12: 33,26], [C13: 44,01], [C14: 39,83], [C15: 49,13], [C16: 156,03], [C17: 103,15], [C18: 29,12], [C19: 184,72], [C20: 15,74]. White crystalline solid, m.p. 178-180°C.

Ent-kaur-9(11),16-dien-19-oic acid, [2]

FT-IR, νmax (cm^{-1}), functional group: 3066, ν(OH); 1693, ν(C=O); 1658 ν(C=C)]. NMR-^{1}H, *M* ($CDCl_3$): ppm]: δ: [$2H_1$, *dt*: 1,9276; 1,2246], [$2H_2$, *m*: 1,8573; 1,4922], [$2H_3$, *dt*: 2,1859; 1,0043 (methylen)], [$1H_5$, *t*: 1,6591 (methyn)], [$2H_6$, *m*: 2,4483; 1,8515 (methylen)], [$2H_7$, *dt*: 1,9684; 1,4465 (methylen)], [$1H_{11}$, *t (broad)*: 5,2250], [$2H_{12}$, *m*: 2,4083; 1,9502 (methylen)], [$1H_{13}$, *t*: 2,7491 (methyn], [$2H_{14}$, *dd*: 1,5965; 1,4803 (methylen)], [$2H_{15}$, *d*: 2,5766; 2,1508 (methylen)], [$2H_{17}$, *s*: 5,2200; 5,2145 (neighbor protons)], [$3H_{18}$, *s*: 1,2246 (methyl)], [$3H_{20}$, *s*: 1,0043 (methyl)]. [RMN-^{13}C, ($CDCl_3$): ppm]: δ: [$C_{1:}$ 40,77], [$C_{2:}$ 20,15], [$C_{3:}$ 38,26], [$C_{4:}$ 44,74], [$C_{5:}$ 46,62], [$C_{6:}$ 18,46], [$C_{7:}$ 29,68], [$C_{8:}$ 42,28], [$C_{9:}$ 155,96], [$C_{10:}$ 38,81], [$C_{11:}$ 114,91], [$C_{12:}$ 37,93], [$C_{13:}$ 41,25], [$C_{14:}$ 44,96], [$C_{15:}$ 50,32], [$C_{17:}$ 105,47], [$C_{18:}$ 28,24], [$C_{19:}$ 184,24], [$C_{20:}$ 23,60]. White crystalline solid, m.p. 159-160°C.

Synthesis of dibutyltin(IV) oxide

Dibutyltin (IV) oxide was prepared following the method reported by Muyiwa et al., 2014, with some changes as follows: dibutyltin dichloride was dissolved in the minimum quantity of diethylether and an aqueous NaOH solution

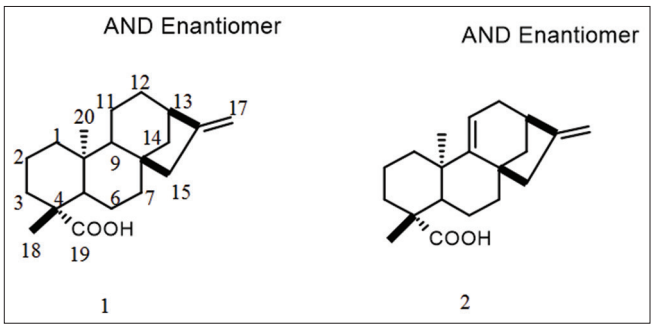

Fig 1. Chemical structure of kaurenic acids [1] and [2].

to form dibutyltin(IV) dihydroxide. The NaCl salt formed was washed completely (evaluated with $AgNO_3$ solution), was filtered and thermally dehydrated at 80 ºC for 48 h, producing the oxide as a white solid.

Synthesis and characterization of the organotin complexes

[Bis(ent-kaur-16-en-19-oate)]dibutyltin(IV), $Sn(C_4H_9)_2[C_{20}H_{29}O_2]_2$, [3]

A mixture of dibutyltin(IV) oxide (2,31 x10^{-4} mol) and the sodium salt of [1] (0,150g, 4,623 x 10^{-4} mol), 1:2 molar relation, was dissolved in 15 mL of methanol and was refluxed for 2 hours. The solution was filtered and allowed to stand, until a white precipitate formed and was re-crystallized in a hexane-chloroform-acetonitrile, 0,5:1:1 mixture forming white needles. (m.p. 85 °C, 55 % yield, soluble in acetone).

FT-IR, νmax (cm^{-1}), functional group: 1544, ν(COO as); 1404, ν(COO s); 1656, ν(C=C)]; 700, ν(OSnO); 562, ν(SnC)]. NMR-^{1}H, *M* (Acetone-D6): ppm (functional group)]: Show the peaks characteristic of [1], plus: δ: [H_a, 0.80 (methylen)], [H_b, 1.48 (methylen)], [H_c, 1,7 (methylen)], [H_d, 1.57 (methyl)]. NMR-^{13}C, (Acetone-D6): ppm (functional group)]: Show the peaks characteristic of [1], plus: δ: [C_a: 14 (methylen)], [C_b: 19.2 (methylen)], [C_c: 21.23 (methylen)], [C_d: 29.41 (methyl).

[Bis(ent-kaur-9(11),16-dien-19-oate)]dibuthyltin(IV), $Sn(C_4H_9)_2[C_{20}H_{27}O_2]_2$, [4]

A mixture of dibutyltin(IV) oxide (2,49 x10^{-4}mol) and the sodium salt of [2] (0,150g, 4,99 x 10^{-4} mol), 1:2 molar relation was dissolved in 15 mL of toluene and refluxed for 2 h. The solution was filtered and allowed to stand until a white gel formed. The compound is soluble in chloroform and hot ethanol. (m.p. >300 °C, 82% yield).

FT-IR, νmax (cm^{-1}), functional group: 1592, ν(COO as); 1464, ν(COO s); 1654, ν(C=C)]; 756, ν(OSnO); 572, ν(SnC). NMR-^{1}H, *M* ($CDCl_3$): ppm (functional group)]: Show the peaks characteristic of [2], plus: δ: [Ha, 0.874 (methyl)], [H_b, 1.433 (methylen)], [H_c, 0.976 (methylen)], [H_d, 2.129 (methyl)]. RMN-^{13}C, ($Cd Cl_3$): ppm (grupo funcional)]: Show the peaks characteristic of [2], plus: δ: [C_a: 13.5 (methylen)], [C_b: 14.08 (methylen)], [C_c: 16.25 (methylen)], [C_d: 27.16 (methyl)].

Anti-microbial activity

Anti-bacterial activity of KA [1], GA [2] and the dibutyltin complexes [3] and [4] against Escherichia coli and Pseudomonas auriginosa

The agar disk diffusion trial was used. For each microorganism, *Escherichia coli* (urinary infection) and *Pseudomonas aeruginosa* (nosocomial infection) 3 to 4 colonies were taken, and placed in 5mL of 0,85% physiological saline solution. The concentrations were adjusted with the standard 0,5 of Mc Farland (1,5 x 10^6 UFC/ml), the adjusted suspension was seeded with an sterile hyssop in the Mueller-Hinton agar, for each micro-organism. To sterile paper disks, were added 10 mL of each compound for each trial with a 30 μg/mL concentration. The disks were allowed to dry on sterile closed Petri dishes; later on, were distributed on Petri dishes that had Mueller-Hinton agar. As a positive control, a disk with Imipenem (10 μg) was used and as negative control, sterile distilled water was used. They were incubated at 37 °C during 24 hours, and after the incubation, the inhibition halos were measured (mm). All the anti-microbial activity trials were done in duplicate. The measurement of the inhibition halos of the anti-microbial activity was done thus: C= A-B (C= size of the inhibition halo, A= size of the halo plus the disk of filter paper, B= size of the filter paper disk (9 mm).

Evaluation of anti-fungus activity of KA [1], GA [2] and the dibutyltin complexes [3] and [4] against Trametes versicolor

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).

Statistical analysis

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

RESULTS AND DISCUSSION

Spectroscopic analysis

In the FTIR spectra of [3] and [4] complexes 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 the [1] and [2] ligands, 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 symmetric and asymmetric vibrations of the acetate group (COO^-). IR spectra studies of acetates, based on the position difference ($\Delta\nu$) of the bands corresponding to the asymmetric (ν_{as}(COO-)) and symmetric (ν_s(COO-)) stretching, reaching the following conclusions: a) When the acetate group acts as a counter-ion, the difference of the position of the symmetric and asymmetric stretching vibrations bands are between 1640 and 1710 cm^{-1}, b) Complexes with monodentate acetates have values for $\Delta[\nu_{as}(COO^-)-(\nu_s(COO^-)]$ much higher than for ionic complexes, normally above 200 cm^{-1}, c) In the complexes that present bands corresponding to acetate groups with Δ values less than 150 cm^{-1}, the acetate group is acting as a chelating ligand or as a bidentate bridge (Chilwal et al., 2014). Generally, the $\Delta\nu = [\nu_{as}(COO-)-(\nu_s(COO-)]$ values are used as a coordination mode indicator of the carboxylate anion with the tin atom (Win et al., 2010). In our case, the $\Delta\nu = [\nu_{as}(COO^-)-(\nu_s(COO^-)]$ values for the [3] and [4] complexes are 140 and 128 cm^{-1}, respectively, that indicates a bidentate coordination. The Sn-O vibration appears in the 300-800 cm^{-1} range (Sawyer, 1971); for the [3] and [4] complexes, this band appear at 700 y 756 cm^{-1}, respectively. The unidimensional and bidimensional NMR spectra analysis for [3] and [4] complexes shows signals that are characteristic of the [1] and [2] natural products; it is noteworthy, two singlets assigns to the methylenic double bond (2H) that correlates with C17, a broad singlet of the methyn (1H) that corresponds to C13 and two singlets of the methyl groups that correlates with carbon 19 and 20; both the singlet of the methyn group H13 and the two methyls H18 and H20, are diagnostic for the ent-kaur-16-ene (Henrick and Jefferies, 1964). However, in the spectra of the complexes, are observed the signals associated with the dibutyltin(IV) alkyl chains. In the NMR-^{13}C spectra analysis it was possible to assign the signal corresponding to C19 of the kaurenic acid ligands: 206.08 ppm [3] and 206.81 ppm [4]. In the HMBC spectra the quaternary carbons are observed (discriminated in the DEPT-135 experiment) in a low field region, that are assigned to the carbon of the carboxylate group (C19) corresponding the the ligand skeleton, giving evidence of complex formation. Additionally, it is not possible to assign signal to the methylene group bonded to the tin atom in complex [4], since the electronic influence of the metal on the hydrogenes and carbon are deshielded and overlap with signals of the GA [2] ligand. The physical and spectroscopic data for the natural products [1] and [2] correspond to the literature values (Brieskorn and Pöhlmann, 1968; Kloss, 1969; Piozzi et al., 1972; Batista et al., 2005; Reynolds et al., 1984; Enriquez et al., 1997; Amaro-Luis, 1993; Silva et al., 1999; Meccia et al., 2010). The physical and spectroscopic data permits the proposition of the structure for the dibutyltin(IV) complexes: [bis(*ent*-kaur-16-en-19-oate)] dibutyltin(IV), $Sn(C_4H_9)_2[C_{20}H_{29}O_2]_2$, [3] and [bis(*ent*-kaur-9(11),16-dien-19-oate)]dibutyltin(IV), $Sn(C_4H_9)_2[C_{20}H_{27}O_2]_2$, [4] (Fig. 2)

Statistical analysis of biological activity

Antibacterial activity

The results in Table 1, show that all the compounds evidenced notable differences in the bacterial growth control *($p< 0.05$)*. The bioactivity of GA [2], against *E. coli* was significantly lower than its dibutyltin derivative [4], but KA [1], showed an important bacterial growth inhibition compared with its derivative [3]. The activity against *P. auriginosa* was interesting, KA [1] and its derivative [3], did not show important statistically significant differences (*$p=0.437$*), but, the activity of GA [2], was significantly superior to that of its derivative [4]. The bioactivity of the evaluated compounds decreases in the following order 4 > 1 >3 > 2 against *E. coli*, and 2 > 1 = 3 > 4, against *P. aeruginosa*.

When the antimicrobial activity is analyzed and compared (Table 1), it is observed that it depends on the type and nature of the compound, as well as the type of bacterial strain evaluated. For example, the bioactivity behavior of KA [1], showed the same efficiency against both bacteria (*$p=1.00$*), however, GA [2] showed a high activity against *P. aeruginosa*, compared with *E. coli* (*$p<0.05$*). On the other hand, the derivative [4], has a superior inhibitory behavior against *E. coli*. Lastly, the inhibition showed by derivative [3], against *P. aeruginosa* was highly significant.

Antifungal activity

In Fig. 3 and Table 2, are shown the percentual results of the micelar growth inhibition and ANOVA test of *T. versicolor* for two concentrations (60 and 120 μg/mL) of the [1], [2], [3] and [4] compounds. The variance analysis (ANOVA) between both concentrations of the evaluated compounds, showed statistically significant differences (*$p<0.05$*), evidencing the antifungal effect or activity of them.

As can be seen (Table 2), with 120 μg/mL concentration, the [1-4] compounds showed a growth inhibition significantly greater (*$p<0.05$*) compared with the percentage inhibition using a 60 μg/mL concentration. The biological activity against *T versicolor*, for compound [4] at 60 μg/mL was significantly superior (85.57%) compared with the other compounds at equal concentration, while the least efficient was compound [3]. While compounds [1] and [2] showed a biological control over 70%, they did not show a significant difference between them (*$p=0.317$*).

Table 1: Comparison of Tukey means for the bacterial inhibition (in mm) for [1-4] compounds at 30 µg/mL concentration

Compounds	N	*Pseudomonas aeuriginosa* Subsets for alfa=0.05			Compounds	*Escherichia coli* Subsets for alfa=0.05			
		1	2	3		1	2	3	4
4	6	13			2	0			
3	6		15		3		13		
1	6		16		1			16	
2	6			18	4				21
Sig.		1.000	0.437	1.000	Sig.	1.000	1.000	1.000	1.000

The means are shown for the groups in the homogeneous subsets. Positive control: Imipenem. Negative control: sterile distilled water

Tabla 2: Growth inhibitión of *Trametes versicolor* and ANOVA using the natural products KA [1], GA [2] and the dibutyltin (IV) [3-4] derivatives

Growth inhibition (%)	ANOVA (60µg/ml)				Growth inhibition (%)	ANOVA (120µg/ml)			
	1	2	3	4		1	2	3	4
1	73.88				1	87.77			
2	72.76	0.317			2	93.33	0.000		
3	68.88	0.014	0.009		3	100.0	0.000	0.001	
4	85.57	0.000	0.000	0.000	4	95.56	0.000	0.241	0.016

The difference between the means is significant $p<0.05$

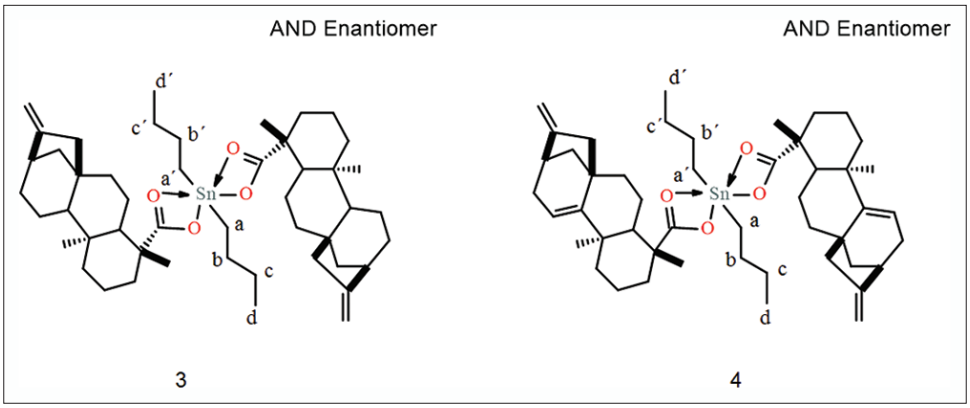

Fig 2. Chemical structure of the dibutyltin(IV) complexes.

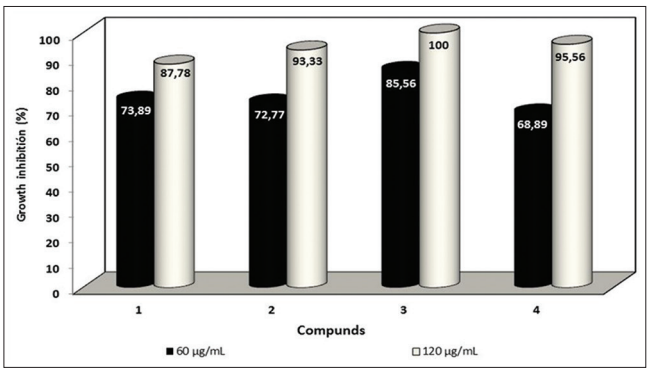

Fig 3. Porcentage micelar growth inhibitión of *Trametes versicolor* using the natural products KA [1], GA [2] and the dibutyltin(IV) [3-4] derivatives.

With the concentración increase (120 µg/mL), some important changes were observed in the evaluation (Fig. 3 and Table 2), for example, the biological activity of compound [3] was significantly superior ($p<0.05$) controlling 100% the growth of *T versicolor*, while compound [1] showed statistically less, giving 83.77 % of micelar growth control. On the other hand, compounds [2] and [4] showed near 95% biological control, without statistically significant difference between them ($p=0.241$).

These results demonstrate that the four compounds evaluated, present biological activity against the fungus that produces white wood rotting, *Trametes versicolor*, indicating also that a slight concentration increase of the natural products or the organotin derivatives, and also an increase in the molecular weight of the [3-4] compounds, increases the micellar growth inhibition activity against the fungus.

The literature shows the priorities in the field for wood preservation, and indicates the need to use new technologies based on sustainable principles, such as the use of pure natural products or natural organic component mixtures with copper or boron salts, which represent a

trustworthy option as fungicides against the wood attacking fungus (González-Laredo et al., 2015). In this sense, with the experimental results in this research, we can propose a basic study of the organotin derivates of kaurenic acids as potential fungicide active molecules.

CONCLUSIONS

In the last years, there has been an important effort in the separation of active molecules from natural sources, and the synthesis of derivatives that widens the action spectrum against micro-organisms that affect mankind directly or indirectly. Natural products have been key molecules in the research field, and the relative natural abundance of species that produce *ent*-kaurenic and grandiflorenic acid have led to important derivatives with interesting biological activity. On the other hand, the synthesis of organotin(IV) derivatives of natural products incorporates the Organometallic Chemistry in the development of Medicinal Chemistry and gives added value to the kaurenic acids and the organotin derivatives, introducing wood preserving molecules, with ecological importance.

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Author contributions

P.Q.R.: Proposal of the research topic, synthesis of complexes, structural characterization and writing of manuscript. B.F.: Design and supervision of experiment with writing of manuscript. Y.F.: Proposal of the research topic and field experiments. F.B.: Critical revision of manuscript. R.C.: Critical revision of manuscript. J.E.V.P.: Proposal of the research topic and field experiments. F.C.: Structural characterization of natural products. A.G.R.: Biological activity with bacterial: *E. coli* and *P. auriginosa*. J.V.: Biological activity with fungi: *T. versicolor*.

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