



US 20180035669A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2018/0035669 A1**  
(43) **Pub. Date: Feb. 8, 2018**  
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(54) **ACETATE DERIVED COMPOUNDS FROM GERANYLORCINOL. SYNTHESIS PROCESS FOR OBTAINING SAID COMPOUNDS AND USE OF SAID COMPOUNDS AS ANTIFUNGAL AGAINST BOTRYTIS CINEREA**

(86) PCT No.: **PCT/CL2016/050004**  
§ 371 (c)(1),  
(2) Date: **Jul. 27, 2017**

(30) **Foreign Application Priority Data**

Jan. 27, 2015 (CL) ..... 200-2015

**Publication Classification**

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(51) **Int. Cl.**  
**A01N 31/16** (2006.01)  
**C07C 39/19** (2006.01)  
**C07C 37/00** (2006.01)

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(52) **U.S. Cl.**  
CPC ..... **A01N 31/16** (2013.01); **C07C 37/00** (2013.01); **C07C 39/19** (2013.01)

(57) **ABSTRACT**

The present invention discloses a synthesis process for obtaining linear derivative compounds from geranylborcinol, said linear derivative compounds from geranylborcinol, the acetylated derivatives compounds therefrom, the method for encapsulating the compounds in a polymer matrix and the use of said encapsulated compounds as antifungal against *Botrytis Cinerea*.

(21) Appl. No.: **15/546,910**

(22) PCT Filed: **Jan. 25, 2016**

**ACETATE DERIVED COMPOUNDS FROM  
GERANYLORCINOL. SYNTHESIS PROCESS  
FOR OBTAINING SAID COMPOUNDS AND  
USE OF SAID COMPOUNDS AS  
ANTIFUNGAL AGAINST BOTRYTIS  
CINEREA**

FIELD OF THE INVENTION

**[0001]** The present invention seeks protection on linear derivative compounds from geranylornicinol, on the acetylated derivative compounds thereof, on the synthesis process for obtaining said compounds and on the use of said compounds as antifungals against *Botrytis cinerea*.

BACKGROUND OF THE INVENTION

**[0002]** Linear geranylphenols are an interesting subclass of secondary metabolites found primarily in marine organisms. They are biosynthesized in a mixed form from the metabolic pathway of shikimic acid and mevalonic acid. The terpene portion may have a length of up to nine isoprene units, which chain originates from the mevalonate biosynthetic pathway while the aromatic portion is derived from the biosynthetic pathway of the shikimic acid. Among marine organisms in which this type of compound has been isolated are brown seaweed (Faulkner, D. J. *Natural Product Reports* 1986, 3, 1-33; Ochi, M.; Kotsuki, H.; Inoue, S.; Taniguchi, M. *Chemistry* 1979, 831-832; Capon, R. J.; Ghisalberti, E. L.; Jefferies, P. R. *Phytochemistry* 1981, 20, 2598-2600; Gerwick, W. H.; Fenical, W. *The Journal of Organic Chemistry* 1981, 46, 22-27), sponges (Rosa, S. De; Crispino, A., Giulio, A. De *Journal of Natural* 1995, 58, 1450-1454; Bifulco, G.; Bruno, I.; Minale, L.; Riccio, R.; Debitus, C.; Bourdy, G.; Vassas, A.; Lavayre, J. *Journal of Natural Products* 1995, 58, 1444-1449), alcyonaceas (Bowden, B. F.; Coll, J. C. *Australian Journal of Chemistry* 1981, 34, 2677-2681), gorgonaceas (Ravi, B. N.; Wells, R. J. *Australian Journal of Chemistry* 1982, 35, 105-112), ascidias (Howard, B. M.; Clarkson, K.; Bernstein, R. L. *Tetrahedron Letters* 1979, 20, 4449-4452; Targett, N. *Journal of Natural Products* 1984, 1696, 1975-1976; Guella, G.; Mancini, I.; Pietra, F. *Helvetica Chimica Acta* 1987, 70, 621-626; Faulkner, D. J. *Natural product reports* 1993, 10, 497-539; Fu, X.; Hossain, M. B.; Helm, D. Van Der; Schmitz, F. J.; Van der Helm, D. *Journal of the American Chemical Society* 1994, 116, 12125-12126; Fu, X.; Hossain, M. B.; Schmitz, F. J.; Helm, D. Van Der *The Journal of Organic Chemistry* 1997, 62, 3810-3819). From the brown algae, compounds with chains of the mono-, sesqui- and di-terpene type have been isolated whereas structures with long linear chains have been obtained from sponges.

**[0003]** For this family of compounds, potent biological activities have been reported including anti-inflammatory (Quang, D. N.; Hashimoto, T.; Arakawa, Y.; Kohchi, C.; Nishizawa, T.; Soma, G.-I.; Asakawa, Y. *Bioorganic & medicinal chemistry* 2006, 14, 164-8; Bauer, J.; Koeberle, A.; Dehm, F.; Pollastro, F.; Appendino, G.; Northoff, H.; Rossi, A.; Sautebin, L.; Werz, O. *Biochemical pharmacology* 2011, 81, 259-68), antifungal (Danelutte, A. P.; Lago, J. H. G.; Young, M. C. M.; Kato, M. J. *Phytochemistry* 2003, 64, 555-559), anti-HIV (Manfredi, K. P.; Vallurupalli, V.; Demidova, M.; Kindscher, K.; Pannell, L. K. *Phytochemistry* 2001, 58, 153-7), antioxidant (Yamaguchi, L. F.; Lago, J. H. G.; Tanizaki, T. M.; Mascio, P. Di; Kato, M. J. *Phyto-*

*chemistry* 2006, 67, 1838-43), the most frequent of reported activities being the antineoplastic activity (Han, Q.-B.; Qiao, C.-F.; Song, J.-Z.; Yang, N.-Y.; Cao, X.-W.; Peng, Y.; Yang, D.-J.; Chen, S.-L.; Xu, H.-X. *Chemistry & biodiversity* 2007, 4, 940-6.; Liu, Q.; Shu, X.; Wang, L.; Sun, A.; Liu, J.; Cao, X. *Cellular & molecular immunology* 2008, 5, 271-8).

**[0004]** Synthesis of Linear Geranylphenols

**[0005]** As we have seen, these compounds, whose structure combines a linear terpene unit with a phenolic portion and which have been isolated from various organisms, have been shown to possess a large number of biological properties. However, the major problem for the study of this group relates to its low yield for obtaining them from natural sources. Clearly this important structural group requires an overall strategy for the preparation of the natural products containing this pharmacophore as well as systems with other aromatic residues (Osorio, M.; Aravena, J.; Vergara, A.; Taborga, L.; Baeza, E.; Catalán, K.; González, C.; Carvajal, M.; Carrasco, H.; Espinoza, L. *Molecules* (Basel, Switzerland) 2012, 17, 556-70).

**[0006]** The most recurrent of the synthetic strategies used for the preparation of terpenylphenols consists, as a first step, of the separate preparation of the appropriate terpene and aromatic fragments for the synthesis. The second step is crucial to the success of the synthesis and involves coupling of the synthetic terpenyl equivalent to the aromatic nucleus. The most commonly used methods for anchoring the two portions are numbered in the following list:

1. Condensation of the terpene and the phenol (Friedel-Crafts allylation) catalysed by a Brønsted acid.
2. Direct terpenylation with terpenyltrialkyltin in the presence of a Lewis acid
3. Rearrangement of terpenylether catalysed by a Lewis acid.
4. Nucleophilic substitution of aryllithium derivatives on alkyl halides.
5. Nucleophilic substitution of aryllithium derivatives on aryl halides.
6. Nucleophilic addition of aryllithium derivatives to carbonyls of the terpenyl unit and subsequent reduction in the presence of a Lewis acid.

Condensation of Geraniol and hydroquinone (Friedel-Crafts allylation) catalysed by a Lewis acid.

Condensation of Geraniol with Hydroquinone and Phenols (Friedel-Crafts Allylation) Catalysed by a Lewis Acid.

**[0007]** The most common direct geranylation schemes involves "π-excedent" aromatic compounds in a Friedel-Crafts alloy under acidic conditions (Keinan, E.; Eren, D. *The Journal of Organic Chemistry* 1987, 18, 3872-3875; Syper, L.; Kloc, K.; Mz.xl; lochowski, J. *Tetrahedron* 1980, 36, 123-129; Eisohly, H. N.; Turner, C. E.; Clark, A. M.; Eisohly, M. A. *Journal of Pharmaceutical Sciences* 1982, 71, 1319-23). Despite the number of modifications, these approaches are limited by the inherent instability of the allylic alcohol in the acidic conditions employed and undesirable side reactions (Stevens, K L, Jurd, L. Manners, G. *Tetrahedron* 1972, 28, 1939-1944). However, when acid catalysis, such as the reaction with oxalic acid, is replaced by the Lewis acid BF<sub>3</sub>.Et<sub>2</sub>O, reaction yields are considerably increased, in addition to using non-aqueous solvents such as ether, dioxane, CH<sub>2</sub>Cl<sub>2</sub> and CCl<sub>4</sub>. For this synthesis strategy, yields close to 60% are obtained (Fedorov, S.; Radchenko, O.; Shubina, L. *Pharmaceutical* 2006, 23, 70-81; Shubina, L. K.; Fedorov, S. N.; Radchenko, O. S.; Balaneva, N. N.;

Kolesnikova, S. a.; Dmitrenok, P. S.; Bode, A.; Dong, Z.; Stonik, V. a. *Tetrahedron Letters* 2005, 46, 559-562). However, in recent publications where this synthetic strategy has been used to couple hydroquinone to geraniol, reaction yields ranges from 28 to 34% (Takenaka, K.; Tanigaki, Y.; Patil, M. L.; Rao, C. V. L.; Takizawa, S.; Suzuki, T.; Sasai, H. *Tetrahedron: Asymmetry* 2010, 21, 767-770; Baeza, E.; Catalán, K.; Peña-Cortés, H.; Espinoza, L. *Quim. Nova* 2012, 35, 523-526), on the other hand when coupling 2,4,5-trimethoxyphenol with geraniol a mixture of mono-coupled compounds is obtained with yields ranging from 13 to 15% (Baeza, E.; Catalan, K.; Villena, J.; Carrasco, H.; Cuellar, M.; Espinoza, L. *Journal of the Chilean Chemical Society* 2012, 57, 1219-1223).

**[0008]** Therefore, there is still a need for a synthesis process for obtaining linear derivative compounds from geranylornicinol with a good yield.

#### SUMMARY OF THE INVENTION

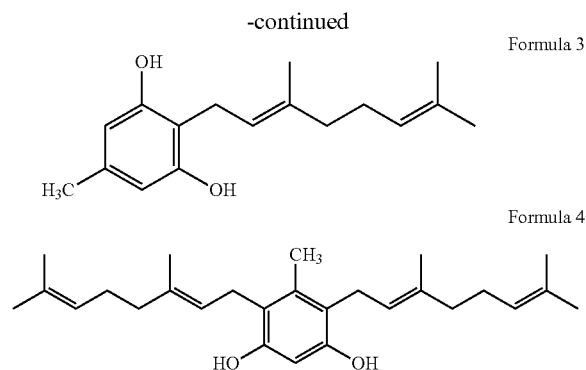
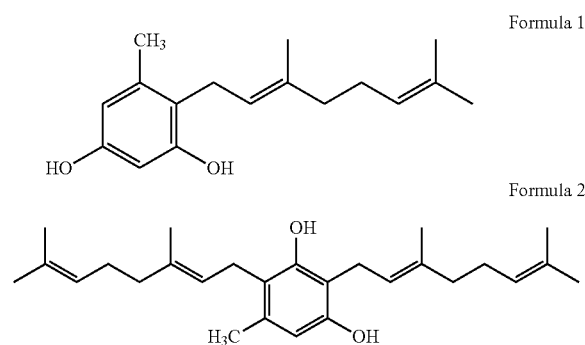
**[0009]** The present invention discloses a synthesis process for obtaining linear derivative compounds from geranylornicinol, said linear derivatives compounds from geranylornicinol, the acetylated derivative compounds thereof, the method for encapsulating the compounds in a polymer matrix and the use of such encapsulated compounds as antifungal against *Botrytis Cinerea*.

#### DETAILED DESCRIPTION OF THE INVENTION

**[0010]** The present invention discloses a synthesis process for obtaining linear derivative compounds from geranylornicinol, said linear derivative compounds from geranylornicinol, the acetylated derivative compounds thereof, the method for encapsulating the compounds in a polymer matrix and the use of said encapsulated compounds as antifungal against *Botrytis cinerea*.

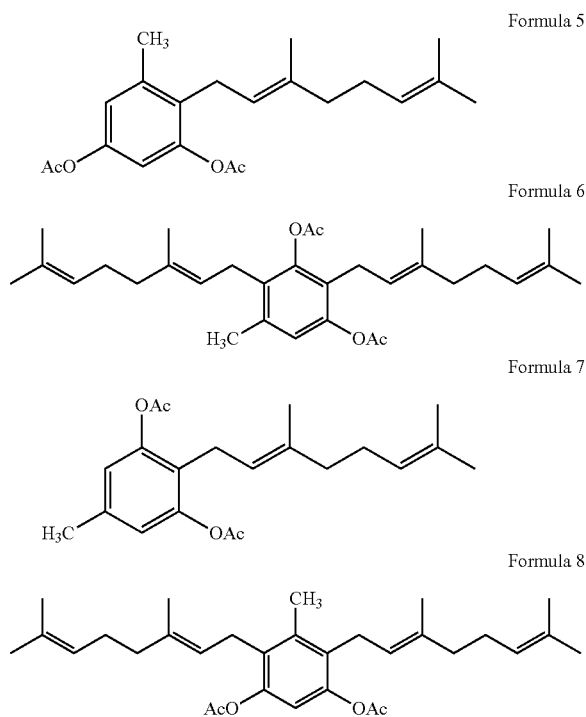
**[0011]** The synthesis process comprises condensation of geraniol with orcinol (Friedel-Crafts allylation) catalysed by a Lewis acid. The process comprises using the Lewis acid  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ —catalyzed direct alkylation technique and the use of a  $\text{AgNO}_3$  secondary catalyst employed for the first time in this type of reactions.

**[0012]** The synthesis process allowed the di-coupled derivative compounds represented by the formulas 1 to 4 to be obtained, from which the compounds of formula 2 and 4 correspond to compounds claimed in the present invention. The structures of the linear geranylornicinols, obtained by the coupling method of the present invention, using the secondary  $\text{AgNO}_3$  catalyst are shown in formulas 1 to 4.



**[0013]** The compounds derived from the formulas 1 and 3, were previously synthesized by other authors (Manners, G.; Jurd, L.; and Stevens, K.; *Tetrahedron*, 1972, 28, 2949-2959; Eisohly, H. N.; Turner, C. E.; Clark, A. M.; and Eisohly, M. A.; *J. Pharmaceutical Sciences*, 1982, 71, 1319-1323) with yields of between 2-18% and also previously described by our research group, with yields of 12.6% and 27.6% for the derived compound 3 and 1 (Taborga, L., Vergara, A., Fernández, MJ, Osorio, M, Carvajal, M, Madrid, A, Marilaf, F, Carrasco, H, and Espinoza, *Journal of the Chilean Chemical Society*, 2013, 58, 1790-1796).

**[0014]** Additionally, the acetylated derivative compounds of the four compounds represented in formulas 1 to 4, which have been represented by formulas 5 to 8, respectively, were prepared. Formulas 5 to 8, correspond to the following structures:



**[0015]** The acetylated derivative compounds 5 to 8 correspond to structures claimed in the present invention. These derivative compounds were obtained from the respective

geranylornicinol derivative compounds of formulas 1 to 4, through acetylation reaction under standard conditions; that is, by acetylation with acetic anhydride, 4-N,N-dimethylaminopyridine (DMAP) as catalyst and dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) as the solvent.

#### Experimental Procedure

##### Synthesis Process of the Geranylornicinol Derived Compounds of the Formulas 1, 2, 3 and 4

**[0016]** 2 mL of acetonitrile was added to a solution of orcinol (1.22 g, 9.8 mmol) and geraniol (1.51 g, 9.8 g), and a solution of acetonitrile saturated in  $\text{AgNO}_3$  (400 mg in 2 mL) was added thereafter, and then stirred at room temperature under a nitrogen atmosphere.  $\text{BF}_3\text{Et}_2\text{O}$  (0.46 g, 3.2 mmol) was directly and slowly (dropwise) injected to this mixture. It was allowed to stir for 48 hours. The term of the reaction was monitored by thin layer chromatography (TLC). To complete the reaction, crushed ice (about 30 g) and abundant salt (about 50 g NaCl) were added, then vacuum filtered and the organic phase was extracted with  $\text{AcOEt}$  (3x20 mL), which phase was then washed with a solution of  $\text{NaHCO}_3$  (15 mL, 5%) and water (2x15 mL), then dried over anhydrous  $\text{Na}_2\text{SO}_4$ , this solution was then filtered and evaporated to dryness. The reaction was performed in quadruplicate and the sum of crude reaction product was purified by column chromatography (CC) using silica gel (silica gel 60 for column chromatography, 0.040-0.063 mm) as the stationary phase and a mixture of acetate as the mobile phase (0:20→6:14:starting with a first addition with a total volume of 20 mL of hexane and 0 mL of ethyl acetate, the second addition of the same total volume, but now with 18.8 mL of hexane and 0.2 mL of ethyl acetate, and so on by increasing the amount of ethyl acetate in 0.2 mL and decreasing the amount of hexane in 0.2 mL in each fraction, until the last addition of 6 mL of hexane and 14 mL of ethyl acetate is reached, always maintaining a total and constant volume of 20 mL). A total of 108 fractions were collected in 10 mL flasks. Then, in order to identify the fractions containing the products, a qualitative analysis was performed by Thin Layer Chromatography (TLC) where fractions from 18-28 contained compound 2. They were combined and labelled as F-I 1,554.8 mg (9.9% yield) of a dark yellow viscous oil (formula 2 compound). Fractions from 38-49 were combined and contained compound 3, labelled F-II 1281.8 mg (12.5% yield) of a yellow viscous oil (formula 3 compound). Fractions from 67-73 were combined and contained the compound 4, labelled F-III 955.3 mg (6.1% yield) of a dark yellow viscous oil (formula 4 compound). Fractions from 74-76 were combined and contained the mixture of compounds 1 and 4, labelled F-IV 415.4 mg of a dark yellow viscous oil (mixture of compounds of formula 1 and 4, in a 0.125:1 ratio respectively). Fractions from 77-87 were combined and contained compound 1, labelled F-V 2816.2 mg (27.6% yield) of a yellow viscous oil (formula 1 compound). From fractions 88-108, the starting material (ornicinol) which did not react (78.6 mg) was recovered.

##### Formula 1 Compound Spectroscopic Characterization:

**[0017]** RMN of  $^1\text{H}$ : 6.26 (s, 1H, H-6); 6.22 (s, 1H, H-2); 5.60 (bs, 2H, OH); 5.13 (t, J=6.8 Hz, 1H, H-2'); 5.05 (t, J=6.7 Hz, 1H, H-6'); 3.28 (d, J=6.7 Hz, 2H, H-1'); 2.21 (s,

3H, H-7); 2.09-2.06 (m, 2H, H-5'); 2.04-2.00 (m, 2H, H-4'); 1.78 (s, 3H,  $\text{CH}_3\text{-C}3'$ ); 1.67 (s, 3H, H-8'); 1.58 (s, 3H,  $\text{CH}_3\text{-C}7'$ ); RMN of  $^{13}\text{C}$ : 155.2 (C-3); 154.1 (C-1); 138.6 (C-5); 137.2 (C-3'); 131.8 (C-7'); 123.9 (C-6'); 122.2 (C-2'); 118.1 (C-4); 109.7 (C-6); 101.0 (C-2); 39.6 (C-4'); 26.4 (C-5'); 25.6 (C-8'); 25.0 (C-1'); 20.0 (C-7); 17.6 ( $\text{CH}_3\text{-C}7'$ ); 16.1 ( $\text{CH}_3\text{-C}3'$ ); IR ( $\text{cm}^{-1}$ ): 3,393, 2,975, 2,925, 2,859, 1,602; EM (m/z, %):  $\text{M}^+$  260 (21.7), 137 (100), 191 (26.9), 177 (13.9), 123 (10.5).

##### Formula 2 Compound Spectroscopic Characterization:

**[0018]** RMN of  $^1\text{H}$ : 6.30 (s, 1H, H-6); 5.50 (s, 1H, OH); 5.44 (bs, 1H, OH); 5.32 (t, J=6.9 Hz, 1H, H-2'); 5.19 (t, J=6.6 Hz, 1H, H-2''); 5.11 (t, J=6.6 Hz, 2H, H-6' y H-6''); 3.46 (d, J=6.9 Hz, 2H, H-1'); 3.35 (d, J=6.6 Hz, 2H, H-1''); 2.25 (s, 3H,  $\text{CH}_3\text{-Ar}$ ); 2.16-2.15 (m, 4H, H-5' y H-5''); 2.12-2.09 (m, 4H, H-4' y H-4''); 1.86 (s, 3H,  $\text{CH}_3\text{-C}3'$ ); 1.84 (s, 3H,  $\text{CH}_3\text{-C}3''$ ); 1.73 (s, 6H,  $\text{CH}_3\text{-C}7'$  y  $\text{C}7''$ ); 1.64 (s, 6H, H-8' y H-8''); RMN of  $^{13}\text{C}$ : 153.4 (C-3); 152.5 (C-1); 138.3 (C-3'); 137.0 (C-3''); 135.1 (C-5); 131.8 (\*C-7'); 131.6 (\*C-7''); 123.9 (\*\*C-6'); 123.8 (\*\*C-6''); 122.5 (C-2''); 122.0 (C-2'); 118.1 (C-4); 111.5 (C-2); 109.7 (C-6); 39.6 (C-4'); 39.5 (C-4''); 26.4 (\*\*\*C-5); 26.3 (\*\*\*C-5''); 25.6 (C-8' y C-8''); 25.4 (C-1''); 22.5 (C-1'); 19.7 ( $\text{CH}_3\text{-Ar}$ ); 17.6 (C-10' y C-10''); 16.1 (C-9'); 16.0 (C-9''); IR ( $\text{cm}^{-1}$ ): 3,454, 2,967, 2,917, 2,855, 1,747, 1,621, 1,592, 1,448, 1,377.

##### Formula 3 Compound Spectroscopic Characterization:

**[0019]** RMN of  $^1\text{H}$ : 6.26 (s, 2H, H-6 y H-4); 5.66 (bs, 2H, OH); 5.29 (t, J=6.6 Hz, 1H, H-2'); 5.08 (t, J=6.1 Hz, 1H, H-6'); 3.42 (d, J=7.0 Hz, 2H, H-1'); 2.20 (s, 3H,  $\text{CH}_3\text{-Ar}$ ); 2.13-2.10 (m, 2H, H-5'); 2.09-2.07 (m, 2H, H-4'); 1.83 (s, 3H,  $\text{CH}_3\text{-C}3'$ ); 1.70 (s, 3H, H-8'); 1.61 (s, 3H,  $\text{CH}_3\text{-C}7'$ ); RMN of  $^{13}\text{C}$ : 154.8 (C-1 y C-3); 138.6 (C-3'); 137.3 (C-5); 131.9 (C-7); 123.7 (C-6'); 121.8 (C-2'); 110.6 (C-2); 109.0 (C-4 y C-6); 39.6 (C-4'); 26.3 (C-5'); 25.6 (C-8'); 22.1 (C-1'); 21.0 ( $\text{CH}_3\text{-Ar}$ ); 17.6 ( $\text{CH}_3\text{-C}7'$ ); 16.0 ( $\text{CH}_3\text{-C}3'$ ); IR ( $\text{cm}^{-1}$ ): 3,455, 2,966, 2,922, 2,852, 1,772.

##### Formula 4 Compound Spectroscopic Characterization:

**[0020]** RMN of  $^1\text{H}$ : 6.25 (s, 1H, H-2); 5.12 (t, J=6.4 Hz, 2H, H-2'); 5.05 (t, J=6.6 Hz, 2H, H-6'); 3.33 (d, J=6.6 Hz, 4H, H-1'); 2.21 (s, 3H,  $\text{CH}_3\text{-Ar}$ ); 2.09-2.06 (m, 4H, H-5'); 2.03-2.00 (m, 4H, H-4'); 1.78 (s, 6H,  $\text{CH}_3\text{-C}3''$ ); 1.66 (s, 6H, H-8'); 1.58 (s, 6H,  $\text{CH}_3\text{-C}7'$ ); RMN of  $^{13}\text{C}$ : 152.9 (C-1 y C-3); 136.7 (C-3'); 136.4 (C-5); 131.7 (C-7); 124.0 (C-6'); 122.6 (C-2'); 118.5 (C-4 y C-6); 101.4 (C-2); 29.6 (C-4'); 26.5 (C-5'); 25.7 (C-8'); 25.6 (C-1'); 17.6 ( $\text{CH}_3\text{-C}7'$ ); 16.2 ( $\text{CH}_3\text{-C}3'$ ); 15.8 ( $\text{CH}_3\text{-Ar}$ ); IR ( $\text{cm}^{-1}$ ): 3,404, 2,967, 2,922, 2,855, 1,649, 1,599, 1,445, 1,376.

##### Synthesis Process of the Geranylornicinol Acetylated Derived Compounds of the Formulas 5, 6, 7 and 8.

**[0021]** From each compound one portion (362 mg of the compound of formula 1; 170 mg of the formula 2 compound; 74 mg of the formula 3 compound; and 245 mg of the formula 4 compound) were taken which were separately placed in four reaction balloons with 50 mL of  $\text{CH}_2\text{Cl}_2$ , and then 1.0 mL of acetic anhydride and 2 crystals of 4-N,N-dimethylaminopyridine (DMAP) were added to each balloon. Then each mixture was allowed to stir for about one hour. After completion of the reaction the solution in each

balloon was evaporated to dryness and re-suspended in 50 mL of ethyl acetate, which was washed with 30 mL of a 5% sodium bicarbonate solution and water (2×20 mL), then dried over anhydrous sodium sulphate (20 g), filtered and evaporated to dryness. Finally, the four compounds represented by formulas 5 to 8 were obtained with the following yields: Compound 5: 444.6 mg (92.8% yield) of a dark yellow viscous oil. Compound 6: 185 mg (90.5% yield) of a dark yellow viscous oil. Compound 7: 93.3 mg (96.4% yield) of a dark yellow viscous oil. Compound 8: 267.5 mg (89.8% yield) of a dark yellow viscous oil.

#### Formula 5 Compound Spectroscopic Characterization:

**[0022]** RMN of  $^1\text{H}$ : 6.80 (s, 1H, H-6); 6.70 (s, 1H, H-2); 5.04 (t, J=6.3 Hz, 1H, H-6'); 4.97 (t, J=6.4 Hz, 1H, H-2'); 3.21 (d, J=6.4 Hz, 2H, H-1'); 2.28 (s, 6H,  $\text{COCH}_3 \times 2$ ); 2.26 (s, 3H,  $\text{CH}_3\text{—Ar}$ ); 2.06-2.02 (m, 2H, H-5'); 1.99-1.95 (m, 2H, H-4'); 1.73 (s, 3H,  $\text{CH}_3\text{—C3'}$ ); 1.65 (s, 3H, H-8'); 1.58 (s, 3H,  $\text{CH}_3\text{—C7'}$ ); RMN of  $^{13}\text{C}$ : 169.3 ( $\text{COCH}_3$ ); 169.2 ( $\text{COCH}_3$ ); 149.0 (C-3); 148.3 (C-1); 139.2 (C-5); 135.7 (C-3'); 131.4 (C-7'); 129.7 (C-4); 124.1 (C-6'); 121.2 (C-2'); 120.8 (C-6); 113.5 (C-2); 39.5 (C-4'); 26.5 (C-5'); 25.8 (C-8'); 25.6 (C-1'); 21.1 ( $\text{COCH}_3$ ); 20.9 ( $\text{COCH}_3$ ); 19.7 ( $\text{CH}_3\text{—Ar}$ ); 17.6 ( $\text{CH}_3\text{—C7'}$ ); 16.2 ( $\text{CH}_3\text{—C3'}$ ). IR ( $\text{cm}^{-1}$ ): 2,968, 2,927, 2,851, 1,774, 1,752, 1,367; EM (m/z, %): 274 (10.8), 259 (4.1), 192 (12.3), 191(100), 176 (8.3).

#### Formula 6 Compound Spectroscopic Characterization:

**[0023]** RMN of  $^1\text{H}$ : 6.79 (s, 1H, H-6); 5.08-5.04 (m, 3H, H-6', H-6'' y H-2'); 5.00 (t, J=7.0 Hz, 1H, H-2''); 3.17 (bs, 4H, H-1' y H-1''); 2.28 (s, 3H,  $\text{COCH}_3$ ); 2.26 (s, 6H,  $\text{COCH}_3$  y  $\text{CH}_3\text{—Ar}$ ); 2.08-2.04 (m, 4H, H-5' y H-5''); 2.00-1.96 (m, 4H, H-4' y H-4''); 1.73 (s, 3H,  $\text{*CH}_3\text{—C3'}$ ); 1.71 (s, 3H,  $\text{*CH}_3\text{—C3''}$ ); 1.67 (s, 6H, H-8' y H-8''); 1.59 (s, 6H,  $\text{CH}_3\text{—C7'}$  y  $\text{CH}_3\text{—C7''}$ ); RMN of  $^{13}\text{C}$ : 169.3 ( $\text{COCH}_3$ ); 169.0 ( $\text{COCH}_3$ ); 148.1 (C-3); 147.2 (C-1); 136.2 (C-5); 135.4 ( $\text{*C-3'}$ ); 135.2 ( $\text{*C-3''}$ ); 131.3 (C-7' y C-7''); 130.2 (C-4); 124.1 (C-6' y C-6''); 123.9 (C-2); 121.8 (C-6); 121.5 ( $\text{**C-2'}$ ); 121.4 ( $\text{**C-2''}$ ); 39.5 (C-4' y C-4''); 26.5 (C-5' y C-5''); 26.4 (C-1''); 25.6 (C-8' y C-8''); 24.2 (C-1'); 20.8 ( $\text{COCH}_3$ ); 20.5 ( $\text{COCH}_3$ ); 19.4 ( $\text{CH}_3\text{—Ar}$ ); 17.6 ( $\text{CH}_3\text{—C7'}$  y  $\text{CH}_3\text{—C7''}$ ); 16.1 ( $\text{***CH}_3\text{—C3'}$ ); 16.0 ( $\text{***CH}_3\text{—C3''}$ ).

#### Formula 7 Compound Spectroscopic Characterization:

**[0024]** RMN of  $^1\text{H}$ : 6.77 (s, 2H, H-4 y H-6); 5.06 (t, J=6.3 Hz, 1H, H-6'); 5.02 (t, J=6.9 Hz, 1H, H-2'); 3.14 (d, J=6.9 Hz, 2H, H-1'); 2.32 (s, 3H,  $\text{CH}_3\text{—Ar}$ ); 2.27 (s, 6H,  $\text{COCH}_3$ ); 2.07-2.01 (m, 2H, H-5'); 1.97-1.93 (m, 2H, H-4'); 1.71 (s, 3H,  $\text{CH}_3\text{—C3'}$ ); 1.66 (s, 3H, H-8'); 1.58 (s, 3H,  $\text{CH}_3\text{—C7'}$ ); RMN of  $^{13}\text{C}$ : 169.1 ( $\text{COCH}_3 \times 2$ ); 149.4 (C-1 y C-3); 137.0 (C-5); 135.5 (C3'); 131.3 (C-7'); 124.0 (C-6'); 123.3 (C-2); 121.2 (C-2'); 120.8 (C-4-C-6); 39.5 (C-4'); 26.5 (C-5'); 25.6 (C-8'); 23.6 (C-1'); 20.9 ( $\text{CH}_3\text{—Ar}$ ); 20.8 ( $\text{COCH}_3 \times 2$ ); 17.6 ( $\text{CH}_3\text{—C7'}$ ); 16.1 ( $\text{CH}_3\text{—C3'}$ ).

#### Formula 8 Compound Spectroscopic Characterization:

**[0025]** RMN of  $^1\text{H}$ : 6.70 (s, 1H, H-2); 5.06 (t, J=6.2 Hz, 2H, H-6'); 5.01 (t, J=5.6 Hz, 2H, H-2'); 3.26 (d, J=6.2 Hz, 4H, H-1'); 2.28 (s, 6H,  $\text{COCH}_3$ ); 2.22 (s, 3H,  $\text{CH}_3\text{—Ar}$ ); 2.08-2.05 (m, 4H, H-5'); 2.01-1.98 (m, 4H, H-4'); 1.75 (s, 6H,  $\text{CH}_3\text{—C3'}$ ); 1.67 (s, 6H, H-8'); 1.60 (s, 6H,  $\text{CH}_3\text{—C7'}$ ); RMN of  $^{13}\text{C}$ : 169.2 ( $\text{COCH}_3 \times 2$ ); 146.8 (C-1 y C-3); 137.9 (C-5); 135.3 (C-3'); 131.2 (C-7'); 130.0 (C-4 y C-6); 124.0

(C-6'); 121.6 (C-2'); 113.9 (C-2); 39.4 (C-4'); 26.4 (C-5'); 26.2 (C-1'); 25.5 (C-8'); 20.7 ( $\text{COCH}_3 \times 2$ ); 17.5 ( $\text{CH}_3\text{—C7'}$ ); 16.0 ( $\text{CH}_3\text{—C3'}$ ); 15.4 ( $\text{CH}_3\text{—Ar}$ ).

Evaluation of the Antifungal Activity of the Compounds Synthesized Against *Botrytis Cinerea* in Free State and Encapsulated System.

**[0026]** Preparation of the Stock Solution of Each Synthesized Compound without Encapsulating.

**[0027]** 20-40 milligrams of each of compounds 1 to 8 were dissolved in 500 microliters of Ethanol to have a stock solution of each compound according to its molecular weight. The stock solution was diluted in 5 mL of water so as to obtain a new stock solution of 5,000 ppm; from which corresponding aliquots were taken to achieve a final concentration on each plate with 50, 150 and 250 ppm potato dextrose agar (PDA) culture medium.

Preparation of Stock Solution of Each Compound Synthesized in a Polymer Solution Pluronic F-127.

**[0028]** The method used for the preparation of polymeric micelles, based on Polycaprolactone (MW: 3.409 g/mol) or Pluronic® F-127 (MW: 12,500 g/mol) corresponds to the direct dissolution method consisting of the addition of the polymer to water with stirring and at room temperature, until the polymer dissolves to give a clear aqueous solution. The amount of polymer and water is chosen so as to have a solution of a concentration of  $1 \times 10^{-3}$  M of polymer chains.

**[0029]** For the preparation of a solution of 50 mL of Pluronic F-127, 0.63 g is used and for the case of polycaprolactone 0.17 g is used.

**[0030]** The physical encapsulation of the compounds in the polymeric micelles ( $1 \times 10^{-3}$  M) is carried out by the emulsion method. The compound is dissolved in a volatile solvent (dichloromethane) and 10 mL of the polymer solution added in 100  $\mu\text{L}$  aliquots to complete 400  $\mu\text{L}$  reaching a concentration of 0.101 M of compound. By stirring in a vortex and applying ultrasound a very homogeneous milky emulsion is formed. The organic solvent is then removed by evaporation by heating the emulsion at 50° C. in a system open to air. Upon removal of the solvent, a clear solution remains containing the polymer forming micelles and the non-polar compounds which have been encapsulated in the hydrophobic micro-domain provided by the core of these polymeric micelles.

**[0031]** Subsequently, the solution is diluted by adding polymer solution corresponding to a  $1 \times 10^{-3}$  M concentration until the compound of interest reaches a stock concentration in the range of 625-1,250 ppm in the micelle to be applied to the plate. This dilution should be done by adding polymer solution so as to maintain the same concentration of micelles and vary only the concentration of active ingredient.

Anti-Phytopathogenic Activity Test Against *B. cinerea*.

**[0032]** In order to determine the inhibition of mycelial growth of *B. cinerea*, both for the free state and encapsulated compounds, the radial growth method in potato dextrose agar (PDA) culture medium was used. The compounds of interest in both free and encapsulated form were added to the PDA at different concentrations of 50, 150 and 250 ppm at a temperature of 50° C. Once the PDA had been solidified, a 4-mm diameter disc was seeded with mycelium of the pathogen in the center of the petri dish. The experiment

consists of a negative control which is a petri dish where PDA culture medium and 1% Ethanol were added, in the case of Negative Control for encapsulated compounds it consists in adding only the micelle (polycaprolactone or Pluronic F-167) free in PDA. In addition, a positive control containing commercially available fungicide Captan in PDA culture medium is included at the same concentrations tested in both its free form and also a positive control of Captan in its encapsulated form. Each treatment consisted of three replicates (n=3 replicates/compound/concentration).

**[0033]** After 48 (free state) and 72 (encapsulated system) hours of incubation at 23° C. under a photoperiod of 16 light hours/8 darkness hours, the mycelial growth inhibition percentage (MGIP) was calculated by assessing the measurement of the diameter of the fungus growth around the disc to calculate the mycelial growth inhibition area. In this way, the mycelial growth inhibition percentage that was compared to the corresponding controls after 48 hours of incubation was determined.

## Results

**[0034]** Table 1 shows that all the compounds derived from formulas 1 to 8 in their free state affect the mycelial growth of the pathogenic fungus *B. cinerea* in vitro between 51-97% at concentrations ranging from 150 to 250 ppm, as compared to the negative control after 48 hours of incubation. Compound 6 was found to be the most active by inhibiting

improves its biological activity by 93% at 50 ppm, whereas the compounds derived from formulas 2, 6 to 8 do not produce any inhibiting effect on the mycelium of the pathogen as they are encapsulated in caprolactone micelles.

**[0036]** In the case of the mono-coupled compound of formula 1 (previously described in the literature) its activity at lower concentrations is also slightly improved when encapsulated in polycaprolactone as compared to the control, unlike the mono-coupled compound of formula 3 (previously described in the literature) which was inactive against the mycelium of the fungus when encapsulated in polycaprolactone.

**[0037]** The results indicate that the biological activity of mycelial inhibition is improved when the compounds are encapsulated in polymeric micelles; depending on its structure, it may be inferred that for the di-coupled compounds derived from formulas 2, 4, 6 and 8 it is more effective to use a polymeric Pluronic F-127 micelle, since its activity becomes equal to the activity of the positive control.

**[0038]** In the case of mono-coupled compounds derived from formulas 1, 3, 5 and 7 their effectiveness will depend on their structural composition when encapsulated in a micelle of polycaprolactone diblock.

TABLE 1

Mycelial inhibition percentage of the compounds of formula 1-8 both in free system and in encapsulated form in Polycaprolactone and Pluronic F-127 at different concentrations in vitro.									
Mycelial inhibition percentage of <i>Botrytis cinerea</i> in vitro (%)									
Compound	Free compound			Compounds encapsulated in Polycaprolactone (Diblock)			Compounds encapsulated in Pluronic F-127 (Triblock)		
	50 ppm	150 ppm	250 ppm	50 ppm	150 ppm	250 ppm	50 ppm	150 ppm	250 ppm
Formula 1	0	25	75	37	61	73	—	—	—
Formula 2	25	61	93	16	36	37	85	91	96
Formula 3	37	65	82	9	32	42	—	—	—
Formula 4	12	56	83	93	94	97	82	92	96
Formula 5	24	55	78	16	39	80	—	—	—
Formula 6	5	69	97	10	31	50	93	99	99
Formula 7	8	51	82	32	35	59	—	—	—
Formula 8	11	54	85	0	36	36	93	99	99
C- (negative control)	0	0	0	0	0	0	0	0	0
C+ (positive control)	87	94	94	93	99	99	98	99	99

(—): Not tested.

mycelial growth by 97% at 250 ppm. The inhibition result is directly dependent on the concentration in which it is applied against the pathogen.

**[0035]** The inhibitory capacity is increased when the compounds are encapsulated in polymeric micelles of different origin. In the case of compounds derived from formulas 2, 4, 6 and 8 the biological activity in vitro increases when encapsulated in micelles formed by Pluronic F-127 to obtain a percentage of mycelial inhibition at 50 ppm between 81-99% as compared to the negative control after 72 hours. However, when these compounds are encapsulated in polycaprolactone micelles, only the compound of formula 4

1- Synthesis process for obtaining compounds derived from linear geranylborcinols, characterized in that it comprises:

- preparing a solution by mixing orcinol (1.22 g, 9.8 mmol), with geraniol (1.51 g, 9.8 mmol) and acetonitrile (2 ml),
- adding the above solution to a solution of acetonitrile saturated in AgNO<sub>3</sub> (2 ml);
- stirring the solution at room temperature under a nitrogen atmosphere;
- slowly and directly injecting BF<sub>3</sub>Et<sub>2</sub>O (0.46 g, 3.2 mmol);
- stirring for 48 hours;

adding crushed ice (approximately 30 g) and abundant salt (NaCl);

filtering under vacuum;

extracting the organic phase with AcOEt (3×20 ml);

washing organic phase with a solution of NaHCO<sub>3</sub> (15 ml, 5%) and water (2×15 ml);

drying over anhydrous Na<sub>2</sub>SO<sub>4</sub>; and

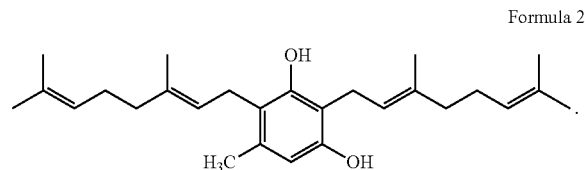
filtering and evaporating to dryness.

2- The synthesis process according to claim 1, wherein the reaction is performed in quadruplicate and the sum of the crude reaction product is purified by column chromatography (CC) using silica gel as the stationary phase and a mixture of ethyl acetate/hexane in increasing polarity gradient (0:20->6:14) as the mobile phase.

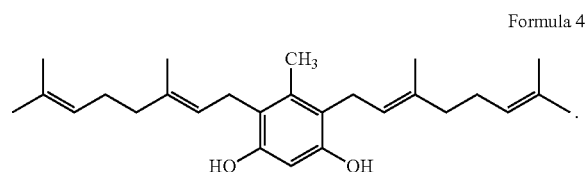
3. The synthesis process according to claim 2, wherein five fractions are obtained, where fraction I is a viscous oil dark yellow corresponding formula 2 compound; fraction II is a viscous yellow oil corresponding to formula 3 compound; the third fraction is a viscous dark yellow oil corresponding to formula 4 compound; the fourth fraction is a viscous dark yellow oil corresponding to a mixture of formula 1 and 4 compounds; the fifth fraction is a viscous yellow oil corresponding to formula 1 compound.

4. The synthesis process according to claim 3, wherein the yield of formula 2 compound is 9.9%; the yield of formula 3 compound is 12.5%; the yield of formula 4 compound is 6.1%; and the yield of formula 1 compound is 27.6%.

5. A compound derived from linear geranylgercinol, wherein it has the formula 2:



6. A compound derived from linear geranylgercinol, wherein it has the formula 4



7. Use of compounds derived from linear geranylgercinols, characterized in that they have an antifungal activity.

8. Use of the compounds according to claim 7, wherein the antifungal activity is against *Botrytis cinerea*.

\* \* \* \* \*