Synthesis of New Hydrated Geranylphenols and \textit{in Vitro} Antifungal Activity against \textit{Botrytis cinerea}

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Abstract: Geranylated hydroquinones and other geranylated compounds isolated from Aplydium species have shown interesting biological activities. This fact has prompted a number of studies where geranylated phenol derivatives have been synthesized in order to assay their bioactivities. In this work, we report the synthesis of a series of new hydrated geranylphenols using two different synthetic approaches and their inhibitory effects on the mycelial growth of \textit{Botrytis cinerea}. Five new hydrated geranylphenols were obtained by direct coupling reaction between geraniol and phenol in dioxane/water and using BF\textsubscript{3}·Et\textsubscript{2}O as the catalyst or by the reaction of a geranylated phenol with BF\textsubscript{3}·Et\textsubscript{2}O. Two new geranylated quinones were also obtained. The synthesis and structural elucidation of all new compounds is presented. All hydrated geranylphenols efficiently inhibit the mycelial growth of \textit{B. cinerea}. Their activity is higher than that observed for non-hydrated compounds. These results indicate that structural modification on the geranyl chain brings about an enhancement of the inhibition effect of geranylated phenol derivatives.

Keywords: geranylated phenol derivatives; hydrated geranyl; synthesis; structural elucidation; growth inhibition effect; \textit{Botrytis cinerea}; fungicide

1. Introduction

The subgroups of linear geranylated quinones, geranylated hydroquinones and meroterpenes are represented by an important number of metabolites isolated from ascidians belonging to the genus Aplydium [1,2]. The first biologically active tunicate metabolites were 2-geranylhydroquinone (1) and 2-geranyl hydroquinone diacetate (2) (Figure 1), isolated from Aplydium sp. and \textit{Phacelia crenulata} [3,4] and \textit{Pyrola japonica} [5], respectively, and later found in many others Aplydium species. It has been shown that these compounds exhibit antitumoral activity [6]. Additionally, several linear quinones/hydroquinones carrying a geranyl type side chain (Compounds 1, 3–11; Figure 1) have been obtained from diverse Aplydium species [7–12]. Compound 1 and 2-geranylbenzoquinone (3) exhibit interesting and various biological activities [3,8,13–17], whereas Compounds 5 and 6 show antioxidant activities [8], and Compound 4 shows cytotoxicity activity against P-388 mouse lymphoma suspension culture [18]. Additionally, linear geranylmethoxyphenol/acetates derivatives isolated from \textit{Phacelia ixodes} [19] are cytotoxic, allergenic and insecticidal.

On the other hand, the anticancer properties, both \textit{in vitro} and \textit{in vivo}, of a group of prenylated quinones, \textit{i.e.}, 3-demethylubiquinone Q2 (9) and its synthetic analogs, were studied as a function of their molecular structure [20,21]. The results indicate that 9 and its derivatives are able to inhibit the
Thus, an increasing number of metabolites isolated from plants, hemisynthetic and synthetic products have been studied as an alternative to chemical fungicide [29,36–38]. Previous work has shown that the anti-fungal activity of geranylated phenols is mainly determined by the presence of the geranyl chain and substitution on the aromatic ring [29,31]. The latter is a facultative phytopathogenic fungus that attacks the flowers, fruits, leaves and stems of more than 200 plant species [33]. In Chile, there is a high incidence of this fungus, and its control by commercial fungicides (dicarboximides and benzimidazoles) is becoming more ineffective due to the appearance of highly resistant strains [34,35]. Thus, an increasing number of metabolites isolated from plants, hemisynthetic and synthetic products have been studied as an alternative to chemical fungicide [29,36–38].

Therefore, in this research, a study of the inhibitory effects on the mycelial growth of plant pathogen B. cinerea of geranylated phenols (Compounds 14–18), geranylated quinones (Compounds 14–18), methoxyconidiol (12) and conitriol (13), have been isolated from Aplidium aff. densum [22] and Aplidium conicum [9], respectively. Methoxyconidiol and its methoxy derivative were synthesized, and their biological activities on human cancer cell lines and sea urchin embryos were assessed [23]. A detailed description of the isolation and biological activities of these and other structures of natural prenylquinones, hydroquinones and meroterpenes can be found in [24,25].

Thus, the interesting biological activity shown by 1 and other prenyl derivatives [17] (see Figure 1) has prompted us to undertake the synthesis of a significant number of linear geranylated phenols, including Compounds 1–3 and some geranylated methoxyphenyl/acetate analogs [26–32], in order to evaluate the in vitro cytotoxic activity on some cancer lines and the inhibitory effects on the mycelial growth of plant pathogen Botrytis cinerea [29–31]. The latter is a facultative phytopathogenic fungus that attacks the flowers, fruits, leaves and stems of more than 200 plant species [33]. In Chile, there is a high incidence of this fungus, and its control by commercial fungicides (dicarboximides and benzimidazoles) is becoming more ineffective due to the appearance of highly resistant strains [34,35].

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19–21) and hydrated geranylphenols derivatives (Compounds 22–26) (see Figure 2) is reported. The synthesis and structural elucidation of the new compounds (14–18, 20, 22–26) is also presented.

**Figure 2.** Chemical structures of geranylated phenols (14–18) geranylated quinones (19–21) and hydrated geranylphenols derivatives (22–26) that have been studied in this work.

### 2. Results and Discussion

#### 2.1. Synthesis

Linear geranylated phenols/methoxyphenols have been synthesized by direct coupling of geraniol with the respective phenol or methoxyphenols. This reaction has been studied for many authors, because it is directly related to the synthesis of biologically-active phenolic terpenoids [11,14,20,26–30]. The coupling is commonly carried out in strong mineral acids or aprotic solvents with Lewis acids, e.g., BF$_3$·Et$_2$O, in dioxane for the synthesis of tocopherols and geranyl and farnesyl analogs of the ubiquinones, p-toluenesulfonic acid in CH$_2$Cl$_2$ for the synthesis of cannabigerol and related marihuana constituents [39]. Alternatively, BF$_3$·Et$_2$O/AgNO$_3$ has been used as a catalyst and acetonitrile as a solvent [29,30]. In this work, Compounds 1, 3, 14, 15, 17 and 19 were synthesized through this reaction, using dioxane as the solvent, BF$_3$·Et$_2$O as the catalyst and in the presence or absence of a nitrogen atmosphere.

Direct coupling between o-cresol and p-cresol with geraniol under a nitrogen atmosphere leads to Compounds 14 and 15 with 3.1% and 12% yields, respectively (Scheme 1).
Scheme 1. Synthesis of Compounds 14, 15 and 16.

Following the same synthetic procedure, Compound 17 is obtained as a unique product by coupling between 2-metoxyhydroquinone and geraniol with 5.9% yield (Scheme 2).

Scheme 2. Synthesis of Compounds 17 and 18.

Standard acetylation (Ac$_2$O/CH$_2$Cl$_2$/DMAP) of 15 and 17 gives the acetylated derivatives 16 and 18 with 94.8% and 98% yields, respectively.

In the search for a synthetic pathway to obtain hydrated geranlyphenols, we have attempted two different approaches. In the first one, we explore the possibility of obtaining both geranylated quinones and hydrated geranlyphenols in a one-pot synthesis. It has been reported that some hydrated geranlyorcinols have been obtained as minor products in the coupling reaction of orcinol and geraniol in the presence of 1% aqueous oxalic acid at 80 °C [40]. Therefore, in this approach, the coupling reaction is carried out under air using dioxane as the solvent, BF$_3$·Et$_2$O as the catalyst and small amounts of added water. Interestingly, the obtained products depend on the chemical nature of the reacting phenol. Thus, coupling between geraniol and 1,4-hydroquinone leads to monogeranylated hydroquinone 1 and quinone 2, as well as digeranylated quinone 19 (Scheme 3); whereas, coupling between 2-metoxyhydroquinine and geraniol gives the disubstituted quinone 20 as the only product (Scheme 4). Finally, hydrated geranlyphenols 22 and 23 were obtained in the coupling reaction between o-cresol and geraniol (Scheme 5).
In this reaction, Compounds 1, 3 and 19 were obtained with 28.0%, 7.6% and 1.9% yields, respectively. When this coupling is carried out in the presence of a nitrogen atmosphere and with no added water, Compound 1 is obtained as the exclusive product [26]. Recently, this reaction has been performed at higher temperatures using aluminum phenoxide as the catalyst, and a completely different pattern of products has been reported. Compound 1 and a mixture of digeranylated quinones (19 and di-ortho-geranylated quinone) were obtained with 40% and 27% yields, respectively, but Compound 3 was not identified [41].

On the other hand, a methoxy substitution in the hydroquinone induces a complete change in the product distribution, i.e., Compound 20 is obtained with 4.1% yield.

It is worth mentioning that geranylated quinones (3, 19, 20) are formed only by coupling geraniol with 1,4-dihydroxybenzene systems. Probably, the oxidation to 1,4-quinone is enhanced by the redox properties of hydroquinone compounds.

Finally, in the coupling of geraniol with o-cresol, hydrated Compounds 22 and 23 were obtained with 9.0% and 10.5% yields, respectively.

The formation of Compounds 22 and 23 may be explained by the proposed mechanism depicted in Scheme 6.

In the first step, an allylic carbocation is formed by the reaction of BF₃·Et₂O with geraniol, which is then coupled with phenol via Electrophilic Aromatic Substitution (EArS) (Step 2). In presence of water, the adduct BF₃·H₂O is presumably formed by nucleophilic displacement of an ether molecule by H₂O (Step 3). Subsequently, this adduct reacts with the geranyl chain by a Markovnikov-type addition, forming a stable tertiary carbocation, which is then hydrated by reaction with a water molecule.
(Steps 3 and 4). Finally, the remaining olefinic bond is hydrated by BF$_3$·H$_2$O, and a completely hydrated geranyl chain is obtained (Step 5). It is worth mentioning that water is added 24 h after the coupling reaction has been started. This means that Step 3 begins when most of the geranylphenol has already been formed.

Scheme 6. Proposed mechanism for the formation of Compounds 22 and 23.

Based on this result, our second approach consists of the direct hydration of the side chain by the reaction of a geranylated phenol with a Lewis acid (BF$_3$·Et$_2$O) in dioxane and in the presence of water. Compound 17 was submitted to this reaction, and compounds 21, 24–26 were obtained with 11.1%, 10.7%, 24% and 18.9% yields, respectively (Scheme 7).

Compounds 24 and 26 may be formed through Steps 3–5 of the mechanism proposed in Scheme 6. However, in this reaction, Compound 25 is formed by cyclization of the tertiary carbocation formed in Step 3, the formation of tertiary carbocation in the C-7' position of geranyl chain, 6-endo-trig cyclization from the C2'C3' double bond and hydration by the subsequent nucleophilic attack of water on the tertiary carbocation in the C-3' position (Scheme 8).

The carbocation intermediates appearing in Schemes 6 and 8 have been proposed for coupling of phenol with geraniol and various reactions of geraniol in acidic aqueous solution [39,40].

Compounds 14–18, 20, 22–26 are new, and their structural characterization is described in the next section.
Further correlations at 6.92 ppm (1H, J = 8.7 Hz, H-3, meta-coupling of H-3 with H-5 was not detected) and two doublet signals at 6.88 ppm (1H, J = 8.7 Hz, H-5); and doublet at 6.69 ppm (1H, J = 8.0, H-6). The position of the geranyl chain on the aromatic ring has been established by two-dimensional (2D) Heteronuclear Multiple Bond Correlation (HMBC) correlations. In this spectrum, a 2JH-C coupling of H-1 with C-4 (δC = 133.9 ppm) and C-2′ (δC = 123.6 ppm) and a 3JH-C coupling between the signals of C-1, C-3, C-3′ and C-5 at δC = 130.9, 135.7 and 126.7 ppm, respectively, were observed. Further correlations at 3JH-C between the CH₃-Ar group (δH = 2.23 ppm) with C-1 and C-3 at δC = 151.8 and 130.9 ppm, respectively, were observed (Figure 3a). On the other hand, the ¹H-NMR spectrum of Compound 15 shows a singlet signal at 6.93 ppm (1H, H-3, meta-coupling of H-3 with H-5 was not detected) and two doublet signals at 6.92 ppm (1H, J = 8.7 Hz, H-5) and 6.73 (1H, J = 8.7 Hz, H-6). Additionally, a signal appearing at 5.07 ppm (s, 1H) was assigned to the OH group. The aromatic substitution pattern shows unequivocally
that the geranyl chain is attached to the ortho position at hydroxyl groups. The position of the geranyl chain on the aromatic ring has been confirmed by 2D HMBC correlations. In this spectrum, the signal at $\delta_H = 3.35$ ppm assigned to H-1' (2H d, $J = 7.2$ Hz) shows $^{3}J_{H-C}$ coupling with C-1 ($\delta_C = 152.1$), C-3 ($\delta_C = 127.8$) and C-3' ($\delta_C = 138.1$ ppm) and $^{2}J_{H-C}$ coupling with C-2 and C-2' ($\delta_C = 126.6$ and 121.8 ppm, respectively; Figure 3b) Additionally, the signal at $\delta_H = 3.35$ ppm (H-1') showed spatial correlations with the signals at $\delta_H = 6.93$, 5.07 and 1.79 ppm, assigned to H-3, OH and CH$_3$-C3', respectively; while the signal at $\delta_H = 2.23$ ppm (s, CH$_3$-Ar) showed spatial correlations with H-3 and H-5 (Figure 3c).

Figure 3. Main observed correlations: 2D Heteronuclear Multiple Bond Correlation (HMBC), Compound 14 (a) and Compound 15 (b); 1D Nuclear Overhauser Effect Spectroscopy (NOESY) Compound 15 (c).

In the $^1$H-NMR spectrum of the acetylated derivative 16, a singlet at $\delta_H = 2.29$ ppm (3H, CH$_3$CO) was observed. Additionally, in the $^{13}$C NMR spectrum, the signals appearing at $\delta_C = 169.6$ (C=O) and 20.8 (CH$_3$) ppm confirmed the presence of monoacetylated derivative 16.

Compound 17: In the $^1$H-NMR spectrum, a pattern characteristic of aromatic tetra-substitution, i.e., two singlet signals at 6.67 (1H, H-3) and 6.43 (1H, H-6), was observed. The position of the geranyl chain on the aromatic ring was established by two-dimensional (2D) HMBC correlations. In this spectrum, a $^{3}J_{H-C}$ coupling of H-1' with C-2 ($\delta_C = 118.5$ ppm) and C-2' ($\delta_C = 121.8$ ppm) and a $^{3}J_{H-C}$ coupling between the signals of C-1, C-3 and C-3' at $\delta_C = 147.6$, 115.3 and 139.2 ppm, respectively, were observed. In addition, a correlation at $^{3}J_{H-C}$ between the CH$_3$O group ($\delta_H = 3.83$ ppm) and C-5 ($\delta_C = 145.4$ ppm) and correlations between $^{3}J_{H-C}$ and $^{3}J_{H-C}$ of the OH-C4 group ($\delta_H = 5.15$ ppm) with C-3 ($\delta_C = 115.3$ ppm) were also observed (Figure 4).

Figure 4. Major 2D HMBC observed correlations for Compound 17.

In the $^1$H-NMR spectrum of the acetylated derivative 18, two singlet signals at $\delta_H = 2.29$ and 2.28 ppm (each 3H, CH$_3$CO) were observed. Additionally, in the $^{13}$C NMR spectrum, the signals appearing at $\delta = 169.2$ (COCH$_3$-C4), 168.9 (COCH$_3$-C1) ppm and $\delta = 20.8$ (CH$_3$COO-C1) and 20.6 (CH$_3$COO-C4) ppm, confirmed the presence of diacetylated derivative 18.

Compound 19: The symmetrical molecular structure was confirmed by the substitution pattern in the olefin zone and by the intensity of integrated signals of hydrogen atoms in quinone and olefinic portion. For instance, the signal at $\delta_H = 6.70$ ppm (s, 2H, H-3 and H-6) indicates the presence of two identical H. In a previous report, two different signals were found and assigned to these H ($\delta_H = 6.48$ ppm, 1H, s, H-13 and $\delta_H = 6.52$ ppm, 1H, s, H-16). A detailed assignment of $^1$H-NMR signals is given in the experimental part, and the corresponding spectrum is shown in the Supplementary Material. Additionally, spatial correlations (NOE) were observed for the signals at $\delta_H = 6.70$ ppm and at $\delta_H = 3.21$ ppm (4H, d, $J = 6.8$, H-1') and for the latter and the signal at $\delta_H = 1.73$ ppm, assigned to
CH3-C3' (Figure 5a). Finally, in the 13C NMR spectrum, only one signal at δC = 187.6 ppm (C-1 and C-4) of the carbonyl group was observed, confirming the symmetrical structure of Compound 19.

![Figure 5. Main observed correlations: 1D NOE Compound 19 (a), 2D HMBC Compound 20 (b).](image)

Compound 20: The presence of double geranyl chain substitution on the quinone nucleus was confirmed by the observation of two doublet signals in the 1H-NMR spectrum at δH = 3.15 ppm (2H, J = 7.4 Hz) and 3.12 ppm (2H, J = 7.0 Hz), which were assigned to the hydrogens H-1' and H-2", respectively. Additionally, the presence of only one hydrogen at δH = 6.32 ppm (1H, s, H-6) demonstrates the degree of tetra-substitution on the quinone moiety. Differentiation between geranyl chains was established by the HMBC correlations observed for H-1' at 3JH-C with C-4 (δC = 187.9 ppm; C=O) and H-1' at 3JH-C with C-2 (δC = 155.1 ppm; C-OCH3) and at 2JH-C with C-3 (δC = 131.9 ppm) (Figure 5b). Similarly, the signal of H-1" showed 3JH-C correlations with C-4 (δC = 187.9 ppm; C=O) and C-6 (δC = 130.5 ppm) and 2JH-C with C-5 (δC = 148.3 ppm) (Figure 5b).

Compound 22: The 1H-NMR spectrum shows a pattern characteristic of aromatic tri-substitution, i.e., doublet signals at δH = 6.95 ppm (J = 7.3 Hz, 1H, H-4') and δH = 6.90 ppm (J = 7.4 Hz, 1H, H-6'), a doublet doublet signal at δH = 6.70 ppm (J = 7.3 and 7.4 Hz, 1H, H-5'). The position of the geranyl chain on the aromatic ring was established by two-dimensional (2D) HMBC correlations. In this spectrum, a 2JH-C coupling of H-8 (δH = 2.78-2.74, m, 2H) with C-1' (δC = 120.4 ppm) and a 3JH-C coupling between the signals of C-2' and C-6' at δC = 151.9 and 126.9 ppm, respectively, were observed. In addition, correlations at 3JH-C between the CH3-Ar group (δH = 2.16 ppm) with C-2' and C-4' at δC = 151.9 and 128.4 ppm, respectively, and a 2JH-C with C-3' (δC = 126.2) were observed (Figure 6a). The presence of two hydroxyl groups in the geranyl chain was confirmed by the observation of two tertiary carbonylic signals at δC = 75.8 and 71.0 ppm in the 13C NMR spectrum. These were assigned to carbons C-6 and C-2, respectively, by two-dimensional (2D) HMBC correlations. Thus, H-8 showed correlation at 3JH-C with carbonylic carbon at C-6 (δC = 75.8 ppm), whereas the methyl groups at δC = 29.2 ppm (CH3-1 and CH3-C2) showed correlations at 2JH-C with C-2 (δC = 71.0 ppm) (Figure 6a).

![Figure 6. Main 2D HMBC observed correlations for: (a) Compound 22; (b) Compound 23.](image)

Compound 23: A similar analysis was conducted to elucidate the structure of this compound. The 1H-NMR spectrum shows a pattern characteristic of aromatic tri-substitution, i.e., a singlet signal at δH = 6.95 ppm (1H, H-2', meta-coupling of H-2' with H-6' was not detected), a doublet signal at δH = 6.90 ppm (J = 8.1 Hz, 1H, H-6') and a doublet signal at δH = 6.68 ppm (J = 8.1 Hz, 1H, H-5'). The position of
the geranyl chain on the aromatic ring was established by two-dimensional (2D) HMBC correlations. In this spectrum, a $^{3}$H-C coupling of H-8 ($\delta_H = 2.65$-$2.52$, m, 2H) with C-1' ($\delta_C = 135.5$ ppm) and a $^{3}$H-C coupling between the signals of C-2' and C-6 at $\delta_C = 130.9$ and 126.7 ppm, respectively, were observed. In addition, correlations at $^{3}$H-C between the CH$_3$-Ar group ($\delta_H = 2.22$ ppm) with C-2' and C-4' at $\delta_C = 130.9$ and 151.6 ppm, respectively, and a $^{3}$H-C with C-3' ($\delta_C = 123.4$) were observed (Figure 6b). The presence of two hydroxyl groups in the geranyl chain was confirmed by the observation of two tertiary carbinolic signals at $\delta_C = 72.9$ and 71.3 ppm in the $^{13}$C NMR spectrum. These signals were assigned to carbons C-6 and C-2, respectively, by two-dimensional (2D) HMBC correlations. Thus, the CH$_3$-C6 group showed correlation at $^{2}$H-C with carbinolic carbon at C-6 ($\delta_C = 72.9$ ppm), while the methyl groups at $\delta_C = 31.2$ and 29.9 ppm (CH$_3$-1 and CH$_3$-C2, respectively) showed correlations at $^{2}$H-C with C-2 ($\delta_C = 71.3$ ppm) (Figure 6b).

Because Compound 24 was obtained from 17 by the hydration reaction of it, the aromatic substitution pattern was maintained for Compounds 24 and 25. Thus, in Compound 24, the presence of two hydroxyl groups in the geranyl chain was confirmed by the observation of two tertiary carbinolic signals at $\delta_C = 75.8$ and 70.9 ppm in the $^{13}$C NMR spectrum. These signals were assigned to carbons C-3' and C-7', respectively, by two-dimensional (2D) HMBC correlations. Thus, the CH$_3$-C7' and CH$_3$-8' groups showed correlation at $^{3}$H-C with carbinolic carbon at C-7' ($\delta_C = 70.9$ ppm), and therefore, the signal at $\delta_C = 75.8$ ppm was unequivocally assigned to C-3' (Figure 7a); while for Compound 25, the methylene group (at $\delta_C = 22.7$ ppm, assigned as C-7') showed a correlation at $^{3}$H-C with C-2 ($\delta_C = 114.2$ ppm) and C-1' ($\delta_C = 48.3$ ppm) and $^{3}$H-C with a tertiary carbinolic carbon at $\delta_C = 76.7$ ppm assigned to C-2'. Additionally, the CH$_3$-C2' group at $\delta_H = 1.20$ ppm (3H, s) showed coupling at $^{2}$H-C with C-2' and $^{3}$H-C with tertiary C-1' ($\delta_C = 48.3$ ppm) (Figure 7b) Thus, the cyclohexane structure is confirmed for geranyl chain. Finally, mono-hydroxylation in the geranyl chain for Compound 26 was mainly established by $^{13}$C NMR data and 2D HMBC correlations. Only one signal of carbinolic carbon at $\delta_C = 70.9$ ppm in the $^{13}$C NMR spectrum was observed, and the methyl groups at $\delta_H = 1.22$ (6H, s, CH$_3$-C7' and H-8') showed $^{3}$H-C correlations with this carbon (C-7', $\delta_C = 70.9$ ppm) (Figure 7c). In addition, these methyl groups showed $^{3}$H-C correlations with C-6' ($\delta_C = 43.3$ ppm) (Figure 7c).

![Figure 7. Major 2D HMBC observed correlations for Compounds 24 (a), 25 (b) and 26 (c).](image)

2.3. In Vitro Antifungal Activity against B. cinerea.

All studied compounds (14–26) were tested for in vitro antifungal activity on the mycelial growth of B. cinerea strain GM7 using the agar radial assay with Potato Dextrose Agar (PDA). Figure 8 shows an assay where the B. cinerea mycelium grows in medium containing only PDA and 1% ethanol (Figure 8a, negative control), Captan at 250 ppm (Figure 8b, used in this study as a positive control) and two different concentrations of Compound 26 (Figure 8c, 150 ppm; Figure 8d, 250 ppm).
The inhibition of mycelial growth is evaluated by measuring colony diameters in the presence and absence of the tested compounds. The results, expressed as the percentage of inhibition, are summarized in Table 1.

### Table 1. Percentage of inhibition of geranylated phenols (14–18), geranylated quinones (19–21) and hydrated geranylated phenols (22–26) on the mycelial growth of *B. cinerea* strain GM7 at 72 h *in vitro*.

| Compounds | Percentage of Inhibition on Mycelial Growth of *B. cinerea* *in Vitro* (%) |
|-----------|---------------------------------------------------------------|
|           | 50 mg/L | 150 mg/L | 250 mg/L |
| 14        | 0 ± 0    | 0 ± 0    | 0 ± 0    |
| 15        | 9 ± 4    | 6 ± 3    | 8 ± 5    |
| 16        | 0 ± 0    | 0 ± 0    | 9 ± 0    |
| 17        | 49 ± 2   | 56 ± 2   | 56 ± 0   |
| 18        | 36 ± 3   | 48 ± 3   | 52 ± 2   |
| 19        | 30 ± 2   | 51 ± 1   | 69 ± 1   |
| 20        | 43 ± 8   | 58 ± 8   | 73 ± 8   |
| 21        | 36 ± 0   | 64 ± 0   | 75 ± 0   |
| 22        | 0 ± 0    | 30 ± 2   | 53 ± 3   |
| 23        | 0 ± 0    | 0 ± 0    | 28 ± 7   |
| 24        | 36 ± 0   | 66 ± 4   | 67 ± 5   |
| 25        | 50 ± 6   | 81 ± 5   | 90 ± 1   |
| 26        | 81 ± 0   | 91 ± 0   | 94 ± 0   |
| C−        | 0 ± 0    | 0 ± 0    | 0 ± 0    |
| C+        | 94 ± 5   | 94 ± 0   | 99 ± 0   |

The percentage of inhibition of mycelial growth is based on colony diameter measurements after 72 h of incubation. Each point represents the mean of at least three independent experiments ± the standard deviation. 

1 C− refers to the negative control; and 2 C+ refers to the positive control (Captan).

The data indicate that geranylated derivatives of o- and p-cresol (14–16) have no effect on the mycelial growth of *B. cinerea*. However, the methoxy derivatives of geranylated p-cresol (17 and 18) exhibit a significant increase in the inhibitory activity. This result is in line with previous work where a family of methoxy geranylated derivatives was studied [30].

On the other hand, geranylated quinones (19–21) show an important activity (greater than 50% at the higher tested concentrations) that is independent of the number of geranyl chains. In the case of geranylated phenols, it was found that antifungal activity decreases with the increasing number of prenyl chains [29,30].

All hydrated geranylated phenols exhibit activities on mycelial growth inhibition that are in the range 30%–95% at 250 ppm. A comparison of the percentages of inhibition measured for geranylated phenol and their respective hydrated compound, i.e., 14 with 23, 17 with 24 and 26, shows that the latter are more active than the parent compound. This effect is larger for compounds carrying only one hydroxyl group in the side chain (25 and 26) than for completely hydrated compounds (22–24). In
other words, incorporation of hydroxyl groups in the side chain, by hydration of the geranyl chain, brings about an enhancing effect on the antifungal activity. Previous work was focused on the effect of substitution in the phenol ring, and the results suggested that the inhibition effect depends mainly on the presence of the prenyl chain [29,31]. In this context, these results are important because, as far as we know, this is the first report of the effect of the side chain structure on the fungicide activity of geranylated compounds.

Finally, it is interesting to stress that Compound 26 stands out as being as active as Captan, a fungicide that is currently used for infection control in some crops.

3. Experimental Section

3.1. General

Chemicals were obtained from Merck (Darmstadt, Germany) or Aldrich (St. Louis, MO, USA) and were used without further purification. A detailed description of conditions used to register Fourier transform infrared (FT-IR) spectra, high resolution mass spectra and 1H, 13C, 13C DEPT-135, selective gradients 1D 1H NOESY, gs-2D Heteronuclear Single Quantum Coherence (HSQC) and gs-2D HMBC spectra has been given elsewhere [30]. Silica gel (Merck 200–300 mesh) was used for Column Chromatography (C.C.) and silica gel plates HF_254 for thin layer chromatography (TLC). TLC spots were detected by heating after spraying with 25% H_2SO_4 in H_2O.

3.2. Synthesis

3.2.1. Coupling Reaction in Presence of Nitrogen

The coupling of geraniol and phenols was carried out using boric trifluoro etherate BF_3·Et_2O as the catalyst and dioxane as the solvent. Experimental details for a typical reaction have been given elsewhere [30].

(E)-4-(3,7-dimethylocta-2,6-dienyl)-2-methylphenol (14):

Coupling of o-cresol (1.0 g, 9.3 mmol) and geraniol (1.5 g, 9.7 mmol) was carried out in dioxane (20 mL) with BF_3·Et_2O (0.9 mL, 7.2 mmol) as the catalyst. Two fractions were obtained from the C.C. Fraction I: Compound 14 (64 mg, 3.1% yield) obtained as a yellow viscous oil; Fraction II: unreacted o-cresol (922 mg) that was recovered. Compound 14: IR (cm⁻¹) 3385, 2966, 2923, 2854, 1668, 1611, 1508, 1453, 1377, 1262, 1205, 1115, 772; 1H-NMR (CDCl₃, 400.1 MHz) δ 6.93 (1H, s, H-3), 6.88 (1H, d, J = 8.0, H-5), 6.69 (1H, d, J = 8.0, H-6), 5.32–5.29 (1H, m, H-2'), 5.11–5.09 (1H, m, H-6'), 4.55 (1H, s, OH), 3.25 (2H, d, J = 7.2, H-1'), 2.23 (3H, s, CH₃-C2), 2.12–2.08 (2H, m, H-5'), 2.06–2.04 (2H, m, H-4'), 1.69 (3H, s, CH₃-C3'), 1.68 (3H, s, H-8'), 1.60 (3H, s, CH₃-C7'); 13C NMR (CDCl₃, 100.6 MHz) δ 151.8 (C-1), 135.7 (C-3'), 133.9 (C-4), 131.4 (C-7'), 130.9 (C-3), 126.7 (C-5), 124.3 (C-6'), 126.3 (C-2'), 123.4 (C-2), 144.6 (C-4), 39.7 (C-4'), 33.3 (C-1'), 26.6 (C-5'), 25.7 (C-8'), 17.7 (CH₃-C7'), 16.1 (CH₃-C3'); MS m/z (%) M⁺ 244 (48.3), 201 (16.7), 175 (100), 160 (36.7), 147 (31.7), 133 (35.0), 121 (68.3), 106 (13.3), 91 (20.0), 69 (28.3), 41 (31.7).

(E)-2-(3,7-dimethylocta-2,6-dienyl)-4-methylphenol (15):

Coupling of p-cresol (1.02 g, 9.4 mmol) and geraniol (1.5 g, 9.7 mmol) was carried out in dioxane (20 mL) with BF_3·Et_2O (1.17 mL, 9.5 mmol) as the catalyst. Two fractions were obtained from the C.C. Fraction I: Compound 15 (264 mg, 12% yield) obtained as a yellow viscous oil; Fraction II: unreacted p-cresol (728 mg) that was recovered. Compound 15: IR (cm⁻¹) 3446, 2966, 2919, 2857, 1611, 1506, 1446, 1376, 1260, 1197, 1040, 924, 810; 1H-NMR (CDCl₃, 400.1 MHz) δ 6.93 (1H, s, H-3), 6.92 (1H, d, J = 8.7, H-5), 6.73 (1H, d, J = 8.7, H-6), 5.36–5.32 (1H, m, H-2'), 5.12–5.09 (1H, m, H-6'), 5.07 (1H, s, OH), 3.35 (2H, d, J = 7.2, H-1'), 2.28 (3H, s, CH₃-C4), 2.16–2.14 (2H, m, H-5'), 2.11–2.09 (2H, m, H-4'), 1.79 (3H, s, CH₃-C3'), 1.71 (3H, s, CH₃-C7'), 1.62 (3H, s, H-8'); 13C NMR (CDCl₃, 100.6 MHz) δ 152.1 (C-1), 138.1 (C-3'), 131.9 (C-7'), 130.5 (C-5), 129.8 (C-4), 127.8 (C-3), 126.6 (C-2), 123.8 (C-7'), 121.8 (C-2'), 115.6
Coupling of 2-methoxyhydroquinone (2.02 g, 14.4 mmol) and geraniol (2.36 mL, 13.2 mmol) was carried out in dioxane (20 mL) with BF$_3$·Et$_2$O (1.62 mL, 12.9 mg) as the catalyst. Two fractions were obtained from the C.C. Fraction I: Compound 17 (233 mg, 5.9% yield) was obtained as a brown viscous oil; Fraction II: unreacted 2-methoxyhydroquinone (1.76 g) that was recovered. Compound 17: IR (cm$^{-1}$) 3420, 2965, 2924, 2852, 1600, 1520, 1446, 1196, 1105, 835; $^1$H-NMR (CDCl$_3$, 400.1 MHz) $\delta$ 6.67 (1H, s, H-3), 6.43 (1H, s, H-6), 5.28 (1H, t, $J = 7.2$ Hz, H-2’), 5.15 (1H, bs, OH-C4), 5.06 (1H, t, $J = 5.5$ Hz, H-6’), 4.87 (1H, bs, OH-C1), 3.83 (3H, s, CH$_3$O), 3.26 (2H, d, $J = 7.2$ Hz, H-1’), 2.12–2.10 (2H, m, H-5), 2.08–2.05 (2H, m, H-4’), 1.76 (3H, s, CH$_3$-C3’), 1.69 (3H, s, H-8’), 1.60 (3H, s, CH$_3$-C7’); $^{13}$C NMR (CDCl$_3$, 100.6 MHz) $\delta$ 147.6 (C-1), 145.4 (C-5), 139.2 (C-3), 138.6 (C-4), 132.1 (C-7’), 123.8 (C-6’), 121.8 (C-2’), 118.5 (C-2), 115.3 (C-3), 100.4 (C-6), 56.1 (CH$_3$O-C5), 39.7 (C-4’), 29.4 (C-1’), 26.4 (C-5’), 25.7 (C-8’), 17.7 (CH$_3$-C7’), 16.2 (CH$_3$-C3’). MS m/z (%): 276 (39.5: M$^+$), 191 (21), 175 (9.9), 153 (100: M$^+$-123 (C$_9$H$_{15}$)), 91 (4.9), 69 (16), 41 (16).

3.2.2. Coupling Reaction in the Absence of Nitrogen and with Added Water

The main difference in the experimental procedure of this reaction is that, after the addition and stirring were completed, 5 mL of H$_2$O were added, and the stirring was continued for another 24 h. The crude was chromatographed on silica gel with petroleum ether/EtOAc mixtures of increasing polarity (19:8:0.2 → 8:0:12.0).

2-Geranylhydroquinone (1), 2-geranylquinone (3) and 2,5-bisgeranylquinone (19):

Coupling of 1,4-hydroquinone (1.01 g, 9.2 mmol) and geraniol (1.35 g, 5.5 mmol) was carried out in dioxane (30 mL) with BF$_3$·Et$_2$O (0.46 g, 3.2 mmol) as the catalyst. Three fractions were obtained from the C.C. Fraction I: Compound 19 (42 mg, 1.9% yield) obtained as a brown viscous oil. Compound 19: $^1$H-NMR (CDCl$_3$, 400.1 MHz) $\delta$ 6.70 (2H, s, H-3 and H-6), 5.03 (2H, t, $J = 6.8$ Hz, H-2’), 4.94 (2H, t, $J = 6.3$ Hz, H-6’), 3.21 (4H, d, $J = 6.8$ Hz, H-1’), 2.07–2.02 (4H, m, H-5’), 1.98–1.95 (4H, m, H-4’), 1.73 (6H, s, CH$_3$-C3’), 1.68 (6H, s, H-8’), 1.57 (6H, s, CH$_3$-C7’); $^{13}$C NMR (CDCl$_3$, 100.6 MHz) $\delta$ 187.6 (C-1 and C-4), 143.6 (C-2 and C-5), 137.5 (C-3’ and C-3”), 136.2 (C-3 and C-6), 131.5 (C-7’ and C-7”), 123.9 (C-6’ and C-6”), 119.5 (C-2’ and C-2”), 39.7 (C-4’ and C-4”), 26.5 (C-1’ and C-1”), 25.7 (C-5’ and C-5”), 25.3 (C-8’ and C-8”), 17.7 (CH$_3$-C7’ and CH$_3$-C7”), 16.4 (CH$_3$-C3’ and CH$_3$-C3”). Fraction II: Compound 3 (166 mg, 7.6% yield) obtained as a brown viscous oil. Fraction III: Compound 1 (616 mg, 28% yield) obtained as a brown viscous oil. The spectroscopic data (IR, MS and NMR) for 1 and 3 were consistent with those previously reported [26].

3,5-Bis(E)-3,7-dimethylocta-2,6-dienyl)-2-methoxy cyclohexa-2,5-diene-1,4-dione (20):

Coupling of 2-methoxyhydroquinone (500 mg, 3.6 mmol) and geraniol (0.65 mL, 3.6 mmol) was carried out in dioxane (20 mL) with BF$_3$·Et$_2$O (0.46 g, 3.2 mmol) as the catalyst. Two fractions were obtained from the C.C. Fraction I: Compound 20 (60 mg, 4.1% yield) was obtained as a brown viscous oil; Fraction II: unreacted 2-methoxyhydroquinone (419 mg) that was recovered. Compound 20: IR (cm$^{-1}$) 2966, 2924, 2854, 1649, 1602, 1446, 1376, 1323, 1207, 1161, 1121, 954, 887; $^1$H-NMR (CDCl$_3$, 400.1 MHz) $\delta$ 6.32 (1H, s, H-6), 5.15–5.13 (1H, m, H-2’), 5.11–5.08 (1H, m, H-6’), 5.07–5.04 (1H, m, H-2”), 5.03–5.01 (1H, m, H-6”), 3.99 (3H, s, CH$_3$O-C2’), 3.15 (2H, d, $J = 7.4$ Hz, H-1’), 3.12 (2H, d, $J = 7.0$ Hz, H-1”), 2.11–2.09” (2H, m, H-5’), 2.07–2.05 (2H, m, H-4’), 2.04–2.02” (2H, m, H-5”), 1.97–1.95 (2H, m, H-4”), 1.73 (3H, s, CH$_3$-C3”), 1.69** (3H, s, H-8’), 1.67** (3H, s, H-8”), 1.64*** (3H, s, CH$_3$-C7”), 1.63 (3H, s, CH$_3$-C3”), 1.60*** (3H, s, CH$_3$-C7”), $^{13}$C NMR (CDCl$_3$, 100.6 MHz) $\delta$ 187.9 (C-4), 184.3 (C-1), 155.1 (C-2), 148.3 (C-5), 139.7 (C-3’), 136.9 (C-3’), 131.9 (C-3), 131.8* (C-7’), 131.4* (C-7”), 130.5 (C-6), 124.1
Coupling of o-cresol (1.0 g, 9.3 mmol) and geraniol (1.55 g; 10.0 mmol) was carried out in dioxane (20 mL) with BF$_3$·Et$_2$O (1.2 mL, 10.0 mmol) as the catalyst. Three fractions were obtained from the C.C. Fraction I: unreacted o-cresol (473 mg) that was recovered. Fraction II: Compound 22 (225 mg, 9.0% yield) obtained as a brown viscous oil. IR (cm$^{-1}$) 3375, 2968, 2866, 1595, 1467, 1376, 1264, 1220, 1152, 1192, 763; $^1$H-NMR (CDCl$_3$, 400.1 MHz) δ 6.95 (1H, d, J = 7.3 Hz, H-4$^\prime$), 6.90 (1H, d, J = 7.4 Hz, H-6$^\prime$), 6.70 (1H, dd, J = 7.4 and 7.3 Hz, H-5$^\prime$), 2.78–2.74 (2H, m, H-8), 2.16 (3H, s, CH$_3$-C$^3$), 1.85–1.73 (2H, m, H-7), 1.68–1.62 (2H, m, H-5), 1.56–1.52 (2H, m, H-4), 1.51–1.47 (2H, m, H-3), 1.29 (3H, s, CH$_3$-C$^6$). 13C NMR (CDCl$_3$, 100.6 MHz) δ 151.9 (C-2$^\prime$), 128.2 (C-4$^\prime$), 126.9 (C-6$^\prime$), 126.2 (C-3$^\prime$), 120.4 (C-1$^\prime$), 118.8 (C-5$^\prime$), 75.8 (C-6), 71.0 (C-2), 44.3 (C-3), 40.5 (C-5), 31.3 (C-7), 29.3 and 29.2 (C-1 and C-3-C$^2$), 24.2 (CH$_3$-C$^2$), 18.4 (C-4), 16.0 (CH$_3$-C$^3$). MS m/z (% M$^+$ 280 (< 1%), 262 (55.4), 244 (12.5), 229 (14.3), 214 (10.5), 197 (37.3), 173 (137.3), 161 (39.2), 121 (100; M$^+$-159 (C$_9$H$_7$O$_2$)), 109 (64.7), 91 (15.7), 69 (19.6), 43 (19.6).

3.2.3. Acetylation of Geranylated Phenols

Geranylated phenols were acetylated by following a described acetylation method [30].

(E)-2-(3,7-dimethylocta-2,6-dienyl)-4-methylphenylacetate (16):

Acetylation of Compound 15 (100 mg, 0.4 mmol) with Ac$_2$O (0.54 g, 5.3 mmol), DMAP (2.0 mg) and pyridine (1.0 mL) in dichloromethane (20 mL) gives Compound 16 as a viscous yellow oil (111.2 mg, 94.8% yield). Compound 16: IR (cm$^{-1}$) 2966, 2923, 1763, 1447, 1496, 1367, 1213, 1191, 1050, 1010, 901, 824; $^1$H-NMR (CDCl$_3$, 400.1 MHz) δ 7.03 (1H, s, H-3), 6.65 (1H, s, H-6), 5.20 (1H, t, J = 7.9 Hz, H-5), 5.13–5.09 (1H, m, H-6), 2.22 (3H, s, CH$_3$-C$^3$), 1.83–1.73 (1H, m, H-7), 1.71–1.65 (2H, m, H-3), 1.62–1.54 (1H, m, H-7), 1.55–1.49 (1H, m, H-5), 1.48–1.42 (2H, m, H-4), 1.41–1.36 (1H, m, H-5), 1.24 (9H, s, CH$_3$-C$^6$), 39.6 (C$_3$), 36.9 (C-4), 34.8 (C-5), 31.2 (C-1), 29.9 (CH$_3$-C$^2$), 29.3 (C-4), 27.7 (CH$_3$-C$^3$), 16.5 (C-3), 15.7 (CH$_3$-C$^3$); MS m/z (% M$^+$ 281 (2.0; M + 1), 262 (23.5), 244 (42.9), 229 (11.8), 201 (15.7), 187 (15.7), 173 (37.3), 161 (39.2), 121 (100; M$^+$-159 (C$_9$H$_7$O$_2$)), 109 (64.7), 91 (15.7), 69 (19.6), 43 (19.6).

(E)-2-(3,7-dimethylocta-2,6-dienyl)-5-methoxy-1,4-phenylene diacetate (18):

Reaction of Compound 17 (65 mg, 0.18 mmol) with Ac$_2$O (0.54 g, 5.3 mmol), DMAP (2.0 mg) and pyridine (1.0 mL) in dichloromethane (20 mL) gives Compound 18 as a viscous yellow oil (83 mg, 98% yield). Compound 18: IR (cm$^{-1}$) 2965, 2919, 2854, 1766, 1509, 1445, 1368, 1201, 1181, 1157, 1012, 906; $^1$H-NMR (CDCl$_3$, 400.1 MHz) δ 6.87 (1H, s, H-3), 6.65 (1H, s, H-6), 5.20 (1H, t, J = 7.2 Hz, H-2$^\prime$),
3.2.4. Reaction of Geranlylated Phenols with BF₃·Et₂O

(E)-2-(3,7-dimethylocta-2,6-dien-1-yl)-5-methoxy cyclohexa-2,5-diene-1,4-dione (21), 2-(3,7-dihydroxy-3,7-dimethyloctyl)-5-methoxy benzene-1,4-diol (24), 2-((2-hydroxy-2,6,6-trimethylcyclohexyl)methyl)-5-methoxy benzene-1,4-diol (25) and (E)-2-(7-hydroxy-3,7-dimethyloct-2-en-1-yl)-5-methoxy benzene-1,4-diol (26):

To a solution of Compound 17 (100 mg; 0.36 mmol) in dioxanne (30 mL) was slowly added dropwise BF₃·Et₂O (0.5 mL, 4.2 mmol) and H₂O (0.5 mL, 27.8 mmol) with stirring at room temperature and without a N₂ atmosphere. The crude of the reaction was washed, extracted and chromatographed on silica gel [30]. Five fractions were obtained. Fraction I: unleached Compound 17 (23 mg) that was recovered. Fraction II: Compound 24 (11 mg, 11.1% yield) obtained as a brown viscous oil. IR (cm⁻¹) 2927, 2853, 1674, 1648, 1603, 1454, 1375, 1207, 1174, 987; ¹H-NMR (CDCl₃, 400.1 MHz) δ 6.46 (1H, s, H-3), 5.94 (1H, s, H-6), 5.17–5.13 (1H, H, H-2'), 5.09–5.06 (1H, H, H-6), 3.82 (3H, s, CH₃-C(O)-C), 3.14 (2H, d, J = 7.0 Hz, H-1'), 2.12–2.08 (2H, m, H-5'), 2.07–2.04 (2H, H, H-5'), 1.69 (3H, s, H-8'), 1.61 (3H, s, CH₃-C(3')), 1.30 (3H, s, CH₃-C(5')); ¹³C NMR (CDCl₃, 100.6 MHz) δ 187.6 (C-1), 182.4 (C-4), 158.7 (C-5), 149.6 (C-2), 140.1 (C-3'), 131.9 (C-7'), 130.3 (C-3), 123.9 (C-6'), 117.8 (C-2'), 107.7 (C-6), 56.2 (CH₂-O(C)), 39.6 (C-5'), 27.3 (C-1'), 26.4 (C-4'), 25.7 (C-8'), 17.7 (CH₃-C(7')), 16.1 (CH₃-C(3')); MS m/z (%) 274 (8.5: M⁺), 259 (5.1), 191 (100: M⁺-83 (C₆H₁₁)), 176 (10.2), 148 (5.1), 91 (3.4), 69 (5.1), 41 (5.1). Fraction III: Compound 25 (25 mg, 24% yield) obtained as a brown viscous oil. IR (cm⁻¹) 355, 3455, 2959, 2927, 2854, 1629, 1509, 1446, 1376, 1278, 1198, 1154, 1121, 1062, 951, 864; ¹H-NMR (CDCl₃, 400.1 MHz) δ 6.61 (1H, s, H-3), 6.33 (1H, s, H-6), 5.12 (1H, s, H-2'), 3.81 (3H, s, CH₃-C(O)-C), 2.65–2.45 (2H, m, CH₂-C₂), 1.93–1.91 (1H, m, H-3'), 1.65–1.64 (1H, H, H-4'), 1.65–1.63 (1H, m, H-1'), 1.60–1.56 (1H, m, H-3'), 1.49–1.46 (1H, m, H-5'), 1.32–1.30 (1H, m, H-5'), 1.20 (3H, s, CH₃-C(2')), 0.98 (3H, s, CH₃-C(6')); ¹³C NMR (CDCl₃, 100.6 MHz) δ 146.2 (C-1), 145.3 (C-5), 138.9 (C-4), 114.3 (C-3), 114.2 (C-2), 100.3 (C-6), 76.7 (C-2'), 55.9 (CH₃-C(O)-C), 48.3 (C-1'), 41.5 (C-5'), 40.0 (C-3'), 33.4 (C-6'), 32.1 (CH₂-C(2)), 22.7 (CH₂-C), 20.7 (CH₃-C(9')), 19.8 (C-4'), 19.6 (CH₃-C(2')); MS m/z (%) 276 (54.9: M⁺-H₂O), 191 (8.2), 153 (100), 69 (8.5), 41 (7.3). Fraction IV: Compound 26 (20 mg, 18.9% yield) obtained as a brown viscous oil. IR (cm⁻¹) 3396, 2985, 2937, 1646, 1603, 1521, 1446, 1374, 1274, 1196, 1018, 867; ¹H-NMR (CDCl₃, 400.1 MHz) δ 6.67 (1H, s, H-3), 6.42 (1H, s, H-6), 5.29 (1H, m, H-2'), 3.82 (3H, s, CH₃-C(O)), 3.26 (2H, d, J = 7.1 Hz, H-1'), 2.07–2.03 (2H, m, H-4'), 1.76 (3H, s, CH₃-C(3')), 1.52–1.48 (2H, m, H-6'), 1.46–1.41 (2H, m, H-5'), 1.22 (6H, s, CH₃-C(9') and H-8'); ¹³C NMR (CDCl₃, 100.6 MHz) δ 147.4 (C-1), 145.4 (C-5), 139.3 (C-4), 138.3 (C-3'), 122.0 (C-2'), 118.5 (C-2), 115.3 (C-3), 100.4 (C-6), 70.9 (C-7'), 56.1 (CH₃-C(O)-C), 43.3 (C-6'), 39.9 (C-4'), 29.2 (CH₃-C(9') and C8'), 28.9 (C-1'), 22.5 (C-5'), 16.1 (CH₃-C(3')); MS m/z (%) 292 (8.9: M⁺-H₂O), 277 (3.6), 219 (1.8), 203 (8.9), 191 (100: M⁺-H₂-101 (C₆H₁₃O)), 176 (8.9), 148 (3.6), 91 (1.8), 69 (1.8), 43 (1.8). Fraction V: Compound 21 (12 mg, 10.7% yield) obtained as brown viscous oil. The spectroscopic data (IR, MS and NMR) were consistent with those previously reported [27].

3.3. In Vitro Effect of the Compounds on the Mycelial Growth of B. cinerea

The antifungal activities of all tested compounds were evaluated using the radial growth test at final concentrations of 50, 150 and 250 mg/L in PDA medium [37]. Captan was used as the positive control, whereas PDA medium containing 1% ethanol was considered as the negative control. The
percentages of inhibitions were determined following a standard method [42]. Experimental conditions have been detailed elsewhere [30].

4. Conclusions

Hydrated geranylphenols were synthesized by following two different synthetic pathways: direct coupling of geraniol with o-cresol in dioxane with added water and using BF$_3$·Et$_2$O as the catalyst; or by the reaction of a geranylated phenol with BF$_3$·Et$_2$O in dioxane and added water. Interestingly, the coupling of geraniol to hydroquinones gives completely different products.

On the other hand, the mycelial growth inhibition of hydrated geranylphenols is in the range of 30%–95% at 250 ppm. The percentages of inhibition induced by hydrated compounds (23 and 26) are higher than those produced by the respective geranylated phenol (14 and 17). The enhancement of the antifungal activity is larger for hydrated compounds carrying only one hydroxyl group in the side chain (25 and 26) than for completely hydrated compounds (22–24). It is worth stressing that the new Compound 26 exhibits antifungal activity similar to Captan, a common fungicide used to control B. cinerea. Finally, as far as we know, this is the first study relating the structure of the geranyl chain with the antifungal activity of geranylated phenols.

Supplementary Materials: Supplementary materials can be found at http://www.mdpi.com/1422-0067/17/6/840/s1.

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References

1. Zubia, E.; Ortega, M.J.; Salva, J. Natural products chemistry in marine ascidians of the genus Aplidium. Mini Rev. Org. Chem. 2005, 2, 389–399. [CrossRef]
2. Marialuisa, M.; Anna, A. Handbook of Marine Natural Products; Fattorusso, E., Gerwick, W.H., Tagliatalata-Scafati, O., Eds.; Springer Science + Business Media: New York, NY, USA, 2012.
3. Reynolds, G.; Rodriguez, E. Geranylhydroquinone: A contact allergen from trichomes of Phacelia crenulata. Phytochemistry 1979, 18, 1567–1568. [CrossRef]
4. Inouye, H.; Tokura, K.; Tohita, S. Uber die inhaltsstoffe von Pirolaceen, XV zur struktur des pirolatins. Chem. Ber. 1968, 101, 4057–4065. (In German) [CrossRef] [PubMed]
5. Reynolds, G.; Epstein, W.L.; Terry, D.; Rodriguez, E. A potent contact allergen of Phacelia (Hydrophyllaceae). Contact Dermat. 1980, 6, 272–274. [CrossRef]
6. Fenical, W. Food-Drugs from the Sea, Proceedings (of the Fourth Food Drugs from the Sea Conference) 1974. In Proceeding of the Food Drugs from the Sea Conference, 17–21 November 1974; Webber, H.H., Ruggieri, G.D., Eds.; Marine Technological Society: Washington, DC, USA.
7. Akinin, M.; Dayan, T.L.A.; Rudi, A.; Kashman, Y.; Gaydou, E.M. Hydroquinone antioxidants from the Indian Ocean tunicate Aplidium savignyi. J. Agric. Food Chem. 1999, 47, 4175–4177. [CrossRef] [PubMed]
8. Sato, A.; Shindo, T.; Kasanuki, N.; Hasegawa, K. Antioxidant metabolites from the tunicate amatnlocum-multiplicatum. J. Nat. Prod. 1989, 52, 975–981. [CrossRef] [PubMed]
9. Carrido, L.; Zubia, E.; Ortega, M.J.; Salva, J. New meroterpenoids from the ascidian Aplidium conicum. J. Nat. Prod. 2002, 65, 1328–1331. [CrossRef] [PubMed]
10. Shubina, L.K.; Fedorov, S.N.; Radchenko, O.S.; Balaneko, N.N.; Kolesnikova, S.A.; Dmitrenok, P.S.; Bode, A.; Dong, Z.; Stonik, V.A. Desmethylubiquinone Q$_2$ from the far-eastern ascidian Aplidium glabrum: Structure and synthesis. Tetrahedron Lett. 2005, 46, 559–562. [CrossRef]
25. Bertanha, C.S.; Januario, A.H.; Alvarenga, T.A.; Pimenta, L.P.; Andrade e Silva, M.L.; Cunha, W.R.; Barker, D.; Copp, B.R. Anti-inflammatory and antimarial meroterpenoid from the New Zealand ascidian Aplidium scabellum. J. Org. Chem. 2011, 76, 9151–9156. [CrossRef] [PubMed]

26. Espinoza, L.; Taborga, L.; Villena, J.; Carrasco, H.; Espinoza, L. Synthesis and cytotoxic activity of linear geranylphenols and their phenyl acetate derivatives. J. Nat. Prod. 2010, 73, 373–376. [CrossRef] [PubMed]

27. Baeza, E.; Catalan, K.; Villena, J.; Carrasco, H. Synthesis of geranylhydroquinone derivatives with potential cytotoxic activity. Quim. Nova 2012, 25, 828–833. [CrossRef] [PubMed]

28. Taborga, L.; Vergara, A.; Osorio, M.; Carvajal, M.; Madrid, A.; Marilaf, F.; Carrasco, H.; Espinoza, L. Synthesis and NMR structure determination of new linear geranylphenols by direct geranylation of activated phenols. J. Chil. Chem. Soc. 2013, 58, 1790–1796. [CrossRef] [PubMed]

29. Espinoza, L.; Taborga, L.; Diaz, K.; Olea, A.F.; Peña-Cortes, H. Synthesis of linear geranylphenols and their effect on mycelial growth of plant pathogen Botrytis cinerea. Molecules 2014, 19, 1512–1526. [CrossRef] [PubMed]

30. Chavez, M.I.; Soto, M.; Taborga, L.; Diaz, K.; Olea, A.F.; Bay, C.; Pena-Cortes, H.; Espinoza, L. Synthesis and in vitro antifungal activity against Botrytis cinerea of geranylated phenols and their phenyl acetate derivatives. Int. J. Mol. Sci. 2015, 16, 19130–19152. [CrossRef] [PubMed]
31. Taborga, L.; Diaz, K.; Olea, A.F.; Reyes-Bravo, P.; Flores, M.E.; Pena-Cortes, H.; Espinoza, L. Effect of polymer micelles on antifungal activity of geranylorcinol compounds against Botrytis cinerea. *J. Agric. Food Chem.* 2015, 63, 6890–6896. [CrossRef] [PubMed]

32. Taborga, L.; Espinoza, L.; Moller, A.; Carrasco, H.; Cuellar, M.; Villena, J. Antiproliferative effect and apoptotic activity of linear geranylphenol derivatives from phloroglucinol and orcinol. *Chem. Biol. Interact.* 2016, 247, 22–29. [CrossRef] [PubMed]

33. Elad, Y.; Evenses, K. Physiological aspects of resistance to Botrytis cinerea. *Phytopathology* 1995, 85, 637–643. [CrossRef]

34. Latorre, B.A.; Flores, V.; Sara, A.M.; Roco, A. Dicarboximide-resistant isolates of Botrytis cinerea from table grape in Chile—survey and characterization. *Plant Dis.* 1994, 78, 990–994. [CrossRef]

35. Latorre, B.A.; Spadaro, I.; Rioja, M.E. Occurrence of resistant strains of Botrytis cinerea to anilinopyrimidine fungicides in table grapes in Chile. *Crop Prot.* 2002, 21, 957–961. [CrossRef]

36. Cotoras, M.; Folch, C.; Mendoza, L. Characterization of the antifungal activity on Botrytis cinerea of the natural diterpenoids kaurenoic acid and 3β-hydroxy-kaurenoic acid. *J. Agric. Food Chem.* 2004, 52, 2821–2826. [CrossRef] [PubMed]

37. Mendoza, L.; Espinoza, P.; Urzua, A.; Vivanco, M.; Cotoras, M. *In vitro* antifungal activity of the diterpenoid 7α-hydroxy-8(17)-labden-15-oic acid and its derivatives against Botrytis cinerea. *Molecules* 2009, 14, 1966–1979. [CrossRef] [PubMed]

38. Mendoza, L.; Araya-Maturana, R.; Cardona, W.; Delgado-Castro, T.; Garcia, C.; Lagos, C.; Cotoras, M. *In vitro* sensitivity of Botrytis cinerea to anthraquinone and anthrahydroquinone derivatives. *J. Agric. Food Chem.* 2005, 53, 10080–10084. [CrossRef] [PubMed]

39. Stevens, K.L.; Jurd, L.; Manners, G. Transformations of geraniol in aqueous acid solutions. *Tetrahedron* 1972, 28, 1939–1944. [CrossRef]

40. Manners, G.; Jurd, L.; Stevens, K. Biogenetic-type syntheses of isoprenoid and diisoprenoid derivatives of orcinol. *Tetrahedron* 1972, 28, 2949–2959. [CrossRef]

41. Chukicheva, I.Y.; Fedorova, I.V.; Koroleva, A.A.; Kuchin, A.V. Synthesis of natural geranydroquinone analogs. *Chem. Nat. Compd.* 2015, 51, 1056–1058. [CrossRef]

42. Hou, Z.; Yang, R.; Zhang, C.; Zhu, L.; Miao, F.; Yang, X.; Zhou, L. 2-(Substituted phenyl)-3,4-dihydroisoquinolin-2-iums as novel antifungal lead compounds: Biological evaluation and structure-activity relationships. *Molecules* 2013, 18, 10413–10424. [CrossRef] [PubMed]