Carvacrol Derivatives with Potential Insecticidal Activity †

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Abstract: Three new carvacrol derivatives 1–3 possessing aliphatic carbon chains with different sizes as hydroxyl group substituents were synthesized in order to evaluate their insecticidal activity against the insect cell line Sf9 (Spodoptera frugiperda).

Keywords: carvacrol; biopesticides; green insecticides; natural products; Spodoptera frugiperda

1. Introduction

Synthetic pesticides have been used for decades to manage pest control in crops, in order to avoid considerable damage and production losses due to pathogens, insects and other pest species [1,2]. Nowadays, because of environmental and health issues, synthetic pesticides are being gradually replaced by botanical pesticides [2,3].

An alternative to pest control is the use of essential oils (EOs) and their major chemical constituents [4]. Essential oils plants’ secondary metabolites of low molecular weight and strong organoleptic properties have been investigated as resources of potentially useful bioactive compounds, and it is known that they have an important role in the interactions between insects and plants [5,6]. Carvacrol (2-methyl-5-(1-methylethyl) phenol) is found in the essential oils of thyme (Thymus vulgaris), marjoram (Origanum majorana), oregano (Origanum vulgari L.), pepperwort (Lepidium sp.), and Alaskan yellow cedar (Callitropsis nootkatensis (D. Don) Oerst. ex D. P. Little) [7]. It is a phenolic compound that displays antimicrobial, antifungal, and insecticidal activities [8,9].

Considering these facts, the present investigation is focused on the synthesis of new carvacrol derivatives possessing aliphatic carbon chains with different sizes as hydroxyl group substituents, and their insecticide activity against the insect cell line Sf9 (Spodoptera frugiperda) was evaluated.

2. Materials and Methods

2.1. Typical Procedure for Carvacrol Derivatives 1–3 (Illustrated for 1)

To a solution of 5-isopropyl-2-methylphenol (0.105 mL, 1 eq.) in acetonitrile (4 mL) was added cesium carbonate (1.0885 g, 5 eq.) and 1-bromopropane (6.7 × 10⁻² mL, 1.1 eq.), and the reaction mixture was heated at 65 °C with stirring for 24 h. The progress of the reaction was monitored by thin-layer chromatography (TLC) (silica: dichloromethane). The reaction mixture was filtered, washed with acetonitrile and then the solvent was
evaporated. 4-Isopropyl-1-methyl-2-propoxybenzene was obtained as an orange oil (0.0693 g; 54% yield). \( R_f = 0.56 \) (petroleum ether). \( ^1H \) NMR (CDCl₃, 400 MHz): \( \delta \) 1.12 (3H, t, \( J \) 7.6 Hz, O(CH₂)₂CH₃), 1.30 (6H, d, \( J \) 6.8 Hz, CH(CH₃)₂), 1.84-1.93 (2H, m, OCH₂CH₂CH₃), 2.26 (3H, s, CH₃), 2.88-2.95 (1H, m, CH(CH₃)₂), 3.99 (2H, t, \( J \) 6 Hz, OCH₂CH₂CH₃), 6.75 (1H, d, \( J \) 1.2 Hz, H-3), 6.78 (1H, dd, \( J \) 7.6 Hz, \( J \) 1.6, H-5), 7.11 (1H, d, \( J \) 7.2 Hz, H-6) ppm. \( ^{13}C \) NMR (CDCl₃, 100.6 MHz): \( \delta \) 10.68 (O(CH₂)₂C₃H₃), 15.77 (CH₃), 22.80 (OCH₂CH₂CH₃), 24.13 (CH(CH₃)₂), 34.14 (CH(CH₃)₂), 69.36 (OCH₂CH₂CH₃), 109.45 (C-3), 117.76 (C-5), 124.13 (C-1), 130.32 (C-6), 147.79 (C-4), 157.15 (C-2) ppm.

2.2. Cell Culture

*Spodoptera frugiperda* cells (Sf9 cell line) were maintained as a suspension culture and cultivated in Grace’s medium with 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin, at 28 °C. Cells were kept in a humidified atmosphere of 5% CO₂.

2.3. Viability Assessment

For the assessment of viability, a resazurin-based method was used. Sf9 cells were plated at a density of \( 3.0 \times 10^4 \), incubated for 24 h and then exposed to the molecules under study for 24 h. After this period, a commercial solution of resazurin was added (1:10) and the kinetic reaction of fluorescence increase was monitored at 560/590 nm, with the results being used after 60 min of incubation.

3. Results and Discussion

3.1. Synthesis of Carvacrol Derivatives 1-3

Carvacrol derivatives 1–3 were synthesized by O-alkylation of carvacrol using alkyl halides possessing carbon chains of various sizes. Starting from carvacrol (5-isopropyl-2-methylphenol) and reacting with 1-bromopropane, 1-bromoctane or 1-bromododecane, in the presence of cesium carbonate as a base, at 65 °C in acetonitrile, 4-isopropyl-1-methyl-2-propoxybenzene 1, 4-isopropyl-1-methyl-2-(octyloxy)benzene 2 and 2-(dodecyloxy)-4-isopropyl-1-methylbenzene 3 were obtained, respectively, as oils in 54%–62% yields (Figure 1).

![Figure 1. Carvacrol derivatives 1-3.](image)

The \( ^1H \) NMR spectra of compounds 1–3 showed the different characteristic signals for the protons of methylene (\( \delta \) 1.35–4.00 ppm) and methyl groups (\( \delta \) 0.90–1.12 ppm) of aliphatic chains, the methyl (\( \delta \) 2.23–2.29 ppm) and isopropyl group of carvacrol (\( \delta \) 1.28–1.35 and 2.85–3.00 ppm), as well as protons of the aromatic ring H-3, H-5 and H-6 as singlet or duplet (\( \delta \) 6.73–6.78 ppm), double doublet (\( \delta \) 6.75–6.82 ppm) and duplet (\( \delta \) 7.08–7.15 ppm), respectively. \( ^{13}C \) NMR spectra of all compounds showed the signals of methylene groups (\( \delta \) 22.68–31.93 ppm), being signals of OCH₂ at higher chemical shifts (\( \delta \) 67.83–
69.36 ppm), methyl groups of aliphatic chains (δ 10.68–14.11 ppm), as well as methyl (δ 15.77–15.81 ppm) and isopropyl (δ 24.13 and 34.14–34.16 ppm) groups of carvacrol moiety, in addition to carbons of the aromatic ring (δ 109.39–157.19 ppm).

3.2. Impact of Carvacrol Derivatives 1–3 in Cell Viability

The impact of carvacrol derivatives 1–3 in the viability of Sf9 cells was evaluated at 100 µg/mL, following 24h of exposure. As shown in Figure 2, the molecules were mostly devoid of toxicity, the exception being 2 that elicited a small reduction in viability, around 20%.

![Figure 2. Viability of Spodoptera frugiperda (Sf9) cells after incubation with the indicated molecules (100 µg/mL) for 24 h.](image)

4. Conclusions

Three new carvacrol derivatives possessing aliphatic carbon chains with different sizes as hydroxyl group substituents were synthesized. The evaluation of their impact in cell viability showed that medium size carbon chains may have some impact on the cell viability of Sf9. Other carvacrol derivatives are being synthesized in order to continue the evaluation of its effect in mixtures with active compounds as potential insecticides.

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