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Synthesis, Insecticidal Activity and Nanoencapsulation Studies of Alkoxy Alcohols from Eugenol †

Catarina M. M. Coelho [1], Maria José G. Fernandes [1], David M. Pereira [2], Renato B. Pereira [2], A. Gil Fortes [1], M. Sameiro T. Gonçalves [1] and Elisabete M. S. Castanheira [3,4,*]

1 Centre of Chemistry (CQ/UM), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; pg40075@alunos.uminho.pt (C.M.M.C.); mjfernandes@quimica.uminho.pt (M.J.G.F.); gilf@quimica.uminho.pt (A.G.F.); msameiro@quimica.uminho.pt (M.S.T.G.)
2 REQUIMTE/LAQV, Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal; dpereira@ff.up.pt (D.M.P.); ren.pereira@gmail.com (R.B.P.)
3 Centre of Physics of Minho and Porto Universities (CF-UM-UP), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal
4 LaPMET Associate Laboratory, University of Minho, 4710-057 Braga, Portugal
* Correspondence: ecoutinho@fisica.uminho.pt
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Abstract: The synthesis of three alkoxy alcohols with different chains was carried out from eugenol oxirane. These derivatives were evaluated against their effect on the viability of the insect cell line *Sf9* (*Spodoptera frugiperda*). The molecules under study displayed mild-cytotoxic activity towards insect cells, equivalent to the commercial insecticide chlorpyrifos. Nanoencapsulation studies of the most active compound against *Sf9* were performed using liposomes of egg-phosphatidylcholine containing 30% cholesterol. The encapsulation efficiency is around 93%, and the release profile for 24 h follows a Korsmeyer–Peppas model.

Keywords: eugenol alkoxy alcohols; nanoencapsulation; bioinsecticides; essential oils; natural products; insecticides

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1. Introduction

Food production and human health are greatly affected by insects. One way to control pests is to use synthetic insecticides. Despite being frequently used, the inappropriate utilization of these compounds is related to the development of insecticide resistance in pests, human diseases, and contamination of food and the environment. Consequently, the biological activity of natural products or semisynthetic compounds with insecticidal activity can be a very important alternative that allows an environmental-friendly way to manage the action of insects and pests without affecting people's health [1].

Essential oils, for example, are a matrix of several phytochemicals, some of which with efficient biopesticide action. However, their fast degradation when exposed to external factors, such as light and air, is a barrier to their wider use [2,3]. Eugenol, the major component of clove oil, is an important insecticide with large efficiency on a wide variety of domestic arthropod pests [4]. It has shown numerous applications in the agricultural, food, pharmaceutical, and cosmetics industries [4–7].

One way to overcome the difficulties related to the easy degradation of biopesticides and the use of (semi)synthetic pesticides is by encapsulating them. This method improves their resistance to biological and physicochemical degradation and increases their effectiveness and controlled release with reduced toxicity [8,9].

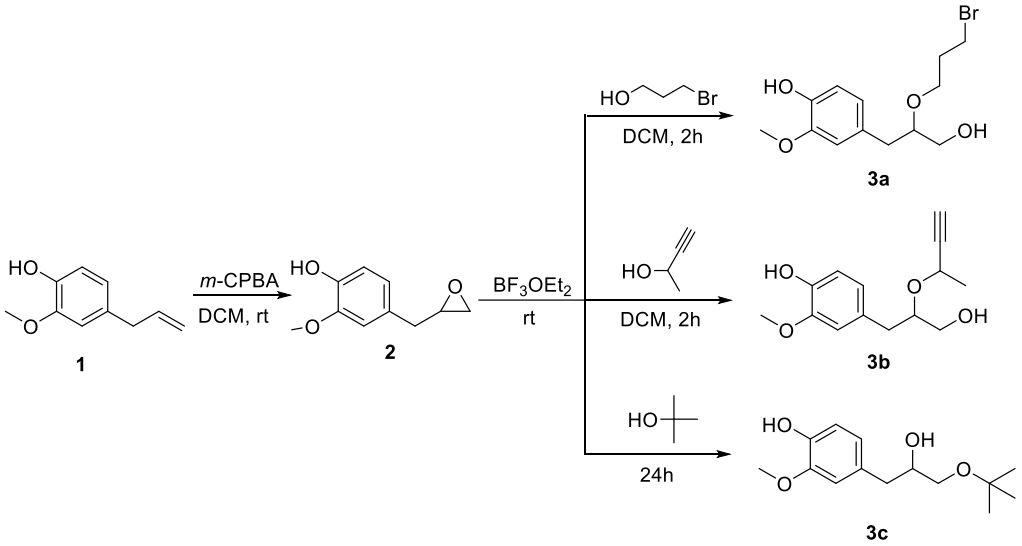
Considering all these facts and our recent interests in alternative pesticides [10–12], the synthesis of three alkoxy alcohols from eugenol oxirane was carried out, followed

by the evaluation of their effect on the viability of the insect cell line *Sf9* (*Spodoptera frugiperda*). Encapsulation assays of the most promising compound were also carried out, using nanoliposomes of egg-phosphatidylcholine/cholesterol (7:3).

2. Results and Discussion

2.1. Synthesis of Alkoxy Alcohols from Eugenol Oxirane

4-Allyl-2-methoxyphenol, eugenol **1**, extracted from clove, was used in the efficient synthesis of 2-methoxy-4-(oxiran-2-ylmethyl)phenol **2**, by reaction with *m*-chloroperbenzoic acid (*m*-CPBA) in DCM following a known procedure [4,7]. Compound **2** was then reacted at room temperature, in the presence of boron trifluoride etherate as Lewis acid, with the corresponding alcohol, namely 3-bromopropan-1-ol, but-3-yn-2-ol, and 2-methylpropan-2-ol. DCM was used as a solvent in the first two cases to give the alkoxy alcohols **3a–c** (Scheme 1).



Scheme 1. Synthesis of eugenol epoxide **2** and alkoxy alcohols **3a–c**.

Compounds **2** and **3a-c** were fully characterized by the usual analytical techniques. Epoxidation of compound **1** was verified by the ^{1}H NMR spectrum, namely through the presence of the protons' signals related to the oxirane ring (δ 2.55–3.12 ppm) and the absence of the protons' signals for the double bond of eugenol skeleton. In the ^{13}C NMR spectrum, the presence of carbons signals relative to the oxirane ring, CH-oxirane (δ 52.67 ppm), and CH_2-oxirane (δ 46.79 ppm) confirmed the structure of compound **2**.

The ^{1}H NMR spectra of compounds **3a–c** showed the absence of protons related to the oxirane ring, and instead, the presence of methine and methylene protons of the 3-hydroxypropyl (**3a**, **3b**) or 2-hydroxypropyl (**3c**) groups as multiplets (δ 2.42–3.94 ppm) or duplet (δ 2.74 ppm). The protons of 3-bromopropoxy (**3a**), but-3-yn-2-yloxy (**3b**), or *tert*-butoxy (**3c**) substituents are also shown, namely the methylene protons as multiplets (δ 2.04–2.85 ppm, **3a**), methyl protons as a doublet (δ 1.37 ppm, **3b**) or a singlet (δ 1.20 ppm, **3c**), and the terminal alkyne proton as a duplet (δ 2.45 ppm, **3b**).

The ^{13}C NMR spectra of compounds **3a–c** confirms the presence of methine and methylene carbons of 3-hydroxypropyl (**3a**, **3b**) or 2-hydroxypropyl (**3c**) groups (δ 27.57–81.62 ppm). The carbons of 3-bromopropoxy (**3a**), but-3-yn-2-yloxy (**3b**) or *tert*-butoxy (**3c**) substituents are also shown, namely the methylene (δ 30.62–67.02 ppm, **3a**), methyl (δ 22.34 –27.57 ppm, **3b**, **3c**), and terminal alkyne carbons (δ 72.97 or 84.41 ppm, **3b**).

2.2. Toxicity of Alkoxy Alcohols Eugenol Derivatives

Aiming to evaluate the insecticidal activity of the synthesized eugenol derivatives, *Spodoptera frugiperda* (*Sf9*) cells, a common pest, were used. For comparison purposes

of their potency, all the molecules under study were screened at the same concentration (100 μg/mL). As can be seen in Figure 1, compounds **3a–c** showed similar toxicity towards insect cells, causing ca. 20% of viability loss (equivalent to the commercial insecticide chlorpyrifos).

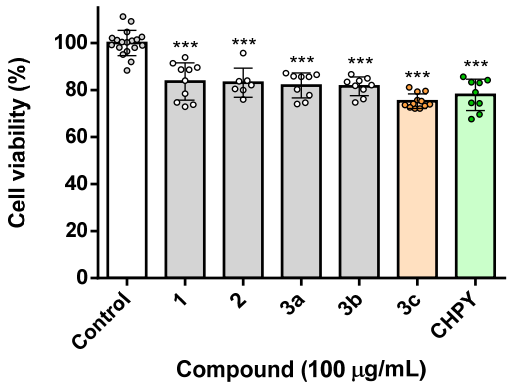


Figure 1. Viability of *Sf9* insect cells exposed to the molecules under study **3a–c** (100 μg/mL), or medium (control), or the reference insecticide chlorpyrifos (CHPY). *** $p < 0.001$.

2.3. Nanoencapsulation Studies

The most active compound against *Sf9* cells, compound **3c**, was encapsulated in liposomal systems of egg-phosphatidylcholine/cholesterol (Egg-PC/Ch) (7:3). The loaded nanosystems display a small uniform size below 120 nm and a low PDI. The encapsulation efficiency of **3c** in these liposomes is very high, above 90% (Table 1).

Table 1. Mean hydrodynamic size, polydispersity index (PDI) and encapsulation efficiency (EE (%)) for **3c**-loaded liposomes (SD: standard deviation from three independent assays).

System	Size ± SD (nm)	PDI ± SD	EE(%) ± SD
3c-loaded Egg-PC/Ch liposomes	114.4 ± 2	0.27 ± 0.01	93.5 ± 0.8

The release profile for 24 h exhibits a delayed release of compound **3c**, well fitted to the Korsmeyer–Peppas model [13] (Figure 2). The release constant determined is 12.94 min^{-1}, and the transport exponent is 0.35, indicating a mechanism of Fickian release (diffusion-controlled transport) [12,13].

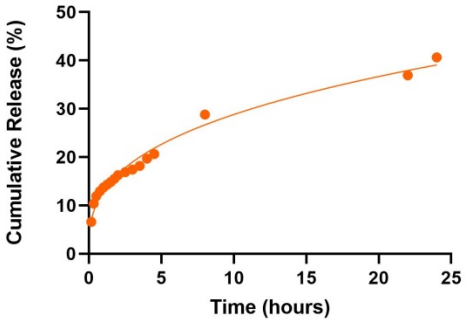


Figure 2. Release profile of compound **3c** from liposomes and fitting to Korsmeyer–Peppas model.

3. Experimental

3.1. Extraction of Eugenol **1** *from Syzygium Aromaticum*

The *Syzygium Aromaticum* (clove) was placed in a round-bottomed flask (25 g), water (250 mL) was added, and the hydrodistillation apparatus was set. The extraction time was

2 h. The distillate was extracted with dichloromethane (2 × 20 mL). The organic phase was dried under anhydrous magnesium sulfate, and the solvent was evaporated in a rotary evaporator to give 4-allyl-2-methoxyphenol, eugenol **1** as a yellowish transparent oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 6.89 (d, *J* = 8.8 Hz, 1H, H-6), 6.71–6.73 (m, 2H, H-3 and H-5), 5.96–6.03 (m, 1H, C*H*=CH_2), 5.60 (broad s, 1H, O*H*), 5.08–5.14 (m, 2H, CH=CH_2), 3.89 (s, 3H, OCH_3), 3.36 (d, *J* = 6.8 Hz, 2H, CH_2Ph) ppm.

3.2. Synthesis of 2-methoxy-4-(oxiran-2-ylmethyl)phenol **2**

In a reaction flask containing *m*-chloroperbenzoic acid (0.750 g, 4.35 mmol, 1 equiv) dissolved in dichloromethane (10 mL), 4-allyl-2-methoxyphenol **1** (0.500 g, 3 mmol, 1 equiv) dissolved in dichloromethane (10 mL) was added dropwise, while stirring at 0 °C (ice bath). After stirring for 1 h, an additional equiv of *m*-chloroperbenzoic acid (0.750 g, 4.35 mmol) was added, and the reaction was kept under stirring for 24 h at room temperature, and its evolution was monitored by ^{1}H NMR ($CDCl_3$). To the final product, dichloromethane (20 mL) and sodium sulfite in solution (2 × 20 mL) were added, and the organic phase was collected and washed with saturated sodium bicarbonate solution (2 × 20 mL). The organic phase was dried over anhydrous magnesium sulfate and the solvent was evaporated giving 2-methoxy-4-(oxiran-2-ylmethyl)phenol **2** as thick dark orange oil residue (0.337 g, 1.87 mmol, 67% yield). R_f = 0.27 (silica: dichloromethane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 6.87 (d, *J* = 7.6 Hz, 1H, H-6), 6.77 (dm, *J* = 2 Hz, 1H, H-3), 6.75 (dd, *J* = 8 Hz and 2 Hz, 1H, H-5), 5.54 (broad s, 1H, O*H*), 3.90 (s, 3H, OCH_3), 3.12–3.16 (m, 1H, C*H*-oxirane), 2.79–2.82 (m, 3H, CH_2Ph and CH_2-oxirane), 2.55 (q, *J* = 2.8 Hz, 1H, CH_2-oxirane) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C 146.46 (C-2), 144.39 (C-1), 129.03 (C-4), 121.64 (C-5), 114.32 (C-6), 111.54 (C-3), 55.90 (OCH_3), 52.67 (*C*H-oxirane), 46.79 (CH_2-oxirane), 38.37 (CH_2Ph) ppm.

3.3. General Procedure for Synthesizing Compounds **3a**–**c** *(Illustrated for* **3c***)*

To a solution of 2-methoxy-4-(oxiran-2-ylmethyl)phenol **2** (0.149 g, 0.83 mmol, 1 equiv) in dichloromethane (7 mL), *tert*-butanol (5 mL) and boron trifluoride etherate (0.117 g, 0.82 mmol, 1 equiv) were added, and the resulting mixture was left stirring under nitrogen atmosphere, at room temperature for 24 h. The progress of the reaction was monitored by ^{1}H NMR ($CDCl_3$) and TLC (silica: ethyl acetate/petroleum ether 1:1). The reaction mixture was dissolved in dichloromethane (5 mL) and water (5 mL) was added. The organic phase was extracted with dichloromethane (2 × 5 mL), dried over anhydrous magnesium sulfate and evaporated in a rotary evaporator. The crude mixture was purified by column chromatography on silica gel using ethyl acetate/petroleum ether (1:1) as eluent giving 4-(3-(*tert*-butoxy)-2-hydroxypropyl)-2-methoxyphenol **3c** as a thick yellowish oil residue (0.018 g, 0.07 mmol, 12% yield). *Rf* = 0.54 (silica: ethyl acetate/petroleum ether 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 6.85 (d, *J* = 8.0 Hz, 1H, H-6), 6.77 (dm, *J* = 1.6 Hz, 1H, H-3), 6.72 (dd, *J* = 8.0 and 2.0 Hz, 1H, H-5), 5.53 (broad s, 1H, O*H*), 3.89–3.94 (m, 1H, C*H*OH), 3.88 (s, 3H, OCH_3), 3.37–3.40 (m, 1H, CH_2OC$(CH_3)_3$), 3.23–3.40 (m, 1H, CH_2OC$(CH_3)_3$), 2.73 (d, *J* = 7.2 Hz, 2H, CH_2Ph), 1.20 (s, 9H, OC(C$H_3)_3$) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C 146.38 (C-2), 144.09 (C-1), 130.12 (C-4), 121.91 (C-5), 114.24 (C-6), 111.77 (C-3), 73.19 (O*C*$(CH_3)_3$), 71.79 (*C*HOH), 64.91 (CH_2OC$(CH_3)_3$), 55.84 (OCH_3), 39.54 (CH_2Ph), 27.57 OC($CH_3)_3$) ppm.

3.4. Insecticidal Studies

3.4.1. Cell Culture

Sf9 (*Spodoptera frugiperda)* cells were acquired from ATCC (Manassas, WV, USA), maintained as a suspension culture, and cultivated in Grace's medium with 10% FBS and 1% penicillin/streptomycin at 28 °C. Cells were used in experiments while in the exponential phase of growth.

3.4.2. Viability Assessment

For the viability assessment, a resazurin-based method was used, similarly to what we described before [10]. *Sf9* cells were plated at a density of 3.0×10^4 cells per well, incubated for 24 h, and then exposed to the molecules studied 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 after 60 min of incubation.

3.5. Nanoencapsulation Studies

Liposomes of egg-phosphatidylcholine/cholesterol (7:3) were prepared by ethanolic injection, as previously reported [11], with simultaneous injection of the lipid mixture and the active compound in phosphate buffer (pH = 7.4). The hydrodynamic diameter and size distribution (polydispersity, PDI) of the loaded liposomes were determined with a Dynamic Light Scattering (DLS) equipment Litesizer 500 from Anton Paar at 25 °C, using a solid-state laser of 648 nm and 40 mW.

Dilutions of compound solutions were carried out to determine a calibration curve (absorbance vs. concentration) and calculate the encapsulation efficiency (EE%) through the relation:

$$EE(\%) = (\text{Total amount} - \text{Amount of non-encapsulated compound})/(\text{Total amount}) \times 100$$

Loaded nanosystems were subjected to centrifugation at 3000 rpm for 10 min using Amicon® Ultra centrifugal filter units 100 kDa. The supernatant (containing the non-encapsulated compound) was removed, and absorbance was measured to calculate compound concentration (using the calibration curve previously obtained). Absorption spectra were measured using a Shimadzu UV-3600 Plus spectrophotometer.

The compound release was followed for 24 h at room temperature towards a phosphate buffer of pH = 7.4. The release profiles were fitted to the Korsmeyer–Peppas model of drug release [13].

4. Conclusions

Three new alkoxy alcohols were synthesized starting from eugenol oxirane. The molecules under study **3a–c** displayed mild-cytotoxic activity towards insect cells. The most active compound was encapsulated in liposomal systems of egg-phosphatidylcholine/cholesterol (7:3), with high encapsulation efficiency and allowing a sustained release in 24 h.

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