

Proceeding Paper

Synthesis of Amino Alcohols from Eugenol and Their Insecticidal Activity against *Sf9* Cell Line [†]

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Abstract: Eugenol is a major constituent of clove essential oil with interesting biological activity, namely antimicrobial and antioxidant. Structural changes of eugenol are a useful strategy in order to improve biological activity and at the same time obtain new analogues with reduced side effects. In this work, a series of amine nucleophiles were reacted with eugenol epoxide so as to obtain the corresponding amino alcohols, considering their potential as biopesticides. These eugenol amino alcohols were evaluated against insect cells, specifically the *Sf9* cell line.

Keywords: eugenol derivatives; amino alcohols; insecticides; biopesticides; natural products

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1. Introduction

Structural changes of natural biologically active compounds are an important strategy to improve biological activity and at the same time to reduce possible side effects [1,2].

Eugenol, a phenylpropanoid, is a major constituent of clove essential oil with several applications in pharmaceutical, food, agricultural, and cosmetics industries [3], showing various types of biological activities, for example antioxidant [4,5], antimicrobial [6], antiviral [7,8] and anti-inflammatory [9]. Additionally, the β -amino alcohol moiety represents an important core in the pharmaceutical industry; drugs such as salbutamol and propranolol are amongst the most important therapeutical agents on the market with this feature [10].

Following previous work in our lab, in which some eugenol derivatives showed high potential as biopesticides in assays using the *Sf9* (*Spodoptera frugiperda*) insect cell line, a new series of eugenol amino alcohol derivatives was obtained and evaluated for insecticidal activity.

2. Materials and Methods

2.1. Synthesis of Eugenol Derivatives

2.1.1. Synthesis of Eugenol Epoxide 2

To a suspension of *m*-CPBA (1.022 g, 55%, 3.27 mmol) in DCM (12 mL) under stirring at 0 °C was added a solution of eugenol (0.5 mL, 3.23 mmol) in DCM (10 mL), and

the reaction mixture was left for 2 h at room temperature, after which another portion of *m*-CPBA was added (1.016 g, 55%, 3.24 mmol). After 23 h, the mixture was washed with NaSO₃ (2 × 10 mL) followed by a saturated solution of NaHCO₃ (2 × 10 mL). The organic phase was dried with MgSO₄, and the solvent evaporated to afford epoxide 2 as yellow oil (0.532 g, 2.95 mmol, 91%). R_f = 0.10 (EtOAc/Pet.Et.-1:5). ¹H-RMN (CDCl₃, 400 MHz) δ_H: 6.86 (d, *J* = 7.6 Hz, 1H, H-6), 6.77 (d, *J* = 2 Hz, 1H, H-3), 6.74 (dd, *J* = 2 Hz, 7.6 Hz, 1H, H-5), 5.56 (s, 1H, OH), 3.90 (s, 3H, OCH₃), 3.14 (tdd, *J* = 2.8 Hz, 4 Hz, 5.6 Hz, 1H, CH₂CHCHH), 2.792–2.82 (m, 3H, CH₂CHCHH), 2.55 (dd, *J* = 2.8 Hz, 4.8 Hz, 1H, CH₂CHCHH) ppm. ¹³C-RMN (CDCl₃, 100.6 MHz) δ_C: 143.5 (C-2), 144.4 (C-1), 129.0 (C-4), 121.6 (C-5), 114.3 (C-6), 111.5 (C-3), 55.9 (OCH₃), 52.7 (-CH₂CHCH₂), 46.8 (-CH₂CHCH₂), 38.4 (-CH₂CHCH₂) ppm.

2.1.2. Typical Procedure for the Preparation of Amino Alcohols 3 and 4 (Illustrated for 3)

To a solution of epoxide 2 (0.204 g, 1.13 mmol) in EtOH/water (2 mL, 1:2, *v/v*) was added aniline (0.4 mL, 4.38 mmol, 3.9 eq.), and the mixture was heated at 50 °C for 5.5 h. Water (2 mL) was added and the resulting mixture was extracted with EtOAc (2 mL); the organic phase was collected, dried with MgSO₄, filtered, and the solvent evaporated to afford a crude as a red oil (0.284 g), which was subjected to column chromatography in silica using DCM/MeOH as eluent of gradient polarity. The pure compound 3 was isolated as yellow oil (0.095 g, 0.35 mmol, 31%). R_f = 0.7 (MeOH/DCM 5:95). ¹H-RMN (CDCl₃, 400 MHz) δ_H: 7.19 (t, *J* = 3.2 Hz, 2H; H-3/H-5 NHC₆H₅OMe), 6.88 (d, *J* = 8 Hz, 1H, H-6), 6.75 (t, *J* = 8 Hz, 1H, H-4 NHC₆H₅OMe), 6.75 (s, 1H, H-3), 6.74 (d, *J* = 7.6 Hz, 1H, H-5), 6.65 (d, *J* = 8 Hz, 2H, H-2/H-6 NHC₆H₅OMe), 4.06 (m, 1H, -CHHCHCHHN), 3.87 (s, 3H, OCH₃), 3.31 (dd, *J* = 12.4 Hz, 7.2 Hz, 1H; -CHHCHCHHN), 3.10 (dd, *J* = 12.8 Hz, 8 Hz, 1H, -CHHCHCHHN), 2.83 (dd, *J* = 14 Hz, 5.2 Hz, 1H, -CHHCHCHHN), 2.75 (dd, *J* = 14 Hz, 8 Hz, 1H, -CHHCHCHHN) ppm. ¹³C-RMN (CDCl₃, 100.6 MHz) δ_C: 147.9 (C-1 NHC₆H₅OMe), 146.6 (C-2), 144.4 (C-1), 129.4 (C-4), 129.3 (C-3/5 NHC₆H₅OMe), 122.0 (C-5), 118.1 (C-4 NHC₆H₅OMe), 114.5 (C-6), 113.5 (C-2/6 NHC₆H₅OMe), 111.8 (C-3), 71.1 (-CH₂CHCH₂N), 55.9 (OCH₃), 49.5 (-CH₂CHCH₂N), 41.2 (-CH₂CHCH₂N) ppm.

2.2. Biological Assays

The potential of compounds 1–4 was evaluated as a biopesticide in assays using the *Sf9* (*Spodoptera frugiperda*) insect cell line. Cells were maintained at 28 °C and cultivated in Grace's medium with 10% FBS.

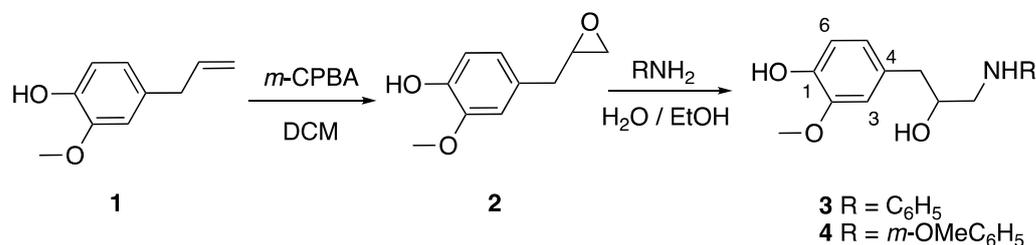
For the evaluation of viability, cells were plated at 3 × 10⁴ cells/well and exposed to the molecules, after which resazurin was added, resulting in being read at 560/590 nm after 60 min of incubation.

3. Results and Discussion

3.1. Synthesis of Eugenol Derivatives

In the present work, a series of amino alcohols were prepared from eugenol epoxide. Eugenol 1, obtained by hydrodistillation of clove essential oil, was reacted by a known procedure [1,11] using *m*-CPBA in DCM, at room temperature, to give epoxide 2 in 91% yield. The reaction of epoxide 2 with two amine nucleophiles, namely aniline and 3-methoxyaniline using an ethanol/water solution as solvent, under heating at 50 °C [12], followed by column chromatography purification afforded the corresponding β-amino alcohols 3 and 4 in 31% and 37% yields, respectively (Scheme 1). The obtained compounds were fully characterized by usual analytical techniques, namely NMR spectroscopy. The main ¹H-NMR features of compounds 3 and 4 are the presence of two doublets of doublets at δ 3.31 and 3.10 ppm and δ 3.27 and 3.05 ppm, respectively corresponding to the CH₂ vicinal to N; the OCH₃ shows as a singlet at δ 3.87 and δ 3.84 ppm in compounds 3 and 4, respectively; at δ 4.06 ppm and δ 4.03 ppm a multiplet corresponding to CHOH. Regarding ¹³C NMR, the main features are the CH₂N at δ 41.2 and δ 41.1 ppm for com-

pounds **3** and **4**, respectively, CHOH at δ 71.1 ppm for both compounds, OCH₃ at δ 55.9 and δ 55.8 ppm, respectively, and CH₂CHOHCH₂N at δ 49.5 and δ 49.3 ppm, respectively. The OCH₃ of the aromatic amine in compound **4** shows at δ 55.0 ppm.



Scheme 1. Synthesis of eugenol derivatives **2–4**.

3.2. Biological Evaluation

Previous work carried out in our lab showed interesting biological activity of some eugenol derivatives. The assessment of the starting molecule **1** showed that it exerted a low effect in the cell viability of *Sf9* cells, causing a loss of viability under 20%. Conversion of **1** into its epoxide **2** resulted in a complete loss of activity. Differently, substitution of the corresponding amino alcohols at the amino function with bulkier groups, such as phenyl, led to a significant increase in the toxicity of the resulting molecule. In fact, amino alcohol **3** was the most active molecule obtained, being twice as toxic as the starting material, eugenol **1**. In the case of compound **4**, its toxicity is mostly comparable to that of eugenol **1**, albeit marginally more toxic (Figure 1).

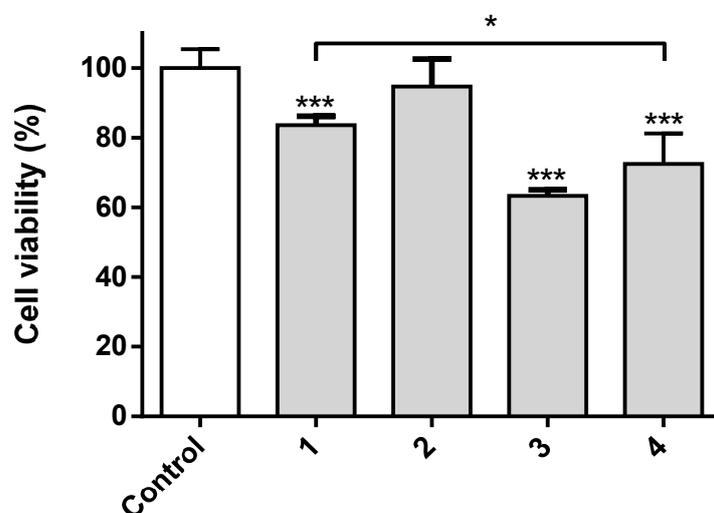


Figure 1. Viability of *Sf9* cells after exposure to the referred molecules for 24 h at the concentration of 100 $\mu\text{g/mL}$. * $p < 0.05$; *** $p < 0.001$.

4. Conclusions

New eugenol derivatives were prepared through the reaction of eugenol epoxide with amine nucleophiles. The compounds obtained were fully characterized and their biological evaluation as insecticides using the *Sf9* (*Spodoptera frugiperda*) insect cell line showed that it was possible to obtain molecules that were more toxic by tuning its structure.

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Conflicts of Interest: The authors declare no conflict of interest.

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