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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

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 phenyl propanoid, 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]. Also the ß-amino alcohol moiety represents an important core in 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 have shown a 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 $NaSO_3$ (2 × 10 mL) followed by a saturated solution of $NaHCO_3$ (2 × 10 mL). The organic phase was dried with $MgSO_4$, 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). ^{1}H-RMN ($CDCl_3$, 400 MHz) δ_H: 6.86 (d, *J* = 7.6 Hz, 1H, H-6), 6.77 (d, *J* = 2Hz, 1H, H-3), 6.74 (dd, *J* = 2 Hz, 7.6 Hz, 1H, H-5), 5.56 (s, 1H, OH), 3.90 (s, 3H, OCH_3), 3.14 (tdd, *J* = 2.8 Hz, 4 Hz, 5.6 Hz, 1H, CH_2CHCHH), 2.792–2.82 (m, 3H, CH_2CHCHH), 2.55 (dd, *J*=2.8 Hz, 4.8 Hz, 1H, CH_2CHCHH) ppm. ^{13}C-RMN($CDCl_3$, 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 (OCH_3), 52.7 (-CH_2CHCH_2), 46.8 (-CH_2CHCH_2), 38.4 (-CH_2CHCH_2) 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 $MgSO_4$, 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 a yellow oil (0.095 g, 0.35 mmol, 31%). R*f* = 0.7 (MeOH/DCM 5:95). ^{1}H-RMN ($CDCl_3$, 400 MHz) δ_H: 7.19 (t, *J* = 3,2 Hz, 2 H; H-3/H-5 NHC_6H_5OMe), 6.88 (d, *J* = 8 Hz, 1H, H-6), 6.75 (t, *J* = 8 Hz, 1H, H-4 NHC_6H_5OMe), 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 NHC_6H_5OMe), 4.06 (m, 1H, -CHHC*H*CHHN), 3.87 (s, 3H, OCH_3), 3.31 (dd, *J* = 12.4 Hz, 7.2 Hz, 1H; -CHHCHC*H*HN), 3.10 (dd, *J* = 12.8 Hz, 8Hz, 1H, -CHHCHCH*H*N), 2.83 (dd, *J* = 14 Hz, 5.2 Hz, 1H, -C*H*HCHCHHN), 2.75 (dd, *J* = 14 Hz, 8 Hz, 1H, -CH*H*CHCHHN) ppm. ^{13}C-RMN ($CDCl_3$, 100.6 MHz) δ_C: 147.9 (C-1 NHC_6H_5OMe), 146.6 (C-2), 144.4 (C-1), 129.4 (C-4), 129.3 (C-3/5 NHC_6H_5OMe), 122.0 (C-5), 118.1 (C-4 NHC_6H_5OMe), 114.5 (C-6), 113.5 (C-2/6 NHC_6H_5OMe), 111.8 (C-3), 71.1 (-CH_2CHCH_2N), 55.9 (OCH_3), 49.5 (-CH_2CHCH_2N), 41.2 (-CH_2CHCH_2N) ppm.

2.2. Biological Assays

The potential of compounds **14**—was evaluated as biopesticides 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 3×10^4 cells/well and exposed to the molecules, after which resazurin was added, resulting 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 ß-amino alcohols **3** and **4** in 31% and 37% yields, respectively (Scheme 1). The obtained compounds were fully characterised by usual analytical techniques, namely NMR spectroscopy. ^{1}H-NMR main features of compounds 3 and 4 are the presence of two doublet of doublets at δ 3.31 and 3.10 ppm and δ 3.27 and 3.05 ppm respectively corresponding to the CH_2 vicinal to N; the OCH_3 shows as a singlet at δ 3.87 ppm and δ 3.84 in compounds 3 and 4 respectively; at δ 4.06 ppm and δ 4.03 ppm a multiplet corresponding to C*H*OH. Regarding ^{13}C NMR, main features are the *C*H_2N at δ 41.2 ppm and δ 41.1 ppm for compounds 3 and 4 respectively, *C*HOH at δ 71.1 ppm for both compounds, O*C*H_3 at δ 55.9 ppm and δ 55.8 ppm respectively, and *C*H_2CHOHCH_2N at δ 49.5 ppm and δ 49.3 ppm respectively. The OCH_3 of the aromatic amine in compound 4 shows at δ 55.0 ppm.

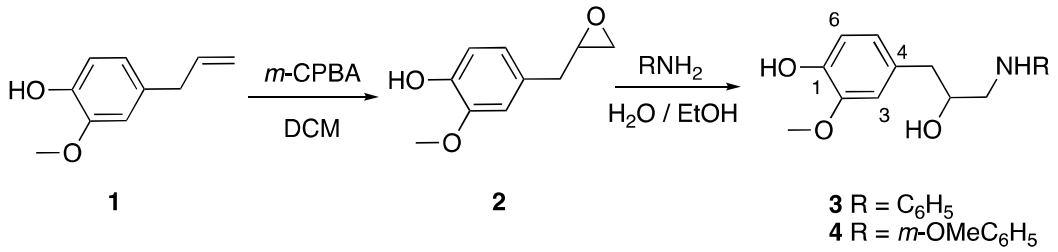

Figure 1. Synthesis of eugenol derivatives **24**–.

3.2. Biological Evaluation

Previous work carried out in our lab has shown 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.

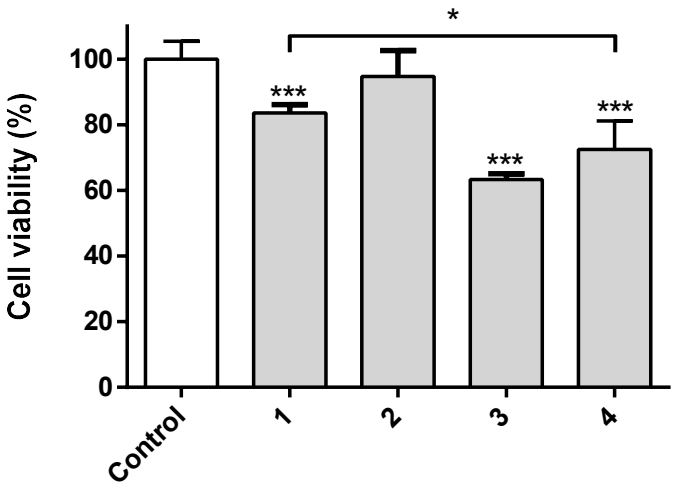

Figure 2. Viability of *Sf9* cells after exposure to the referred molecules for 24 h at the concentration of 100 μg/mL. * $p < 0.05$; *** $p < 0.001$.

4. Conclusions

New eugenol derivatives were prepared through 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 have shown that it was possible to obtained 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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