Abstract: The synthesis of three O-alkylated eugenol derivatives, bearing a hydroxypropyl chain and propyl esters, were synthesized and further converted into the corresponding oxiranes. Oxirane derivatives were then evaluated against their effect upon the viability of the insect cell line Sf9 (Spodoptera frugiperda), in comparison with the starting O-alkylates. The results pointing to their potential as bioinsecticides, with structural changes eliciting significant effects in terms of potency.

Keywords: essential oils; eugenol derivatives; eugenol epoxide; bioinsecticides; natural products

1. Introduction

In the last few decades, the need to prevent diseases and damage caused by the attack of various pests on plants has led to the application of high amounts of synthetic pesticides, including insecticides, which has resulted in the development of resistance to them by several harmful organisms [1]. As an alternative, natural products with insecticidal activity have shown promise for insect control in agriculture [2,3]. The use of bioactive compounds of plants presents many advantages as insecticide, e.g., they are less hazardous to human and animal health, less expensive, non-toxic to non-target species, less resistant in the target organism, and are environmentally friendly [4]. Essential oils (EOs) exhibit antimicrobial activities with particular potential as insecticides [5]. Structural modifications in the constituents of EOs can further enhance their biocidal effect [2,4,6,7], being the best alternatives of synthetic chemicals and can be utilized as biopesticides or green pesticides [8–10].

Eugenol, the major component of clove oil, presents numerous applications, including in pharmaceutical, food and agricultural industries. It is an important insecticide that is widely efficient in a broad variety of domestic arthropod pests [6,11].

Considering all these facts, the synthesis of three O-alkylated eugenol derivatives and their respective oxiranes was carried out and then evaluated against the effect upon the viability of the insect cell line Sf9 (Spodoptera frugiperda), in comparison with the starting eugenol O-alkylates.
2. Results and Discussion

2.1. Synthesis of Eugenol Derivatives

4- Allyl-2-methoxyphenol, eugenol, was extracted from clove and used in the synthesis of three O-alkylated derivatives 1a–c, which were then converted in the respective oxiranes 2a–c as shown in Scheme 1. Alkylation of the hydroxyl group of 4-allyl-2-methoxyphenol with 3-bromopropan-1-ol using cesium carbonate as a base, by heating at 65 °C in acetonitrile, gave methoxyphenoxy)propan-1-ol 1a. This compound was further reacted with acetic anhydride by heating at 65 °C to obtain 3-(4-allyl-2-methoxyphenoxy)propyl acetate 1b. 4-Allyl-2-methoxyphenol was also alkylated with ethyl 4-bromobutanoate by following the same method mentioned above to yield ethyl 4-(4-allyl-2-methoxyphenoxy)butanoate 1c. Compounds 1a–c were obtained as oils in 53 to 84% yields. Their 1H NMR spectra showed the different characteristic signals for the aliphatic protons of methylene and methyl groups (δ 1.21–4.27 ppm), as well as the expected protons for the eugenol’s double bond as multiplets, CH (δ 5.01–5.14 ppm) and CH (δ 5.91–6.01 ppm). 13C NMR spectra of all compounds showed signals of the aliphatic carbons from the methylene (δ 24.57–68.69 ppm) and methyl groups (δ 14.14–20.66 ppm), in addition to signals of the ester carbonyl groups (δ 170.76 and 173.20 ppm, respectively) for compounds 1b and 1c.

![Scheme 1. Synthesis of eugenol derivatives 2a–c.](image)

To perform epoxidation of the double bond of eugenol derivatives 1a–c, they were allowed to react with m-chloroperbenzoic acid in dichloromethane at room temperature, and the respective derivatives, namely, 3-(2-methoxy-4-(oxiran-2-ylmethyl)phenoxy)propan-1-ol 2a, 3-(2-methoxy-4-(oxiran-2-ylmethyl)phenoxy)propyl acetate 2b, and ethyl 4-(2-methoxy-4-(oxiran-2-ylmethyl)phenoxy)butanoate 2c, were obtained. Compounds 2a–c were isolated as yellow oils in yields of 13 to 57% and were fully characterized by the usual analytical techniques. It stands out that epoxidation of compounds 2a–c was verified by the presence of the protons’ signals related to the oxirane ring (δ 2.52–3.17 ppm) and the absence of the signals of protons for the double bond of eugenol skeleton. The presence of carbon signals relative to the oxirane rings, CH: (δ 46.77–46.78 ppm) and CH: (δ 52.55–52.56 ppm), also confirmed the structure of expected eugenol derivatives 2a–c.

2.2. Toxicity of Eugenol Derivatives

In a general way, all molecules of the 1 and 2 series presented the same activity profile, namely, a mild toxic effect around 30–35% of viability reduction. The exception was 2c, which elicited a loss of around 50% of viability (Figure 1). Considering that 2b and 2c present a rather similar structure, the only difference being the ester type, this feature seems to be of importance, given that the latter was more toxic than the former.
3. Experimental

3.1. Typical Procedure for the Preparation of Compounds 2a–c (Illustrated for 2a)

3-(4-Allyl-2-methoxyphenoxy)propan-1-ol (0.156 g, 7.03 × 10^{-4} mol, 1 eq.) (4 mL) dissolved in dichloromethane was added dropwise to a solution of m-chloroperbenzoic acid (0.346 g, 2.0 × 10^{-3} mol, 1 eq.) in dichloromethane (6 mL) at 0 °C. After stirring for 1 h, a new portion of m-chloroperbenzoic acid was added (1 eq.), and the reaction mixture was stirred for further 12 h. A 10% aqueous solution of sodium sulfate (10 mL) was added and the resulting solution was washed with 5% aqueous solution of sodium hydrogen carbonate (2 × 10 mL). The organic phase was dried with anhydrous magnesium sulfate, and solvent was evaporated giving 3-(2-methoxy-4-(oxiran-2-ylmethyl)phenoxy)propan-1-ol 2a as a yellow oil (0.096 g, 57% yield). \( R_f = 0.58 \) (silica; ethyl acetate). \( ^1H \) NMR (CDCl₃, 400 MHz): \( \delta \)H 2.08 (2H, quint, \( J \) 6.0 Hz, OCH₂C₆H₂CH₂OH), 2.55 (1H, q, C₆H₂oxirane), 2.80–2.85 (3H, m, C₆H₂Ph and C₆H₂oxirane), 3.13–3.17 (1H, m, C₆H oxirane), 3.86 (3H, s, OCH₃), 3.89 (2H, t, \( J \) 5.6 Hz, OCH₂CH₂C₆H₂OH), 4.19 (2H, t, \( J \) 5.6 Hz, OCH₂CH₂OH), 6.71–6.73 (2H, m, H-3 and H-5), 6.84 (1H, d, \( J \) 8.4, H-6) ppm. \( ^1C \) NMR (CDCl₃, 100.6 MHz): \( \delta \)C 31.75 (OCH₂C₆H₂CH₂OH), 38.34 (CH₃Ph), 46.78 (CH₂ oxirane), 52.56 (CH oxirane) 55.83 (OCH₃), 61.57 (OCH₂CH₂CH₂OH), 68.64 (OCH₂CH₂CH₂OH), 112.50 (C-3), 113.45 (C-6), 120.96 (C-5), 130.52 (C-4), 146.95 (C-1), 149.41 (C-2) ppm. HRMS: \( m/z \) (ESI): Calcd. for C₁₃H₁₈NaO₄ [M+Na]+ 261.1097; found 261.1098.

3.2. Procedure for Insecticidal Activity

3.2.1. Cell Culture

Sf9 (Spodoptera frugiperda) cells were cultivated in Grace’s medium with 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin, at 28 °C.

3.2.2. Viability Assessment

For the assessment of viability, a resazurin assay was used. Sf9 cells were plated at a density of 3.0 × 10⁶ cells/well and incubated for 24 h with each molecule. 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. We applied 60 min of incubation.

![Figure 1](image-url)
4. Conclusions

Three new O-alkylated eugenol derivatives, bearing a propyl chain with hydroxyl, methyl and ethyl esters as terminals and further converted into the corresponding oxiranes, were successfully synthetized.

We conducted an evaluation of all derivatives against their effect upon the viability of insect cell line Sf9 (Spodoptera frugiperda), and all molecules of the 1 and 2 series presented a mild toxic effect around 30–35% of viability reduction. Upon comparing all of the compounds, 2c exhibited a loss of around 50% of viability, presenting promising insecticidal activity.

Funding: FCT under project PTDC/ASP-AGR/30154/2017 (POCI-01-0145-FEDER-030154) of COMPETE 2020, co-financed by FEDER and EU. FCT- Portugal and FEDER-COMPETE/QREN-EU for financial support to the research centers CQ/UM (UIDB/00686/2020), CF-UM-UP (UIDB/04650/2020) and REQUIMTE (UIDB/50006/2020). The NMR spectrometer Bruker Avance III 400 (part of the National NMR Network) was financed by FCT and FEDER.

Institutional Review Board Statement: N/A.

Informed Consent Statement: N/A.

Data Availability Statement: N/A.

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

References

1. Vargas-Méndez, L.Y.; Sanabria-Flórez, P.L.; Saavedra-Reyes, L.M.; Merchan-Arenas, D.R.; Kouznetsov, V.V. Bioactivity of semisynthetic eugenol derivatives against Spodoptera frugiperda (Lepidoptera: Noctuidae) larvae infesting maize in Colombia. *Saudi J. Biol. Sci.* 2019, 26, 1613–1620, doi:10.1016/j.sjbs.2018.09.010.

2. Filomeno, C.A.; Barbosa, L.C.A.; Teixeira, R.R.; Pinheiro, A.L.; Farias, E.S.; Ferreira, J.S.; Picanço, M.C. Chemical diversity of essential oils of Myrtaceae species and their insecticidal activity against Rhyzopertha dominica. *Crop. Prot.* 2020, 137, 105309, doi:10.1016/j.cropro.2020.105309.

3. Pavela, R.; Benelli, G.; Canale, A.; Maggi, F.; Mártonfi, P. Exploring essential oils of Slovak medicinal plants for insecticidal activity: The case of Thymus alternans and Teucrium montanum subsp. jailae. *Food Chem. Toxicol.* 2020, 138, 111203, doi:10.1016/j.fct.2020.111203.

4. Mwanauta, R.W.; Mtei, K.A.; Ndakidemi, P.A. Prospective Bioactive Compounds from Vernonia amygdalina, Lippia javanica, Dysphania ambrosioides and Tithonia diversifolia in Controlling Legume Insect Pests. *Agric. Sci.* 2014, 5, 1129–1139, doi:10.4236/as.2014.512123.

5. Ainane, A.; Khammour, F.; Charaf, S.; Elabboubi, M.; Elkouali, M.; Talbi, M.; Benhima, R.; Cherroud, S.; Ainane, T. Chemical composition and insecticidal activity of five essential oils: Cedrus atlantica, Citrus limonum, Rosmarinus officinalis, Syzygium aromaticum and Eucalyptus globules. In Proceedings of the International Conference on Materials and Environmental Science, IC MES 2018 IC MES2018, Mohammed Premier University, Oujda, Morocco, 26–28 April 2018.

6. Silva, F.F.M.; Monte, F.J.Q.; Lemos, T.L.G.; Nascimento, P.G.G.; Costa, A.K.M.; Paiva, L.M.M. Eugenol derivatives: Synthesis, characterization, and evaluation of antibacterial and antioxidant activities. *Chem. Cent. J.* 2018, 12, 34, doi:10.1186/s13065-018-0407-4.

7. Xie, Y.; Huang, Q.; Wang, Z.; Cao, H.; Zhang, D. Structure-activity relationships of cinnamaldehyde and eugenol derivatives against plant pathogenic fungi. *Ind. Crops Prod.* 2017, 97, 388–394, doi:10.1016/j.indcrop.2016.12.043.

8. Saroj, A.; Oryomi, O.V.; Nayak, A.K.; Haider, S.Z. Phytochemicals of plant-derived essential oils: A novel green approach against pests. In *Natural Remedies for Pest, Disease and Weed Control*, Egbru, C., Sawicka, B., Eds.; Elsevier Science: Amsterdam, The Netherlands, 2020; pp. 65–79, doi:10.1016/B978-0-12-819304-4.00006-3.

9. Novato, T.; Gomes, G.A.; Zeringóta, V.; Franco, C.T.; Oliveira, D.R.; Melo, D.; Carvalho, M.G.; Daemon, E.; Monteiro, C.M.O. In vitro assessment of the acaricidal activity of carbacol, thymol, eugenol and their acetylated derivatives on Rhipicephalus microplus (Acari: Ixodidae). *Vet. Parasitol.* 2018, 260, 1–4, doi:10.1016/j.vetpar.2018.07.009.

10. Chen, C.-H.; Tung, S.-H.; Jeng, R.-J.; Abu-Omar, M.M.; Lin, C.-H. A facile strategy to achieve fully bio-based epoxy thermosets from eugenol. *Green Chem.* 2019, 21, 4475–4488, doi:10.1039/C9GC01184F.

11. Maurya, A.K.; Agarwal, K.; Gupta, A.C.; Saxena, A.; Nooreen, Z.; Tandon, S.; Ahmad, A.; Bawankule, D.U. Synthesis of eugenol derivatives and its antiinflammatory activity against skin inflammation. *Nat. Prod. Res.* 2020, 34, 251–260, doi:10.1080/14786419.2018.1528585.