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Original Research Article

Iodination of Eugenol Using Chloramine T as Oxidator

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ABSTRACT

The activity of Eugenol in diagnosing and treating cancers has been investigated. This study aims to synthesise a Eugenol derivative by reacting it with iodide in buffer solvents at pH 5.8, 7 and 8 with chloramine T at 40 mg, 50 mg and 60 mg. The derivatives were further purified by liquid-liquid extraction with aquabidest (double purified distilled water) as the polar phase and chloroform as the organic phase. The extraction was monitored with Thin Layer Chromatography (TLC) and purified with PTLC. The characterisation was done using UV and IR spectrophotometers. The product's organoleptic properties showed that it is a colourless liquid with a chloroform odour. The optimal conditions for the synthesis are pH 8 with 40 mg of chloramine T. The product showed a UV signal at λmax263.1 nm and the FTIR spectrum of C-I at 534.28 cm^{-1}. In addition, the C-O band was observed at 1230.58 cm^{-1} and 1303.88 cm^{-1}. The aromatic C = C at 1452.4 cm^{-1}, 1500.62 cm^{-1} and 1527.62 cm^{-1}, while C = C of an aliphatic alkene band at 1597.06 cm^{-1}. The C-H band was observed at 3259.7 3 cm^{-1}, while the free OH gave a signal at 3356.14 cm^{-1}. Substituted Eugenol compounds can be derived with iodide at an optimum pH of 8 and chloramine T at 40 mg.

Keywords: Eugenol, Iodoeugenol, Chloramine T, Oxidator, Anticancer.

Introduction

In organic synthesis, iodinated compounds are frequently used as reagents. Recently, studies have shown the iodination of organic compounds with elemental iodine or iodides.[1] Iodine isotopes have long been used in imaging and radiation therapy in clinical nuclear medicine.[2] Radioisotopes are isotopes of radioactive substances capable of emitting radiation, can occur naturally (natural radioisotopes) or deliberately (made by humans), and can be made as needed.[3] Isotopic labelling of biomolecules with stable isotope-containing molecules or radioactive compounds are commonly used for detection, assays, mass spectrometry analysis, and imaging.[4] Iodides isotopes include ^{123}I, ^{124}I,^{125}I, ^{129}I and ^{131}I. Some of the isotopes can be used in diagnosis or therapy.[5] For the long-term tracking and imaging of radiolabeled NPs, γ-emitter ^{125}I can be used.[2]

Eugenol (Figure 1) is an aromatic compound with known anticancer activity. It has a pro-apoptotic effect in breast cancer and an anti-proliferative effect in melanoma and colon cancers. [6,7]

Eugenol can be effective and selective in killing cancer cells by incorporating iodide as a ligand.[7,8] The oxidising agent used in this study is chloramine T which enables eugenol to react with iodide. Chloramine has an efficiency of up to 90%. However, chloramine T deficiency can denature protein.[9] This research aims to determine the iodination of eugenol using chloramine T as an oxidant.

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Material and Methods

Materials

Eugenol (Merck), Chloramine T (Merck), Natrium Iodide (Merck), Sodium Metabisulfite (Merck). Chloroform, Ethyl Acetate, potassium Dihydrogen Phosphate, and Sodium Hydroxide are analytical grades, TLC-Silica Gel F_{254} (Merck).

Methods

Eugenolin Buffer Solution

To prepare buffer solutions with pH of 5.8; 7; and 8, 50 mL of 0.2M potassium dihydrogen phosphate was taken into a separate 200 mL volumetric flask. Sodium hydroxide of 3.6 mL, 29.1 mL, and 46.1 mL was added to each flask, as shown in Table 1, and distilled water was added to make up to the mark.

Then, to make eugenol in buffer solution, 2.0 µL of eugenol was added to a beaker with 12.5 mL of phosphate buffer solution to varying pH values (pH 5.8, 7, and 8), and the mixture was swirled until homogenous.

Chloramine T Solution

About 60, 50, and 40 mg chloramine T were dissolved in 12.5 mL phosphate buffer pH 5.8, 7, and 8 to obtain 4.8, 4, and 3.2 mg/mL concentrations.

Sodium Metabisulfite Solution

120 mg of sodium metabisulfite was dissolved in 50 mL phosphate buffer pH 5.8, 7, and 8, respectively, to obtain a 2.4 mg/mL concentration.

NaI Solution

Similarly, 4.75 mg of NaI were dissolved in 0.5 mL of phosphate buffer pH 5.8, 7, and 8.

Iodination of Eugenol

Eugenol solution in phosphate buffer with pH variations of 5.8, 7, and 8, respectively, was taken into a beaker, followed by 0.5 mL of sodium iodide solution, and then chloramine T at various variations, 60 mg/12.5 mL, 50 mg/12.5 mL, and 40 mg/12.5 mL, respectively. The solutions were stirred with a magnetic stirrer for 30 seconds. After the mixture

was homogeneous, 50 mL of sodium metabisulfite was added and stirred with a magnetic stirrer for 10 seconds. The resulting solutions were extracted two times by partitioning into chloroform and water. The organic phase containing the product was collected for further purification and characterisation.

Thin Layer Chromatography
The product was spotted using a capillary tube on a GF 254 silica TLC plate and developed in a solvent system of chloroform: ethyl acetate (1:1).

Preparative Thin Layer Chromatography
Bands of the product solution were spotted on the plate and eluted using ethyl acetate (1:1) chloroform. The resolved band on the PTLC was scraped and dissolved in chloroform.

UV Spectroscopy
The absorbance of an aliquot of the product solution was measured in a UV-1700 Pharma (Shimadzu) spectrophotometer against chloroform.

IR Spectroscopy
A few mL of the product solution was evaporated and dispersed in 198 mg of previously dried KBr. The disc %T was measured using FTIR (IRAffinity-1 Shimadzu).

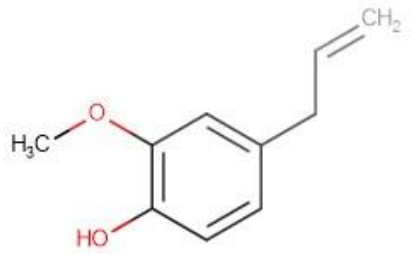

Figure 1: The Structure of eugenol (Marvin sketch 19.8)

Table 1: The Volume of NaOH into Buffer Solution[9]

pH	5.8	7	8
NaOH (mL)	3.6	29.1	46.1

Result and Discussion

The electrophilic substitution in an iodination reaction, specifically, the positively charged iodine (I+), attacks a system with a high electron density, such as an aromatic ring or double bonds, forming a carbon-iodide bond to release positively charged species. Because of its high electron density, the iodide atom has a high propensity to attack alpha carbon in aromatic compounds due to its high electronegativity value and bond strength.[10] An OH group also functions as an ortho and para-driving substituent.[11] Figure 2 depicts the predicted reaction.[9,10]
The reaction results were monitored using Thin Layer Chromatography (TLC) which uses the retention factor (Rf). The best Rf value was between 0.2-0.8. The optimisation of the mobile phase chosen was chloroform because it gave an Rf value of 0.67 for all the derivatives, while for the standard of eugenol, the Rf value was 0.9. The chloroform fraction has not yet got a spot from TLC monitoring from the ECC results (Figure 3).
The products from PTCL were scraped off and identified by UV/Vis spectrophotometer using eugenol as standard for comparison (Figure 4). Table 2 shows that Chloramine T at 40 mg and pH 8 produced the highest product yield. It is assumed that iodo-eugenol will develop under the previously described conditions. Chloramine T tends to be substituted in iodine above pH 8.
The IR spectrum of the product (Figure 5) showed a specific broad band of C-I at 534.28 cm-1, C-O at 1230.58 cm-1 and 1303.88 cm-1, C = C aromatic at wave numbers 1452.4cm^{-1}, 1500.62 cm-1 and 1527.62 cm-1, C = C alkenes at 1597.06 cm-1, C-H at 3259.7 cm^{-1}, and free OH at 3356.14 cm-1. These peaks are comparable to that of standard Eugenol.[11]
TLC results show a difference between the Rf of the synthetic compounds and the eugenol standard, and the UV-Vis and IR spectra show a similar pattern with eugenol. However, a new peak, specifically the C-I bond, was observed. Based on this, it is reasonable to assume that the synthesised compounds differ from eugenol.

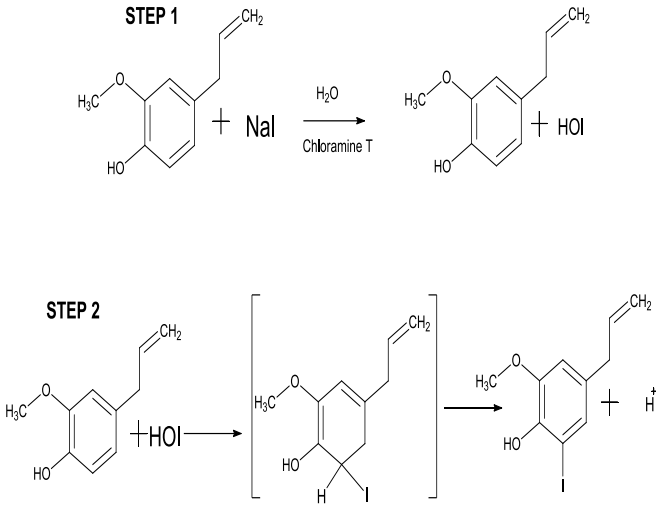

Figure 2: Reaction scheme of iodination of eugenol

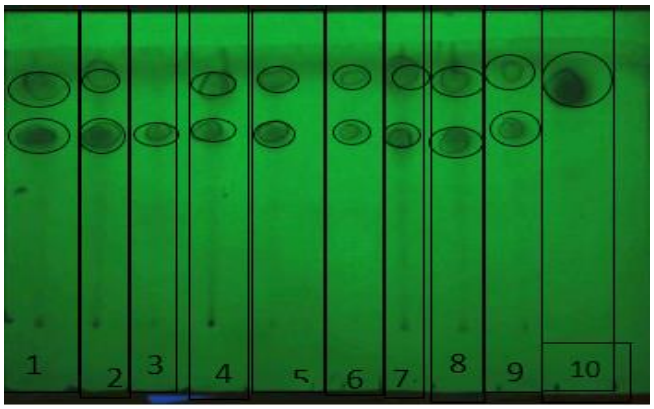

Figure 3: TLC profile of the products using chloroform as eluting solvent
Description:
1 = the results of the synthesis pH 5.8 with chloramine T 40mg
2 = the results of the synthesis pH 5.8 with chloramine T 50mg
3 = the results of the synthesis pH 5.8 with chloramine T 60 mg
4 = the results of the synthesis pH 7 with chloramine T 40mg
5 = the results of the synthesis pH 7 with chloramine T 50mg
6 = the results of the synthesis pH 7 with chloramine T 60mg
7 = the results of the synthesis pH 8 with chloramine T 40 mg
8 = the results of the synthesis pH 8 with chloramine T 50mg
9 = the results of the synthesis pH 8 with chloramine T 60mg
10= Standard of Eugenol

Conclusion

Substituted Eugenol compounds can be derived with iodide at an optimum pH of 8 and chloramine T at 40 mg. Based on the data obtained, it can be concluded that eugenol's potential can be improved by reacting it with iodine. This research can be used as a reference in developing an anticancer product with an iodine isotope. The chemical reaction between eugenol, non-isotope, and isotope iodine is an electrophilic substitution reaction. This study suggests that cancer treatment with the isotope iodine marker eugenol can be achieved in the future.

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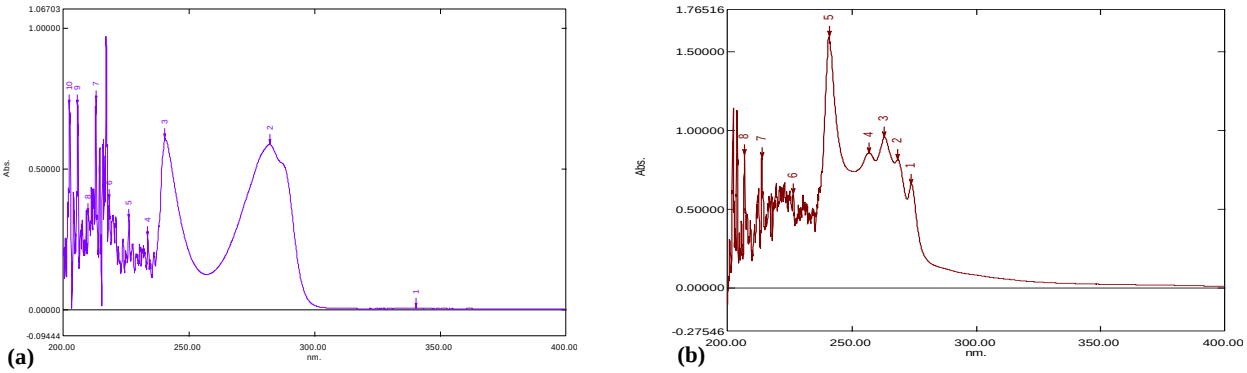

Figure 4: (a) Spectrum of Eugenol in Chloroform; (b) Spectrum of derived product at pH 8 and Chloramine T at 40 mg.

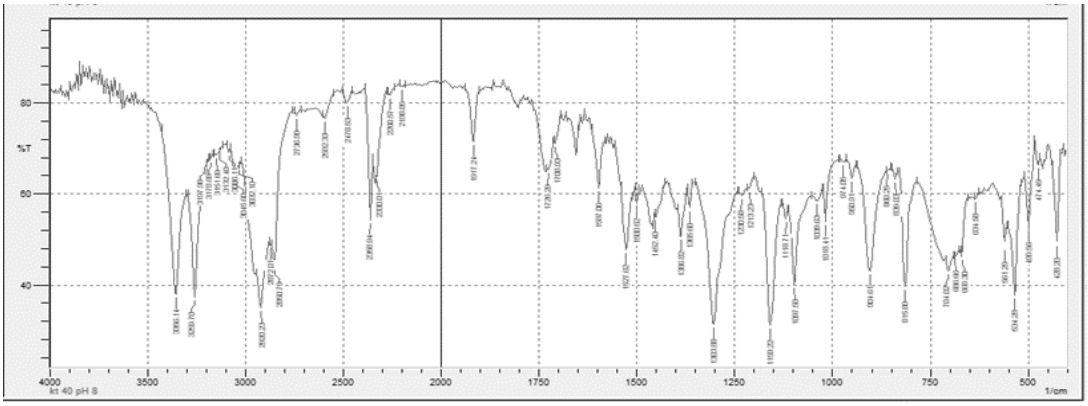

Figure 5: (a) Spectrum of Eugenol in Chloroform; (b) Spectrum of synthesis result pH 8 Chloramine T 40 mg.

Table 2: Synthetic product yields at various reaction conditions.

pH	Chloramine T (mg)	Yield (%)
5.8	40	12.41
	50	17.34
	60	20.06
7	40	8.50
	50	9.25
	60	6.375
8	40	162.87
	50	12.92
	60	9.69

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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