Oxidation reactions of cyclopent-2-en-1-yl thiophene derivatives

Reacciones de oxidación de derivados de ciclopent-2-en-1-iltiofeno

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ABSTRACT

Introduction. Thiophene derivatives are common in a large number of natural compounds. In addition, their biological activity provides great opportunities for synthetic organic chemistry. In particular, cyclopentenylthiophenes have high antimicrobial activity, can affect various inflammatory mechanisms, and can be effective in the treatment of cancer. However, in research, it is essential to first learn how to obtain compounds in pure form and to select such synthesis conditions that, when scaled up and introduced into widespread production, the affordability and safety of the processes would meet the needs of both consumers and pharmaceutical companies. **Material and Methods.** As an oxidant, meta-chloroperoxybenzoic acid was used, which allowed exploring its oxidative properties and advantages of use in more detail. **Results and Discussion.** In the course of this work, several cyclopentenylthiophene derivatives were synthesised, the ratio of isomers in the synthesis of these compounds was analysed, and the advantages and disadvantages of the synthesis methods were discussed. On the other hand, there are several issues that require additional research, including the effect of temperature conditions, electrophilicity of substituents and other factors on the course of the epoxidation reaction for cyclopentenylthiophenes. **Conclusion.** The research of thiophene derivatives is relevant both from the standpoint of exploring electronic effects when substituents with different acceptor properties are introduced into the heterocyclic aromatic system and from the standpoint of medicinal chemistry, as they are interesting biologically active objects with high chemical stability, good penetration of cell membranes, ability to affect enzyme activity, bind to receptors.

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INTRODUCTION

Thiophene (C_4H_4S) is considered one of the basic representatives of five-membered heterocycles with one heteroatom. Its stability and high resistance to acid solutions enable most conventional reactions to be performed with ease. The sulphur atom stabilises the aromatic system due to its large size and moderate electronegativity, giving it new properties. In addition, this effect is observed in biological systems, namely in the possibility of easy binding to the functional groups of various macromolecules. It is important to note that thiophene derivatives have applications in the dye, pharmaceutical, and agrochemical industries. They have been investigated for various bioactivities, including antimicrobial, antiviral, anti-inflammatory, larvicidal, antioxidant, insecticidal, cytotoxic, and nematicidal effects.

Medicines containing thiophene fragments are already actively used in medical practice, in the review by R. Shah and P.K. Verma [1] reviewed its therapeutic benefits as antibiotics (Cephalothin), anti-inflammatory drugs, in particular, for the prevention of rheumatoid arthritis (Tinoridine), and non-steroidal anti-inflammatory drugs (Tenoxicam). In addition, in their research, R.M.D. da Cruz et al. [2] note the ability of several thiophene derivatives to inhibit the production of inflammatory cytokines, adding relevant *in vivo* and *in vitro* experimental data. The modification of the latter and the search for new drugs designed to solve problems associated with dangerous diseases at this stage of the development of medicinal chemistry can be called the primary tasks, as noted in their review by S. Chawla et al. [3]. In addition, cyclopentenylthiophenes have been found to have significant anticancer activity. Some studies indicate their ability to inhibit the growth and spread of cancer cells in the laboratory. In particular, the research group of N. Auld et al. [4] explored the ability of compounds with a thiophene fragment to exhibit cytotoxicity in myeloid leukaemia, noting a number of positive results. It may indicate the possibility of using these compounds in the fight against particular types of cancer.

In particular, it is essential to expand the variety of functional groups introduced into thiophene substrates, analysing both their effect on the biological activity of molecules and the direct change in the chemical properties of such compounds for their further modernisation [5]. Molecules containing an epoxy group play a leading role in modern research in chemistry and pharmaceuticals. Their unique structure gives them several physicochemical and biological properties that are of great interest to researchers. Epoxides can interact with enzymes, which leads to modification of their activity [6]. They can act as inhibitors or activators of enzymatic reactions, which will affect various biochemical processes, including metabolism, and establish covalent bonds with DNA and RNA nucleotides, which can lead to modification of genetic material and significantly affect the processes of replication, transcription and translation of genetic information [7].

From a chemical standpoint, epoxides are typical electrophiles that are capable of establishing open-ring products with the participation of nucleophiles [8]. In the context of organic synthesis, using epoxides as electrophilic intermediates with induced ring deformation presents great opportunities for stereoselective and specific development of key bonds in complex molecules, examples of which are given in F. Moshona et al. [8]. It makes them excellent reactive intermediates in organic synthesis. It will help to expand the functionality of thiophene derivatives for further molecular docking.

This research work was designed to introduce new approaches and solutions for the epoxidation of cyclopentenylthiophenes and can make a significant contribution not only to the development of modern chemistry but also to pharmaceutical science. The significance of this research is the possibility of identifying new biologically active compounds and disseminating knowledge about their structure, activity, methods of preparation and analysis. Its results may be of great significance for the further development of new drugs and therapeutic strategies.

The purpose of the research is to identify the most effective catalyst capable of providing high activity and selectivity of the reaction. The main tasks of the study are:

- to determine optimal oxidation conditions for cyclopent-2-en-1-yl thiophene;
- to explore catalyst effects on oxidation of thiophene derivatives;
- to evaluate how structural changes impact reaction activity;
- to identify key structural features influencing product selection.

MATERIALS AND METHODS

Purchase and storage of reagents

The reagents used for the experiments were purchased from Sigma-Aldrich, a chemical company with a good reputation for providing high quality products. However, the storage and transportation of some of the compounds required particular attention. In particular, mCPBA had an initial purity of 77%, which is a reasonable option for the safety of transporting this reagent. Due to the sensitivity of the epoxidation reaction, the reagent was further purified by recrystallisation in acetone. After the mCPBA was dissolved, the solution was cooled to 0 ℃ to facilitate the precipitation of pure mCPBA as crystals. The crystals were filtered and washed with chilled acetone. The purity was checked by ^{1}H NMR in deuterated chloroform. The mCPBA was stored in a specially equipped refrigerator protected from direct sunlight and heat at -20℃. In addition, it was essential to store it in a hermetically sealed container to avoid interaction with air and moisture, which can reduce its activity.

Analysis of the structures of the obtained substances

The structures of the obtained substances were determined using ^{13}C and ^{1}H NMR spectroscopy, 2D methods, namely NOSY spectra, deuterated dimethyl sulfoxide, chloroform, trifluoroacetic acid on a DRX-400 "Bruker" device, tetramethylsilane was used as solvents, and tetramethylsilane was used as an internal standard. Gas chromatography-mass spectrometry was performed using dichloromethane as a solvent, flame ionisation detector, Agilent Technologies. The current control of the reactions was conducted using TLC on Silufol-UV254 silica gel plates. Benzene, acetone and diethyl ether were dried and purified by distillation at atmospheric pressure before use. Isopropanol-2, dichloromethane, ethanol, hexane, methanol, acetic acid and chloroform were not purified before use. For the chromatographic separation of epimers, a glass column 20 mm in diameter and 500 mm long filled with 50 g of Silicagel 60 (0.063-0.2 mm), pH 6.5-7.5 (MERCK) was used to separate the mixture. The obtained results of the research indicate the successful determination of the structures of substances using NMR spectroscopy and GCMC methods. The solvents and instruments used, such as deuterated dimethyl sulfoxide, chloroform, trifluoroacetic acid and DRX-400 (Bruker), respectively, allowed obtaining reliable data.

Processing of research results

The research results were processed using software: MestReNova, ChemOffice, namely ChemDraw, Bruker TopSpin and MATLAB. Working with MestReNova allowed for fast and high-quality processing of NMR spectra, in particular through a wide range of tools for processing, phase correction, integration, analysis and determination of chemical shifts and structural parameters of the compounds presented in it. Intermediate control of the reaction mixtures was performed by ^{1}H NMR and GCMS. These methods are effective for the analysis of organic compounds with low molecular weight and aliphatic structure. Solvents were selected in which the reaction products were more stable, namely $CDCl_3$, CH_2Cl_2, CH_3OH, and CH_3CN.

Based on the results of the analyses, appropriate decisions were made regarding further purification of the

substrate. If the purity of the product was satisfactory, it was used either for the next process or for the final analytical report (purity, appearance, weight).

List of abbreviations

mCPBA – meta-Chloroperoxybenzoic acid; NOSY – Nuclear Overhauser Effect Spectroscopy; HNMR – Hydrogen Nuclear Magnetic Resonance; GCMS – Gas Chromatography Mass Spectrometry; $CDCl_3$ – deuterated chloroform; CH_2Cl_2 – dichloromethane; CH_3OH – methanol; CH_3CN– acetonitrile; DMDO – dimethyldioxirane.

RESULTS

Experiment course

The determination of the oxidation reaction of cyclopent-2-en-1-yl thiophene derivatives was performed in several stages.

2-(Cyclopent-1-en-3-yl)thiophene (3). To a solution of 8 mL (0.1 mol) of thiophene (1) in 5.12 mL of 85% phosphoric acid and 10 mL of chloroform was added over 2.5 hours under stirring and at 18-30□ a solution of 8.21 mL (0.1 mol) of cyclopentadiene (2) and 7.81 mL (0.1 mol) of thiophene (1) in 10 mL of chloroform. After addition, stirred for another 1.5 hours. It was left for 22 hours. Next, the reaction mixture was washed with water, 5% soda solution and water again until the aqueous layer reacted neutrally. It was dried with anhydrous sodium sulfate. The solvent was removed under vacuum. GCMS: product (3) 5.677 min, m/z 150, 83.85%. Distillation was performed under 28 mmHg at 100-112 ^{0}C. ^{1}H NMR (400 MHz, Chloroform-d) δ 1.85 (m, 1H), 2.40 (qd, 2H), 2.51 (m, 1H), 4.17 (td, 1H), 5.82 (m, 1H), 5.91 (dt, 1H), 6.79 (d, 1H), 6.91 (dd, 1H), 7.11 (d, 1H). Yield 5.47 g (36.5%).

2-(Cyclopent-1-en-3-yl)-5-methylthiophene (14). To a solution of 9.7 mL of methylthiophene (13) in 5.12 mL of 85% phosphoric acid and 10 mL of chloroform was added over 2.5 hours under stirring and at 19-30□ a solution of 8.21 mL (0.1 mol) of cyclopentadiene (2) and 9.6 mL (0.1 mol) of thiophene (1) in 10 mL of chloroform. It was left for 22 hours. The reaction mixture was washed with water, 5% soda solution and water until the aqueous layer was neutral. It was dried with anhydrous sodium sulfate. The solvent was removed in vacuo. GCMS: product (14) 6.4 min, m/z 164. Distilled at 30 mmHg, collecting the fraction at 102-122□. ^{1}H NMR (400 MHz, Chloroform-d) δ 1.85 (m, 1H), 2.4 (m, 2H), 2.44 (s, 3H), 2.51 (m, 1H), 4.09 (m, 1H), 5.81 (dt, 1H), 5.9 (m, 1H), 6.57 (m, 2H). Output 6.29 g (38.3%).

Oxidation of 2-(Cyclopent-1-en-3-yl)thiophene (3). To a solution of 0.5 g (0.0033 mol) (3) in 10 mL of methylene chloride was added a solution of 1.21 g (0.007 mol) of m-chloroacetic acid (mCPBA) in 20 mL of methylene chloride under ice and stirring for 2 hours. The solution was stirred at 0 ^{0}C for another 0.5 hours. The precipitated m-chlorobenzoic acid was filtered off. The filtrate was washed with 5% solutions of sodium sulfite, sodium hydroxide and water until the aqueous layer reacted neutrally. The solvent was removed under atmospheric pressure. The yield was 1.67 g. GCMS: compound (3) 5.68 min, m/z 150 – 21.8%; compound (6a) 7.33 min, m/z 166 – 35.29%; compound (6b) 7.65 min, m/z 166 – 43.53%. Similarly, (Experiment I) with the ratio (3): mCPBA=1:1.5, m-chlorobenzoic acid precipitated very slowly, thus, after addition, the reaction mixture was kept at 17□ for 2 hours. Similarly, (Experiment I) with the ratio (3): mCPBA=1:0.5.

Chromatographic separation of a mixture of epimers of (1S, 2S, 5S)-2-(thiophene-2-yl)-6-oxabicyclo[3.1.0] hexane (6a) and (1R, 2S, 5S)-2-(thiophene-2-yl)-6-oxabicyclo[3.1.0]hexane (6b). The mixture of substances obtained in Experiment I (0.5 g) was preliminarily analysed by TLC (silufol, eluent hexane/ethyl acetate

10:1): alkene (3) R_f=0.85, epoxy (6a) R_f=0.37, epoxy (6b) R_f=0.46. A 20-mm-diameter, 500-mm-long glass column filled with 50 g of Silicagel 60 (0.063-0.2 mm), pH 6.5-7.5 (MERCK) was used to separate the mixture. Fractions:

- 0.05 g, R_f=0.85, starting alkene (3);
- 0.12 g, R_f=0.46, epoxy (6b); PMR: ^{1}H NMR (400 MHz, Chloroform-d) δ 1.67 (d, 1H), 1.87 (m, 2H), 2.08 (q, 1H), 3.53 (s, 1H), 3.61 (s, 1H), 3.71 (d, 1H), 6.81 (d, 1H), 6.94 (t, 1H), 7.16 (d, 1H).0.17 g, mixture of (6a) and (6b);
- 0.16 g, R_f=0.37, epoxy (6a). PMR: ^{1}H NMR (400 MHz, Chloroform-d) δ 1.53 (m, 1H), 1.75 (m, 1H), 1.96 (m, 1H), 2.19 (dq, 1H), 3.42 (t, 1H), 3.56 (d, 2H), 6.95 (m, 2H), 7.16 (d, 1H).

Epoxidation of 2-(Cyclopent-1-en-3-yl)-5-methyl-thiophene (14). To a solution of 0.82 g (0.005 mol) (14) in 10 mL of methylene chloride was added a solution of 0.86 g (0.005 mol) of m-chloro-pentobenzoic acid (mCPBA) in 10 mL of methylene chloride under ice and stirring for 2 hours. The solution was stirred at 0 for another 0.5 hours. The precipitated m-chlorobenzoic acid was filtered off. The filtrate was washed with 5% solutions of sodium sulfite, sodium hydroxide and water until the aqueous layer reacted neutrally. The solvent was removed under atmospheric pressure.

Chromatographic separation of a mixture of epimers (1S, 2S, 5S)-2-(5-methyl-thiophen-2-yl)-6-oxabicyclo[3.1.0]hexane (16a) and (1R, 2S, 5S)-2-(5-methyl-thiophen-2-yl)-6-oxabicyclo[3.1.0]hexane (16b). The mixture of substances obtained in experiment 1 (0.5 g) was preliminarily analysed by thin-layer chromatography (silufol, eluent hexane/ethyl acetate 10:1): alkene (14) R_f=0.89, epoxy (16a) R_f=0.37, epoxy (16b) R_f=0.54, sulfoxide (15) R_f=0.16. For the separation of the mixture, a glass column with a diameter of 20 mm and a length of 500 mm filled with 50 g of Silicagel 60 (0.063-0.2 mm), pH 6.5-7.5 (MERCK) was used. Fractions:

- 0.18 g, R_f=0.89, starting alkene (14); PMR: ^{1}H NMR (500 MHz, Chloroform-d) δ 1.86 (dp, 1H), 2.39 (h, 2H), 2.45 (s, 3H), 2.53 (dtd, 2H), 4.11 (dd, 1H), 5.82 (t, 1H), 5.91 (dd, 1H), 6.57 (d, 1H), 6.60 (d, 1H);
- 0.07 g, R_f=0.46, epoxide (16b); PMR: ^{1}H NMR (500 MHz, Chloroform-d) δ 1.68 (p, 1H), 1.87 (m, 2H), 2.09 (q, 1H), 2.46 (s, 3H), 3.53 (d, 1H), 3.63 (dd, 2H), 6.61 (t, 2H);
- 0.07 g, R_f=0.37, epoxide (16a). PMR: ^{1}H NMR (500 MHz, Chloroform-d) δ 1.53 (m, 1H), 1.75 (m, 1H), 1.94 (dd, 1H), 2.2 (dd, 1H), 2.46 (s, 3H), 3.36 (t, 1H), 3.56 (m, 2H), 6.60 (d, 1H), 6.75 (t, 1H);
- 0.05 g, R_f = 0.16, sulphoxide (15).

2-Iodo-thiophene (7). Synthesised according to the method of H.Y. Lew and C.R. Noller [9]. 2-(Cyclopent-1-en-1-yl)thiophene (10). Synthesis of Grignard reagent (8). To the pellets of 2.5 g (0.1 mol) of metallic magnesium in 50 mL of dry diethyl ether was added ≈2 mL of a solution of 2-iodo-thiophene (7) in 20 mL of diethyl ether and heated to the beginning of boiling. After the reaction started, the heat was removed and the solution (7) was added under stirring for ≈30 minutes. Stirred at boiling until all magnesium was dissolved. Synthesis of 2-(cyclopent-1-hydroxy-1-yl)thiophene (9) and 2-(cyclopent-1-en-1-yl)thiophene (10). A solution of 7.1 ml (0.08 mol) of cyclopentanone was added slowly to the solution of 2-thiophene magnesium iodide (8), stirring and cooling with cold water. After adding the entire solution, the mixture was boiled for 1 hour. While cooling with ice, water and 1:1 dilute hydrochloric acid were added to the reaction mixture until the precipitate was completely dissolved. The organic layer was separated and washed with water, 5% soda ash solution and water until the aqueous layer was neutral. It was dried over sodium sulfate. The solvent was removed under atmospheric pressure from the flask for vacuum distillation. The residue

was distilled at 23 mmHg to give a fraction of 111-115□. ^{1}H NMR (500 MHz, DMSO-d_6) **δ** 1.64 (p, 2H), 2.47 (qt, 2H), 3.01 (tq, 2H), 5.99 (tt, 1H), 7.07 (dd, 1H), 7.26 (dd, 1H), 7.48 (dd, 1H). ^{13}C NMR (125 MHz, Chloroform-d) δ 26.17, 30.87, 33.49, 124.08, 124.16, 126.72, 127.82, 137.40, 140.15. Output of alkene (10) 6.84 g (45.6%).

2-Iodo-5-methyl-thiophene (17). Synthesised according to the method of H.Y. Lew and C.R. Noller [9]. 2-(Cyclopent-1-en-1-yl)-5-methyl-thiophene (20). Synthesis of Grignard reagent (18). To the pellets of 2.5 g (0.10 mol) of metallic magnesium in 50 ml of dry diethyl ether was added ≈2 ml of a solution of 2-iodo-5-methyl-thiophene (17) in 20 ml of diethyl ether and heated to the beginning of boiling.

After the reaction started, the heat was removed and the solution (17) was added under stirring for ≈30 minutes. Stirred at boiling until all magnesium was dissolved. Synthesis of 2-(cyclopent-1-hydroxy-1-yl)-5-methyl-thiophene (19) and 2-(cyclopent-1-en-1-yl)-5-methyl-thiophene (20). A solution of 7.1 ml (0.08 mol) of cyclopentanone was slowly added to the solution of 5-methyl-thiophene-2-magnesium iodide (18) under stirring and cooling with cold water. After adding the entire solution, the mixture was boiled for 1 hour. While cooling with ice, water and 1:1 dilute hydrochloric acid were added to the reaction mixture until the precipitate was completely dissolved.

The organic layer was separated and washed with water, 5% soda ash solution and water until the aqueous layer was neutral. It was dried over sodium sulfate. The solvent was removed under atmospheric pressure from the flask for vacuum distillation. The residue was distilled at 22 mmHg, taking the fraction at 115-125□. Output of alkene (20) was 9.4 g (57.3%). GCMS: product (20) 7.33 min, m/z 164.1. ^{1}H NMR (400 MHz, Chloroform-d) **δ** 1.98 (m, 2H), 2.44 (s, 3H), 2.47 (d, 2H), 2.65 (m, 2H), 5.88 (m, 1H), 6.59 (d, 1H), 6.68 (d, 1H).

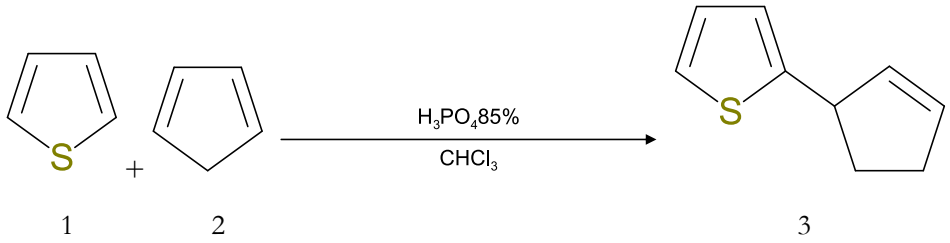
Epoxidation of 2-(Cyclopent-1-en-1-yl)thiophene (10). To a solution of 0.5 g (0.0033 mol) (10) in 10 mL of methylene chloride was added a solution of 1.13 g (0.0066 mol) mCPBA in 20 mL of methylene chloride under ice and stirring for 2 hours. The solution was stirred at 0 ^{0}C for another 0.5 hours. The precipitated mCPBA was filtered off. The filtrate was washed with 5% solutions of sodium sulfite, sodium hydroxide, and water until the aqueous layer was neutral. The solvent was removed under atmospheric pressure. Output 0.79 g, of which sulfoxide epoxide (11) is 24% and ketone (12) is 29%.

Epoxidation of 2-(Cyclopent-1-en-1-yl)-5-methyl-thiophene (20). To a solution of 0.82 g (0.005 mol) (20) in 10 mL of methylene chloride was added a solution of 0.86 g (0.005 mol) mCPBA in 10 mL of methylene chloride under ice and stirring for 2 hours. The solution was stirred at 0□ for another 0.5 hours. The precipitated mCPBA was filtered off. The filtrate was washed with 5% solutions of sodium sulfite and sodium hydroxide, then with water until the aqueous layer was neutral. The solvent was removed under atmospheric pressure. Output 0.85 g.

Comments on the experiment stages

To obtain the epoxides, the synthesis of the corresponding alkenes from commercially available compounds was performed first. In particular, the preparation of 2-(cyclopent-1-en-3-yl)thiophene (3) was performed under acid catalysis. The establishment of a stable intermediate contributed to the development of a new bond between cyclopentadiene and thiophene, due to which the chemical equilibrium was shifted towards the establishment of the target product without an additional temperature increase **(Figure 1)**.

Figure 1. Obtaining 2-(cyclopent-1-en-3-yl)thiophene



Source: compiled by the authors.

This type of reaction is a Diels-Alder reaction, which is a cycloaddition where a diene (in this case, cyclopentadiene) reacts with a dienophile (in this case, the thiophene) to form a six-membered ring. The product has a sulfure atom in the five-membered ring, which is attached to a six-membered carbon ring. The double bond in the six-membered ring is positioned between the two carbon atoms that were not part of the original cyclopentadiene molecule.

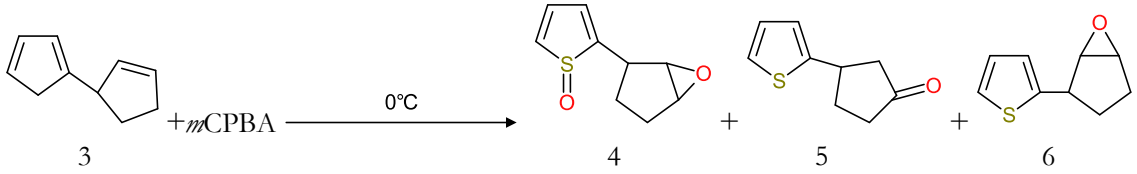
One of the key tasks of the research was to select the optimal oxidant that would meet several criteria. In addition to selectivity, the safety of such a reagent and its ease of use in practical reactions were of great importance. In addition, it was essential to choose a compound that is commercially available to ensure the possibility of obtaining a variety of derivatives and scaling up the synthesis for further implementation of these methods for the synthesis of medicines.

Considering the previous paragraphs, mCPBA was used for the research. In contrast to hydrogen peroxide syntheses, in which the process of isolating the target product involves the evaporation of an explosive mixture, the reaction with mCPBA is much safer [10]. Another advantage is the difference in physical properties between the superacid and the corresponding acid. Considering that the epoxidation of alkenes is mainly performed in dichloromethane or chloroform, the high solubility of mCPBA in these solvents allows for homogeneity of the solution, while reduced 3-chlorobenzoic acid demonstrates low solubility and precipitates [11]. Thus, it is very easy to separate the reaction by-product by filtration and additional washing with water or extraction with alkaline aqueous solutions.

On the other hand, such oxidation demonstrates low selectivity, but using expensive catalysts can significantly increase the cost of the final product. During the reaction of compound (3) with different molar ratios of mCPBA, reaction mixtures with different product ratios were obtained, which were then isolated in pure form by chromatographic separation.

When the synthesis was performed with an excess of mCPBA (2 mol) at n.c., the establishment of sulfoxide (4), cyclopentenone (5) and a mixture of epimers (1S, 2S, 5S)-2-(thiophene-2-yl)-6-oxabicyclo[3.1.0]hexane (6a) and (1R, 2S, 5S)-2-(thiophen-2-yl)-6-oxabicyclo[3.1.0]hexane (6b) **(Figure 2)**. However, despite the excess of the oxidising agent, the equilibrium between the starting compound (3) and the reaction products was maintained. For more stable substrates, it is advisable to increase the temperature of the reaction, but in the case of 2-(cyclopent-1-en-3-yl)thiophene, such an increase in temperature will promote the polymerisation of the compound.

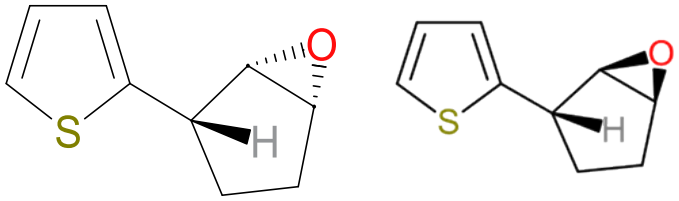
Figure 2. Oxidation of 2-(cyclopent-1-en-3-yl)thiophene from 2 mol of metha-chloroperoxybenzoic acid



Source: compiled by the authors.

The racemic composition of 2-(thiophene-2-yl)-6-oxabicyclo[3.1.0]hexane (6) indicates a synchronous attack of the oxidising agent on the double bond, which in the present work allows isolating and exploring both epimers **(Figure 3)**.

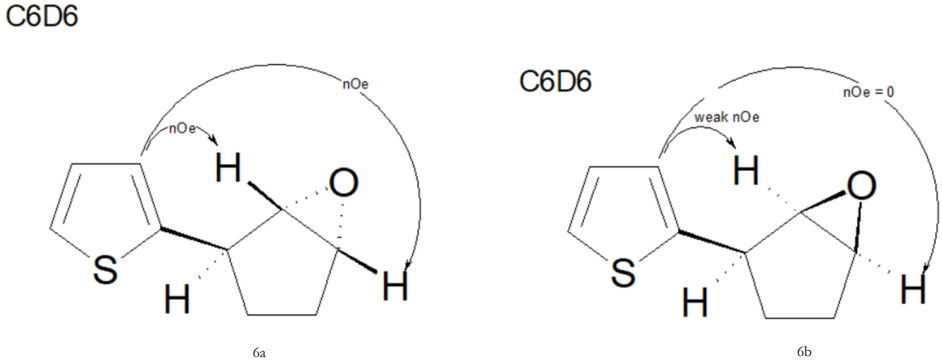
Figure 3. Structures of epimers



Note: 6a) (1S, 2S, 5S)-2-(thiophen-2-yl)-6-oxabicyclo[3.1.0]hexane; 6b) (1R, 2S, 5S)-2-(thiophen-2-yl)-6-oxabicyclo[3.1.0]hexane.
Source: compiled by the authors.

To explore the exact structures of the epoxies and confirm them according to the previous assumptions, 2D-NOESY spectroscopy was used, which is a powerful tool for elucidating the spatial structure, as it can be used to identify pairs of nuclei that are close to each other. The spectrum demonstrated correlations between the aromatic proton of thiophene and the corresponding protons near the epoxide. Notably, for the compound **(Figure 4, 6a)**, such correlations were observed for both protons at the epoxy group, while for (1R, 2S, 5S)-2-(thiophen-2-yl)-6-oxabicyclo[3.1.0]hexane **(Figure 4, 6b)** the interaction was weak and almost imperceptible.

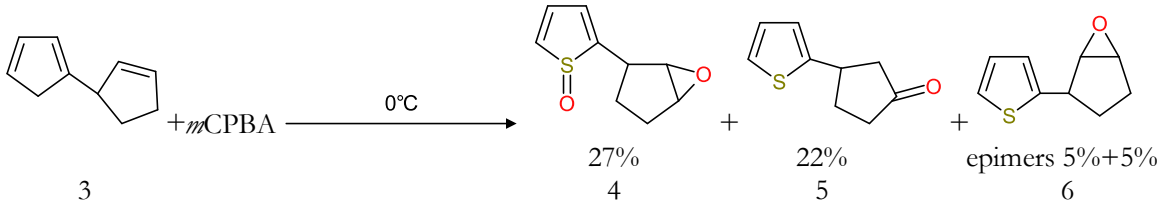
Figure 4. Protons between which the nuclear Overhauser effect occurs



Source: compiled by the authors.

When the excess of mCPBA was reduced to 1.5 mol per 1 mol of 2-(cyclopent-1-en-3-yl)thiophene, an increase in the establishment of sulfoxide (4) was observed due to a decrease in the establishment of cyclopentenone (5), but the equilibrium was maintained **(Figure 5)**.

Figure 5. Oxidation of 2-(cyclopent-1-en-3-yl)thiophene from 1.5 mol of metha-chloroperoxybenzoic acid

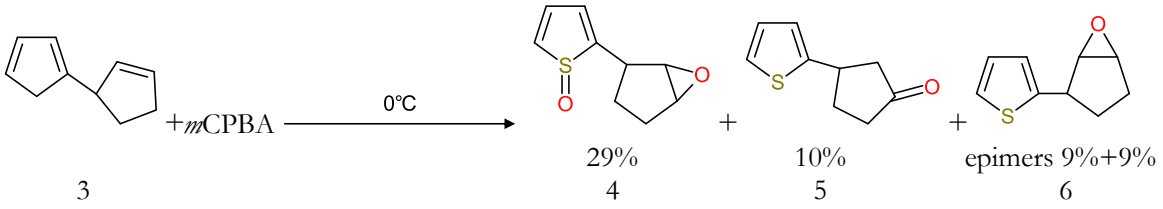


Source: compiled by the authors.

In this scheme, the starting material is an organic compound with a cyclic structure that includes a benzene ring (a hexagon with alternating double bonds) attached to a five-membered ring. The reagent used is mCPBA, which stands for meta-chloroperoxybenzoic acid, a compound often used to introduce an oxygen atom into organic molecules, typically creating epoxides or converting double bonds to epoxides in a reaction known as epoxidation. The reaction is carried out at 0 degrees Celsius, which suggests that the reaction conditions require low temperature, possibly to control the reaction rate or to stabilize the products.

When the reaction is conducted with an oxidant deficiency (0.5 mol), the ratio of products shifts towards the establishment of sulfoxide (4), but the proportion of epoxide (6) increases while maintaining the racemic composition **(Figure 6)**.

Figure 6. Oxidation of 2-(cyclopent-1-en-3-yl)thiophene from 0.5 mol of metha-chloroperoxybenzoic acid



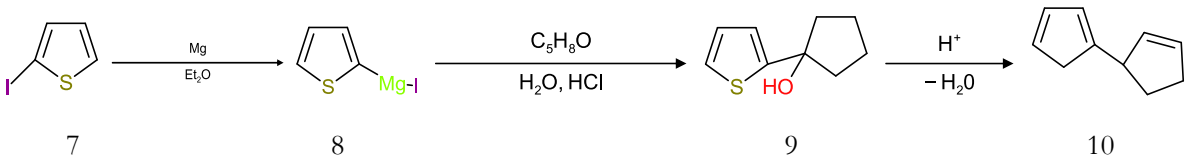
Source: compiled by the authors.

The figure 6 shows a chemical reaction involving compound 3 and mCPBA at 0°C, producing three products with varying yields. Compound 4 is the main product, while compound 5 results from a side reaction. Compound 6 has two major stereoisomers, indicating the influence of reaction conditions and stereochemistry on product formation.

In addition, the position of the double bond in the cycle has a significant impact on the establishment of epoxy products. Thus, for 2-(cyclopent-1-en-1-yl)thiophene, obtained from 2-iodo-thiophene **(Figure 7)**,

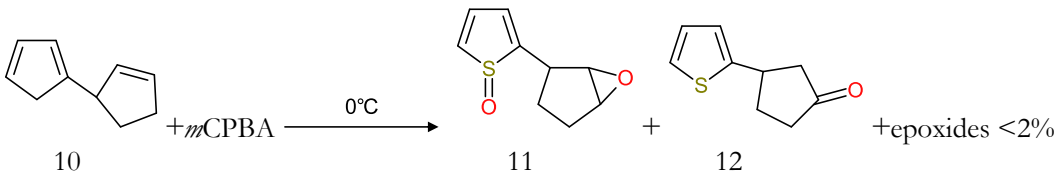
only trace amounts of epoxide were established, and the main product was cyclopentenone (12) **(Figure 8)**. It was caused by steric hindrances that can arise during the establishment of a stressed cycle and the intersection of electron pairs of the oxygen atom with the aromatic π-system of thiophene.

Figure 7. Synthesis of 2-(cyclopent-1-en-1-yl)thiophene



Source: compiled by the authors.

Figure 8. Oxidation of 2-(cyclopent-1-en-1-yl)thiophene

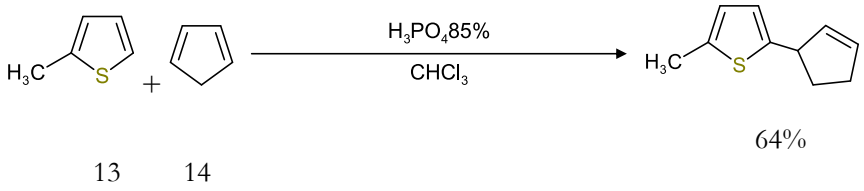


Source: compiled by the authors.

In the synthetic scheme outlined in Figure 7, a multi-step process is employed to prepare compound 10. The sequence begins with the conversion of compound 7 into a Grignard reagent using magnesium in ether, resulting in compound 8. In Figure 8, a different set of reactions takes place using mCPBA as the reagent. The outcomes diverge from the expected epoxidation, leading to the formation of compounds 11 and 12.

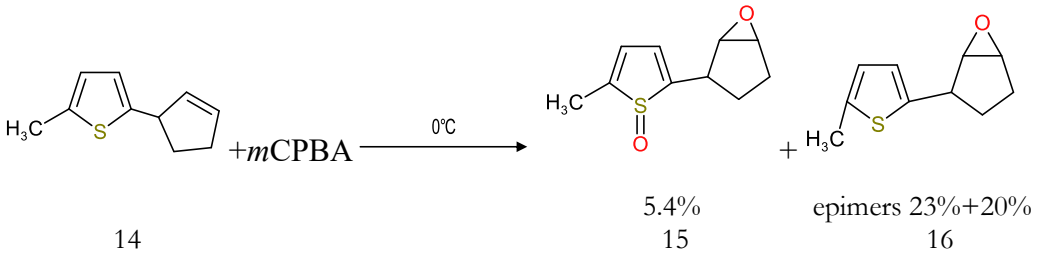
However, when electrophilic substituents are introduced at the 5 positions, the opposite situation is observed, where the main product is epoxide. Thus, upon oxidation of 2-(cyclopent-1-en-3-yl)-5-methyl-thiophene with an excess of mCPBA (2 mol), which was obtained from 2-methyl-thiophene similarly to the synthesis of (3) **(Figure 9)**, an equilibrium system was established where the epoxide content was 43% **(Figure 10)**.

Figure 9. Obtaining 2-(cyclopent-1-en-3-yl)-5-methyl-thiophene



Source: compiled by the authors.

Figure 10. Oxidation of 2-(cyclopent-1-en-3-yl)-5-methyl-thiophene

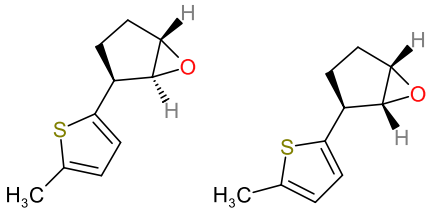


14 — 5.4% 15 — epimers 23%+20% 16

Source: compiled by the authors.

By chromatographic separation, 2 epimers (16) were isolated **(Figure 11)**, and the starting alkene (14) was re-entered into the oxidation reaction to ensure maximum process efficiency.

Figure 11. Structures of epimers



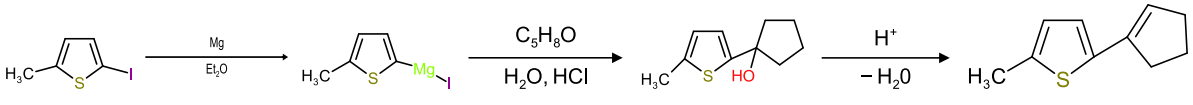
16a 16b

Note: 16a) (1S, 2S, 5S)-2-(5-methyl-thiophen-2-yl)-6-oxabicyclo[3.1.0]hexane; 16b) (1R, 2S, 5S)-2-(5-methyl-thiophen-2-yl)-6-oxabicyclo[3.1.0]hexane.
Source: compiled by the authors.

According to Figure 11, both molecules feature a thiophene ring with a methyl group, giving them their methylthiophene classification. They also share a bicyclic structure with an epoxide ring formed by an oxygen bridge between two carbon atoms.

A similar trend was observed for the methyl derivative cyclopent-1-en-1-yl-thiophene synthesised from 2-iodo-5-methyl-thiophene **(Figure 12)**, where the oxidation of (20) resulted in the main product sulfoxide (21) **(Figure 13)**. However, the epoxide content increased 6.5-fold with the introduction of the methyl group.

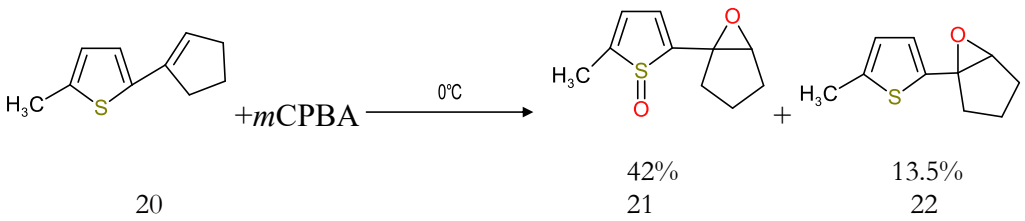
Figure 12. Synthesis of 2-(cyclopent-1-en-1-yl)-5-methyl-thiophene



17 — 18 — 19 — 57.3% 20

Source: compiled by the authors.

Figure 13. Oxidation of 2-(cyclopent-1-en-1-yl)-5-methyl-thiophene

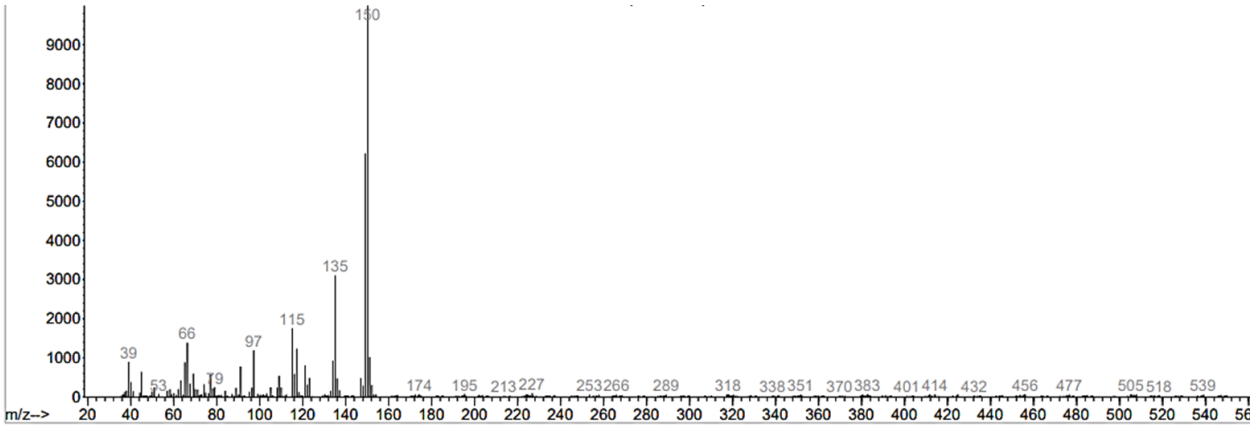


Source: compiled by the authors.

When analysing the reaction mixtures by gas chromatography and mass spectrometry on a calibrated Agilent Technologies instrument, characteristic fragmentation was observed, which is inherent in the compounds in the mixture.

Thus, in molecule (3), fragmentation with cleavage of the methyl group occurred during ionisation **(Figure 14)**.

Figure 14. Fragment of the GCMS spectrum of compound (3)

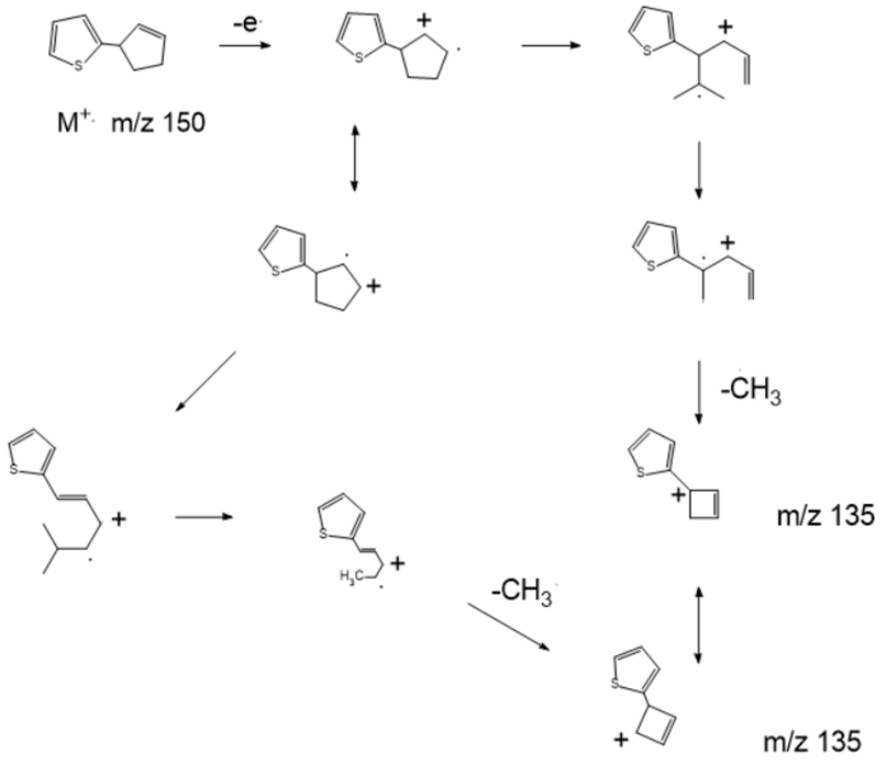


Source: compiled by the authors.

This fragmentation has several pathways involving different intermediates. First of all, the cycle is opened, and a branched radical carbocation is developed.

Then, to stabilise the carbocation, the cycle is re-closed by the cleavage of the methyl group to establish an unsaturated four-membered cycle **(Figure 15)**.

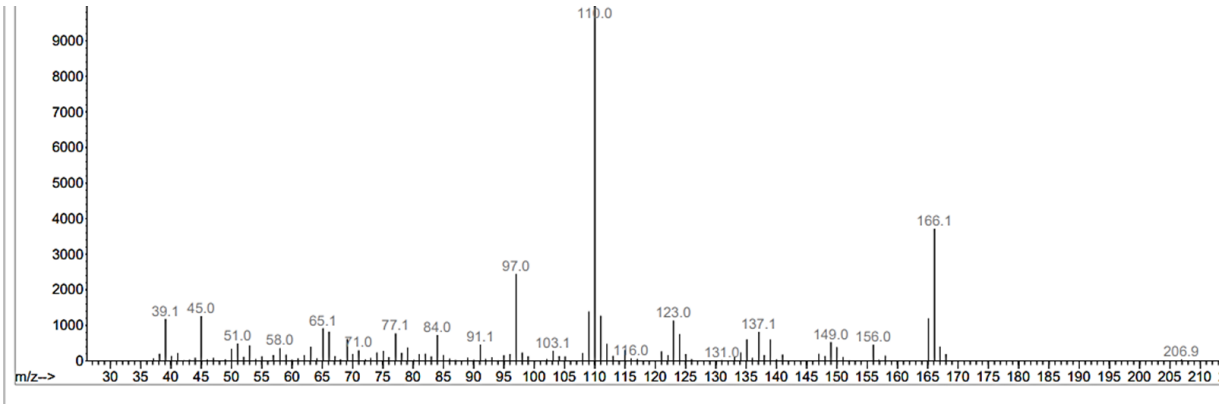
Figure 15. Decomposition of 2-(Cyclopent-1-en-3-yl)thiophene (3)



Source: compiled by the authors.

However, epoxides are subject to fragmentation, even further **(Figure 16)**.

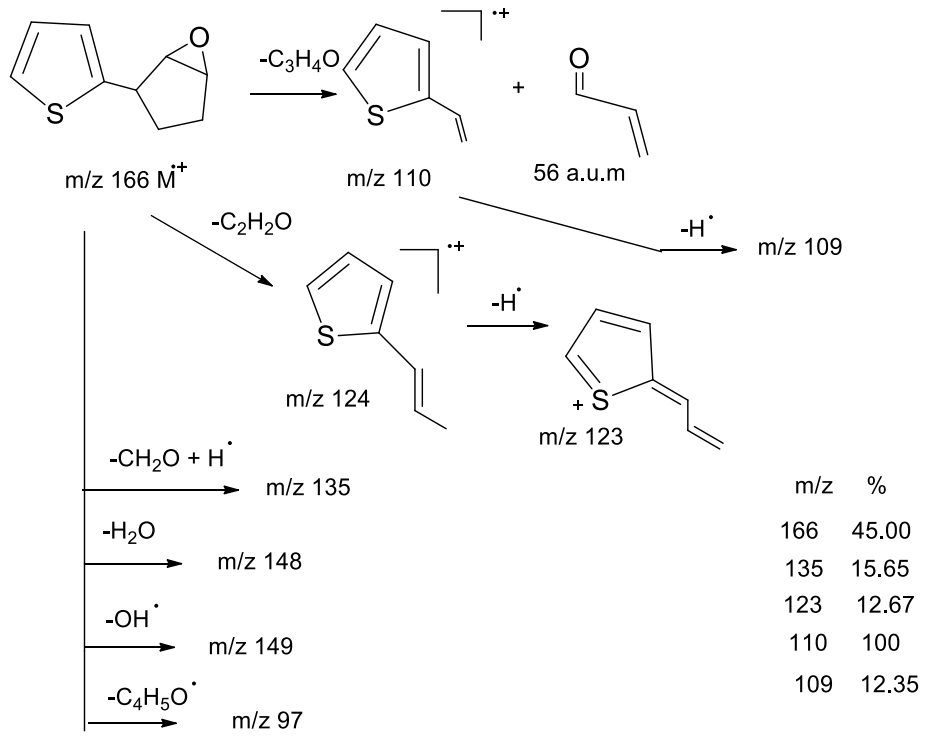
Figure 16. Fragment of the GCMS spectrum of compound (6)



Source: compiled by the authors.

The cleavage of C_3H_4O from (6) results in the establishment of a stable cationic radical, thus, the main peak on the GCMS spectrum is observed at the corresponding *m/z*, while partial fragmentation with the cleavage of the formaldehyde fragment may occur in parallel **(Figure 17)**.

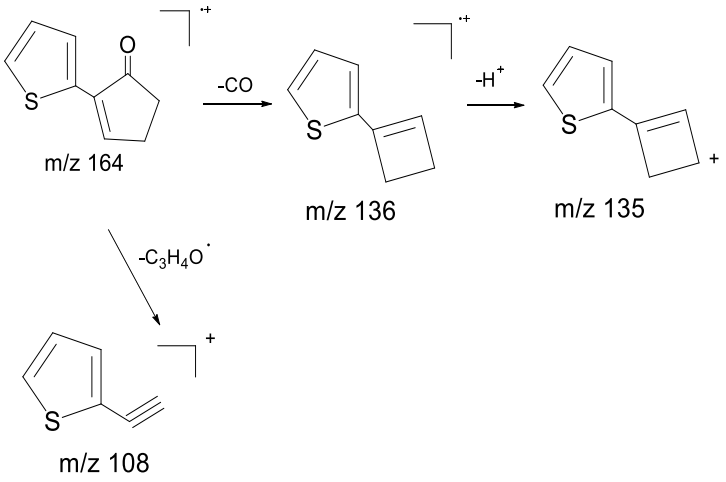
Figure 17. Decomposition of epoxides (6)



Source: compiled by the authors.

In addition, identification of 2-(cyclopent-1-en-5-one-1-yl)thiophenes can be made by the characteristic fragmentation in the mass spectrum. For such a compound, the carbonyl component will be predominantly cleaved during ionisation **(Figure 18)**.

Figure 18. Decomposition of 2-(Cyclopent-1-en-5-one-1-yl)thiophene (12)



Source: compiled by the authors.

Previous studies have demonstrated a significant effect of the electrophilic substituent and steric factors on the epoxidation reaction [1]. Thus, when an electrophilic substituent is introduced into the aromatic core, the electron density shift promotes the dominance of the epoxidation process, i.e., prevents the side oxidation of the aromatic core to sulfoxide. In addition, the position of the double bond plays a significant role in the ratio of reaction products. The more distant double bond is characterised by the establishment of epoxides during oxidation, while the conjugated double bond is oxidised mainly to cyclopentenones in the absence of an electrophilic substituent in the nucleus. In the presence of such a substituent, parallel oxidation of the thiophene core and double bond will be observed.

DISCUSSION

Before conducting the practical part of the research work, several critical factors were analysed, such as the relevance, toxicity and commercial availability of the compounds examined.

Thiophenes occur in nature in a variety of contexts and may have unique functions and properties in biological systems. Thiophenes can be established during the natural decomposition of organic materials such as plant residues and the low-temperature pyrolysis of certain sulphur-containing hydrocarbons. As noted in the review by F. Abedinifar et al. [12], the presence of thiophene in a molecule can affect its biological activity and physicochemical properties. Among the most common natural sources, notably, the presence of its derivatives in some types of coffee, garlic, onions, and rosemary. On the other hand, anti-inflammatory and antimicrobial pharmaceuticals with a thiophene fragment in their structure are highly competitive medicines that save the lives of thousands of people [13; 14].

Several studies have highlighted the high antimicrobial activity of these compounds. T. Harit et al. [15] explored the antibacterial and antifungal effects of several thiophene derivatives *in vitro*. Their effectiveness was recorded both against the gram-positive bacteria *Micrococcus luteus* and *Bacillus subtilis* and the gram-negative bacteria *Escherichia coli* and fungi *Candida pelliculosa*. Similar results were obtained by scientists from the Institute of Molecular Chemistry in Romania [16]. Archna et al. [17] noted that Thiophene-based compounds have garnered significant attention in medicinal chemistry due to their diverse biological activities, particularly in the development of potential anticancer agents. Researchers have explored the potential of thiophene analogues to bind with cancer-specific protein targets, leading to the inhibition of various signalling pathways involved in cancer.

For the oxidation of the double bond to epoxide, both cheap reagents such as hydrogen peroxide, benzoyl peroxide, and DMDO, which are not enantioselective, and more expensive ones are used. The advantage of such oxidants is their low cost the simplicity of the reactions, and the possibility of their adaptation to industrial scale. Although, some studies describe the modification of oxidation processes to increase their selectivity, such as K. Khosravi and Sh. Naserifar [18], the need to use water as a solvent, even in small amounts, and the need to heat the peroxides to produce the reaction is both dangerous and not advisable for more sensitive substrates.

A. Wells [19] examined the effect and efficiency of reactions under various varying conditions of temperature, concentration, and catalysts. Raising the temperature of a chemical reaction increases the reaction rate. This is because the reactant particles move faster and collide with each other more frequently, resulting in a greater frequency of effective collisions. Increasing the concentration of one or more reacting substances generally increases the reaction rate. This is because a greater number of collisions occur between particles, resulting in a higher frequency of effective collisions.

In turn, A catalyst is a substance that increases the rate of a chemical reaction without being used up in the reaction. It works by providing an alternate reaction pathway with a lower activation energy barrier, which increases the percentage of effective collisions.

Although using more expensive and sophisticated methods to obtain the desired compounds in enantioselective Sharpless epoxidation is a more highly selective process, it is not commercially available due to complex catalysts [20]. The research team of M. Majdecki et al. [21] considered the possibility of using a natural extract from the hin tree as a catalyst. This solution is quite effective for improving the environmental friendliness of oxidation processes [22], but in the framework of this research, it can cause several experimental difficulties during the purification and analysis of target products.

Considering all the above factors, m-chloro-nonbenzoic acid was chosen as an oxidant for this research. On the one hand, this method is non-selective, but once several compounds are obtained, they can be easily separated using chromatographic methods [23; 24]. Frequently, this method is cheaper than the preparation of specific catalysts and subsequent purification of the target products [25-27]. On the other hand, for spatially hindered substrates, using *m*CPBA, as noted in Ref. Ikuma et al. [28], the product of electrophilic addition rather than epoxidation will be the major product, as it is more energetically advantageous. However, the opposite situation is observed in the oxidation of allylic amines. The work of S.G. Davies et al. [29] illustrates a case where high-purity epoxides are established by a modified Hanbest transition. In practice, the possibility of obtaining thiophene oxabicyclohexane derivatives varied depending on the structure of the starting materials. Thus, for 2-(cyclopent-1-en-3-yl)thiophenes, the oxidation of the double bond was easier, while for 2-(cyclopent-1-en-1-yl)-5-methyl-thiophenes it was difficult or occurred synchronously with the oxidation of sulfur in the aromatic core [30-33].

Modern laboratories use molecular docking for preliminary monitoring of the biological activity of compounds examined. It allows predicting the spatial interaction between ligands and biomolecular targets using software tools. In addition, determining the optimal position and orientation of the ligand in the active site helps to predict the mechanism of their affinity [34]. The work of S.M. Cohen et al. [35] raised the issue of the possibility of using thiophene derivatives as food additives. Based on the work of A. Sagaama and N. Issaoui [36] at the Laboratory of Quantum and Static Physics, it can be concluded that the research of intermolecular interactions of thiophene derivatives is a promising niche for the development of new synthetic drugs, in particular for cancer. In addition, the toxicological aspect of the examined compounds is very significant [37-39]. Another more recent research by A.V. Khilkovets and I.M. Bilai [40] indicates a sufficient level of safety of thiophene-containing compounds.

In general, thiophene oxybicyclohexane demonstrates significant potential in various fields, including medicine and pharmaceuticals. Its anti-inflammatory, antibacterial and antifungal properties make it an attractive research target for the further development of new drugs and antimicrobials. Additional research into the mechanisms of action and optimisation of the structure of thiophene oxabicyclohexane may lead to the development of new, more effective and safer treatments for various diseases, including inflammatory processes, and bacterial and fungal infections.

CONCLUSIONS

The position of the double bond in the cyclic structure significantly affects epoxide formation. When the double bond is conjugated to the thiophene ring, very little epoxide is formed upon oxidation with *m*CPBA. However, when the double bond is on a distal carbon, epoxidation readily occurs. Introducing an electrophilic methyl substituent at the 5-position of the thiophene ring favours epoxide formation.

The ratio of oxidized products can be controlled by the amount of *m*CPBA used. With excess *m*CPBA, multiple oxidation products are obtained. Reducing the amount shifts the ratio towards sulfoxide formation while still enabling some epoxide production. The epoxidation occurs with low stereoselectivity, resulting in a pair of epimers that can be separated by chromatography. Their structures were confirmed by 2D-NOESY NMR experiments. GC-MS analysis provided insight into the characteristic fragmentation patterns of the starting materials, epoxides, and other oxidation products that aided in structural elucidation.

In summary, the position of the double bond, inclusion of electrophilic substituents on the thiophene ring, and quantity of oxidant used allow controlling the product distribution between sulfoxides, epoxides, and cyclopentenones upon *m*CPBA oxidation.

This study contributes by synthesizing cyclopentenylthiophene derivatives, exploring their oxidation process with *m*CPBA, and highlighting their potential for medicinal applications. The research identifies synthesis advantages and areas for further investigation, promising new compounds and potential pharmaceutical prospects. Further studies can delve into the effects of different oxidants, reaction conditions, and the influence of substituents on product ratios in these reactions. Additionally, exploring the biological activity of the synthesized compounds offers promising avenues for potential pharmaceutical and medical applications.

REFERENCES

1. **Shah R, Verma PK.** Therapeutic importance of synthetic thiophene. Chem Cent J. 2018;12(1):137. https://doi.org/10.1186/s13065-018-0511-5

2. **Da Cruz RMD, Mendonça Junior FJB, de Mélo NB, Scotti L, de Araújo RSA, de Almeida RN, de Moura RO.** Thiophene-based compounds with potential anti-inflammatory activity. Pharm. 2021;14(7):692. https://doi.org/10.3390/ph14070692

3. **Chawla S, Sharma S, Kashid S, Kumar Verma P, Sapra A.** Therapeutic potential of thiophene compounds: a mini-review. Mini Rev Med Chem. 2023. https://doi.org/10.2174/1389557523666230206104257

4. **Auld N, Flood K, Kesharwani T, Cavnar PJ.** A study on the cellular and cytotoxic effects of S and Se heterocycles on the myeloid leukemia cell line PLB-985. Phosphorus Sulfur Silicon Relat Elem. 2022;197(8):876-884. https://doi.org/10.1080/10426507.2022.2085272

5. **Chacín Zambrano CA.** La biotecnología vegetal como herramienta para la conservación de la biodiversidad. innovaciencia. Innovac. 2017;5(1S1):5-6. https://doi.org/10.15649/2346075X.450

6. **Ermiş E, Berber H, Güllü M.** Synthesis of some alkylaminothiophene derivatives from 3,4-dibromothiophene and their theoretical calculations. Tetrahedron. 2019;75(33):4577-4590. http://dx.doi.org/10.1016/j.tet.2019.06.049

7. **Jaszczewska-Adamczak JA, Mlynarski J.** Asymmetric epoxidation of enones promoted by dinuclear magnesium catalyst. Adv Synth Catalysis. 2021;363(17):4247-4255. https://doi.org/10.1002/adsc.202100482

8. **Moschona F, Savvopoulou I, Tsitopoulou M, Tataraki M, Rassias G.** Epoxide syntheses and ring-opening reactions in drug development. Catalysts. 2020;10(10):1117. https://doi.org/10.3390/catal10101117

9. **Lew HY, Noller CR.** A thiophene analog of sulfanilamide. J Am Chem Soc. 1950;72(12):5715-5717. https://doi.org/10.1021/ja01168a092

10. **Caballero R, Cohen B, Gutiérrez M.** Thiophene-based covalent organic frameworks: Synthesis, photophysics and light-driven applications. Molecules. 2021;26(24):7666. https://doi.org/10.3390/molecules26247666

11. **Nasir HM, Saki T, Al-Luaibi MY.** Synthesis, identification and thermal study of some new inorganic polymers based on bis-dithiocarbamate ligands with silicone, tellurium and some transition metals. Innovac. 2019;7(1):1–13. https://doi.org/10.15649/2346075X.507

12. **Abedinifar F, Babazadeh Rezaei E, Biglar M, Larijani B, Hamedifar H, Ansari S, Mahdavi M.** Recent strategies in the synthesis of thiophene derivatives: Highlights from the 2012-2020 literature. Mol Divers. 2021;25(4):2571-2604. https://doi.org/10.1007/s11030-020-10128-9

13. **Xuan DD.** Recent achievement in the synthesis of thiophenes. Mini Rev Org Chem. 2021;18(1):110-134. http://dx.doi.org/10.2174/1570193X17999200507095224

14. **Mabkhot YN, Alatibi F, El-Sayed NN, Al-Showiman S, Kheder NA, Wadood A, Rauf A, Bawazeer S, Hadda TB.** Antimicrobial activity of some novel armed thiophene derivatives and Petra/Osiris/Molinspiration (POM) analyses. Mol. 2016;21(2):222. https://doi.org/10.3390/molecules21020222

15. **Harit T, Bellaouchi R, Asehraou A, Rahal M, Bouabdallah I, Malek F.** Synthesis, characterization, antimicrobial activity and theoretical studies of new thiophene-based tripodal ligands. J Mol Structure. 2017;1133:74-79. https://doi.org/10.1016/j.molstruc.2016.11.051

16. **Roman, G.** Thiophene-containing compounds with antimicrobial activity. Arch Pharm. 2022;355(6):2100462. https://doi.org/10.1002/ardp.202100462

17. **Archna, Pathania S, Chawla PA.** Thiophene-based derivatives as anticancer agents: An overview on decade's work. Bioorg Chemistry. 2020;101:104026. https://doi.org/10.1016/j.bioorg.2020.104026

18. **Khosravi K, Naserifar S.** Facile epoxidation of α, β-unsaturated ketones with urea-2,2-dihydroperoxypropane as a new oxidant. J Iran Chem Soc. 2017;14(2):323-328. https://doi.org/10.1007/s13738-016-0980-1

19. **Wells A.** 10.3: Effects of temperature, concentration, and catalysts on reaction rates. In: *Introduction to Genral, Organic and Biochemistry*. Davis: LibreTexts, pp. 10.3.1-10.3.2.

20. **Sawano T, Yamamoto H.** Regio- and enantioselective substrate-directed epoxidation. Eur J Org Chem. 2020;2020(16):2369-2378. https://doi.org/10.1002/ejoc.201901656

21. **Majdecki M, Tyszka-Gumkowska A, Jurczak J.** Highly enantioselective epoxidation of α,β-unsaturated ketones using amide-based cinchona alkaloids as hybrid phase-transfer catalysts. Org Lett. 2020;22(21):8687-8691. https://doi.org/10.1021/acs.orglett.0c03272

22. **Okassova AK, Ilderbayev OZ, Nursafina AZh, Zharmakhanova GM, Rakhimova BB, Bayan YT, Bayan DN.** Evaluation of lipid peroxidation under immobilization stress in irradiated animals in experiment. Open Access Macedon J Med Sci. 2021;9:119-122.

23. **Tkach VV, Kushnir MV, de Oliveira SC, Zavhorodnii MP, Brazhko OA, Kornet MM, Luganska OV, Kopiika VV, Ivanushko YG, Mytchenok MP, Ahafonova OV, Yagodynets´ PI, Kormosh ZO, Dos Reis LV.** The theoretical description for a sucralose electrochemical cathodical determination over a 9-9´-diacridyl-modified electrode. Orbital. 2021;13(3):219-222.

24. **Pavliuk L.** Electron modelling in conjunction witch vacuum modelling. Sci Her Uzhhor Univ. Ser "Phys". 2022;(52):27-35. https://doi.org/10.54919/2415-8038.2022.52.27-35

25. **Zhurakovsky YA, Korzhik VN, Borisov YS.** Fine-structure of roentgen emission-spectra of alloy Ni-Nb in amorphous and crystalline states. Izv Vyssh Uchebn Zaved. Fiz. 1988;31(7):108-110.

26. **Kunitskii YA, Korzhik VN, Nemirovskii AV.** Transformations in the plasma-sprayed Fe67Ti7B24C2 alloy in heating. Sov Mater Sci. 1990;26(1):87-90. https://doi.org/10.1007/BF00734547

27. **Aghbalyan S, Simonyan V.** Study of hardening and structure of maraging powder steel grade PS-H18K9M5TR (18%Ni+9%Co+5%Mo+1%Ti+1%Re+66%Fe). Sci Her Uzhhor Univ. Ser "Phys". 2022;52:46-55. https://doi.org/10.54919/2415-8038.2022.52.46-55

28. **Ikuma N, Sumioka S, Asahara H, Oshima T.** Regioselective electrophilic addition vs epoxidation of mCPBA towards anti-Bredt olefin of fulleroid. Tetrahedron Lett. 2012;53(28):3581-3584. https://doi.org/10.1016/j.tetlet.2012.05.007

29. **Davies SG, Fletcher AM, Thomson JE.** Hydrogen bond directed epoxidation: diastereoselective olefinic oxidation of allylic alcohols and amines. Org Biomol Chem. 2014;12(26):4544-4549. https://doi.org/10.1039/c4ob00616j

30. **Salikova NS, Bektemissova AU, Nazarova VD, Begenova BE, Ostafeichuk NV.** Preparation of mixed hydrogels based on biopolymers and the study of their rheological properties. Period Tche Quim. 2020;17(35):23-40.

31. **Ristic L, Dakovic D, Postic S, Lazic Z, Bacevic M, Vucevic D.** Clinical Characteristics of Abutment Teeth with Gingival Discoloration. J Prosthodont-Implant Esthet Reconstr Dent. 2019;28(1):E45-E50. https://doi.org/10.1111/jopr.12612

32. **Bulhakova A.** Mass spectrometric studies of valine molecules by electron shock in the gas phase. Sci Her Uzhhor Univ. Ser "Phys". 2022;(51):9-17. https://doi.org/10.54919/2415-8038.2022.51.9-17

33. **Likhanov A, Klyuvadenko A, Subin O, Shevchuk M, Dubchak M.** Gallic acid as a non-specific regulator of phenol synthesis and growth of regenerate plants of Corylus avellana (L.) H. Karst. and Salix alba L. in vitro. Ukrainian J Forest Wood Sci. 2022;13(4):52-63. https://doi.org/10.31548/forest.13(4).2022.52-63

34. **Nazarova VD, Salikova NS, Bektemissova AU, Begenova BE, Aubakirova GB.** Isolation of miricetine-containing fractions from linosyris villosa plant and their application as antianemic agent. Period Tche Quim. 2020;17(35):998-1012.

35. **Cohen SM, Fukushima S, Gooderham NJ, Guengerich FP, Hecht SS, Rietjens IMCM, Smith RL, Bastaki M, Harman CL, McGowen MM, Valerio Jr, LG, Taylor SV.** Safety evaluation of substituted thiophenes used as flavoring ingredients. Food Chem Toxicol. 2017;99:40-59. https://doi.org/10.1016/j.fct.2016.10.023

36. **Sagaama A, Issaoui N.** Design, molecular docking analysis of an anti-inflammatory drug, computational analysis and intermolecular interactions energy studies of 1-benzothiophene-2-carboxylic acid. Comput Biol Chem. 2020;88:107348. https://doi.org/10.1016/j.compbiolchem.2020.107348

37. **Wrobel A, Zapala L, Kluz T, Rogowski A, Misiek M, Juszczak K, Sienko J, Gold D, Stangel-Wojcikiewicz K, Poleszak E, Radziszewski P.** The Potential of Asiatic Acid in the Reversion of Cyclophosphamide-Induced Hemorrhagic Cystitis in Rats. Int J Mol Sci. 2021;22(11):5853. https://doi.org/10.3390/ijms22115853

38. **Postic SD.** X-Ray Diffraction Technique in the Analysis of Phases of Hydroxylapatite and Calcium Phosphate in a Human Jaw. Int J Biomed. 2014;4(2):109-113.

39. **Bigan Z, Derechkei P, Mazur V, Pop O.** Study of the cross-section for the 122Te(γ, n)121Te reaction by the activation method. Sci Her Uzhhor Univ. Ser "Phys". 2022;51:18-23. https://doi.org/10.54919/2415-8038.2022.51.18-23

40. **Khilkovets AV, Bilai IM.** Study of acute toxicity of new thiophene-containing derivatives of 1,2,4-triazole. Zaporozhye Med J. 2023;25(1):46-49. https://doi.org/10.14739/2310-1210.2023.1.266318