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Synthesis, Characterisation of C-Methoxy Phenyl Calix[4]resorcinaryl Octacinnamate and Their Antibacterial Activity

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Abstract

The issue of antibiotic resistance has become a serious problem in the medical world. This resistance leads to reduced effectiveness of treatment for microbial infections and increases treatment costs. To address this issue, the discovery of new, non-resistant antibiotics is necessary. The aim of this study was to synthesize and evaluate the antibacterial activity of the compound C-methoxy phenylcalix[4]resorcinaryl octacinnamate (CMPCROC). The synthesis of CMPCROC was carried out by esterifying C-methoxy phenyl calix[4]resorcinarene (CMPCR) with cinnamoyl chloride. The product was characterized using IR spectroscopy, ^{1}H-NMR, and MS. The antibacterial activity was tested using the Kirby-Bauer disc diffusion method with amoxicillin as the positive control and water as the negative control. The synthesized compound was a yellow solid with a melting point of 215–220 °C and a yield of 67.05 %. The antibacterial test results showed that the inhibition zone of CMPCROC against *Escherichia coli* at a concentration of 50 ppm was 12.40 mm, while the positive control had a diameter of 15.30 mm. The inhibition zone of CMPCROC against *Staphylococcus aureus* was 11.63 mm, compared to 14.50 mm for the positive control. These results indicate that CMPCROC has strong antibacterial activity against *E. coli* and *S. aureus* and has potential for development as a new antibiotic in the future.

Keywords: Synthesis; Characterization; Antibacterial activity; Resistance.

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1. Introduction

Antibiotics are powerful medicines that are taken to kill or inhibit the growth of bacteria in the body [1]. The current problem of antibiotic use in medicine is antibiotic resistance, because it is associated with increased mortality, length of treatment and higher cost. This problem is a serious problem, especially in developing countries because it has caused high mortality rates [2,3]. Inappropriate use of antibiotics causes pathogenic microorganisms to become resistant so that infection treatment becomes ineffective. One of the bacteria that experiences resistance is Streptococcus anginosus [4]. In 2019 alone, an estimated 1.27

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million people died due to antibiotic resistance and the African continent had the highest cases. Success in overcoming the problem of bacterial resistance is still quite far away. Antibiotic resistance is defined as the ability of microorganisms to withstand exposure to antibiotics, which normally kill or inhibit their growth [5,6]. Antibiotic resistance makes antibacterial drugs less effective, making treatment of patients more difficult, expensive, or even impossible to cure [3]. The rising of a phenotype resistant to antimicrobial agents depends on several factors such as degree of resistance expression of the microorganism or its capability to tolerate resistance mechanism, to cite a few [7,8]. Bacteria may have intrinsic resistance or acquire resistance from either mutations in cell genes (chromosomal mutation) leading to cross-resistance, or gene transfer from one microorganism to another mediated by plasmids, transposons, integrons and bacteriophages [9,10]. The problem of microbial resistance is increasing and seems to cause the use of antimicrobial drugs in the future to be increasingly uncertain. Therefore, several steps must be taken to overcome these problems, such as controlling the use of antibiotics, developing research to better understand the genetic mechanisms of resistance and continuing studies to develop new antibiotic drugs [11]. The discovery of new antibiotic compounds that have not experienced resistance is one alternative solution that can be done immediately to overcome the problem.

Antibacterial agents can be obtained through the isolation of natural compounds or through the synthesis of new compounds which are the development of antibiotic compounds that are experiencing resistance. The use of natural materials to treat diseases caused by bacteria has increased recently [11-14]. However, the use of natural compounds, especially those derived from plants, has several challenges such as the difficulty in obtaining pure compounds, their low concentration in plants and their strength in killing or inhibiting bacterial growth is still low. So, the synthesis of new antimicrobial compounds is the most promising alternative because they can be obtained in larger quantities, have stronger activity and a wider spectrum. One of the studies on the discovery of new antibiotics is the synthesis of chalcone derivatives with a 6-sided ring containing nitrogen atoms. The compound is reported to be not active against fungal strains, but it recorded an antibacterial potential against Gram-negative and Gram-positive bacteria [15]. However, this compound has weaknesses, namely a complicated synthesis pathway and low yield.

One group of compounds that has the potential to be developed as a new antibiotic is the calix[4]resorcinarenes group of compounds and their derivatives. Research on the biological activity of calixarenes, resorcinarenes, and pillararenes is increasingly being reported. Research on the potential of calixarenes as antimicrobials was first conducted in 2002 [15], then continued with research on calixarenes in other applications [17]. Calixarenes can cause biological responses through three main mechanisms, namely (1) disrupting the integrity of cell membranes, (2) acting as precursor drugs to release biologically active molecules, or (3) transporting drug molecules in their macrocyclic cavities [18]. These unique properties make calixarenes valuable candidates for the development of antibacterials. Calix[4]resorcinarenes are cyclic macromolecular compounds consisting of a number (*n*) of phenol or resorcinol units and connected by methylene bridges in the ortho position to the hydroxyl group. The synthesis of this group

of compounds is through the condensation reaction of phenol or resorcinol or its derivatives with aldehyde compounds. [18-20].

Previous research conducted by [16] has found calix[4]resorcinarene containing ester groups, but did not further investigate its antibacterial activity. It was only a few years later that it was discovered that C-Alkyl calix[4]resorcinarene containing ester groups such as cinnamate and its derivatives showed significant antibacterial activity [22-24]. In this study, the synthesis of C-methoxy phenylcalix[4]resorcinaryl octacinnamate (CMPCROC) was carried out and its activity as an antibacterial was tested. The novelty of this study compared to previous studies is the coupling of methoxy phenyl groups. The methoxy phenyl group has strong basic properties, so it will have the ability to inhibit bacterial growth compared to alkyl groups. In addition, the presence of eight cinnamate groups can also increase the strength to inhibit bacterial growth. This has been proven [24], just by including one cinnamate group, it can increase the inhibition significantly. The compound made in this study contains eight cinnamate groups as shown in Fig. 1.

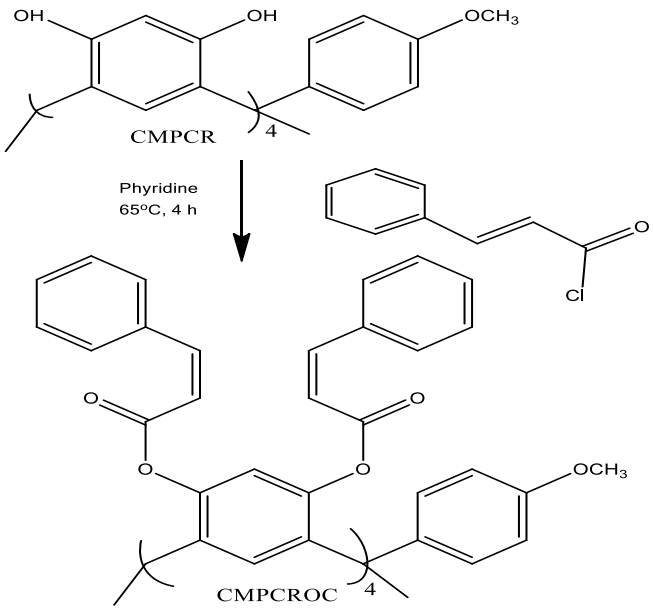

Fig. 1. Synthesis pathway of CMPCROC.

2. Material and Methods

2.1. *Material*

The C-methoxy phenylcalix[4]resorcinarene (CMPCR), were synthesized from the chemicals without further purification. All analytical (AR) grade required chemicals were purchased. Cinnamoyl chloride, pyridine and ethanol were purchased from Sigma enterprises.

2.2. *Synthesis of C-methoxy phenylcalix[4]-resorcinaryl octacinnamates* (CMPCROC)

A mixture of 1 g of C-(4-methoxy) phenylcalix[4]resorcinarene and 1 g of cinnamoyl chloride in 25 mL of pyridine was heated at 65 °C for 4.5 h. After the reaction, the mixture was cooled, and the precipitate formed was collected by filtration. The solid product was washed three times with distilled water, then dried and weighed. The melting point of the synthesized compound was determined using a melting point apparatus. Further characterization was performed using IR spectroscopy, ^{1}H-NMR, and mass spectrometry.

2.3. *Antibacterial activity test*

Antibacterial activity testing in this study used the agar diffusion method with the Kirby-Bauer method using Mueller Hinton Agar (MHA) medium carried out in a Biological Safety Cabinet (BSC). MHA was poured into a petri dish and left to solidify. The petri dish was streaked with a microbial suspension using a sterile cotton swab with the swab method and left for ±5 minutes. *Amoxicillin* antibiotic was used as a positive control and distilled water as a negative control. The concentration of CMPCROC samples was 10 ppm, 30, 50, 70 and 90 ppm. Paper discs (diameter = 6 mm) were soaked in antibiotic solution, distilled water and samples of each concentration for ±10 min. Then, the paper disc was placed on the medium that had been streaked with the previous test microbial suspension. Furthermore, the petri dish was wrapped in plastic wrap and incubated for 24 h at a temperature of 37 ºC. Furthermore, observations and calculations of the inhibition zone were carried out. Measurement of Bacterial Inhibition Zone were made after 24 h of incubation. The diameter of the inhibition zone is indicated by the clear zone produced around the disc paper, then measured using a caliper. The category of the inhibition zone can be classified based on the results of the measurement of the diameter of the clear zone.

3. Results and Discussion

3.1. *Synthesis and characterization of CMPCROC*

The target compound was synthesized through a single-stage reaction, namely the esterification of CMPCR compounds with cinnamoyl chloride. The reaction results in the form of a yellow solid with 215–220 °C and a yield of 67.05 %. The melting point value is lower than the melting point value of calix[4]resorcinarene compounds in general (> 400 °C), indicating that the esterification reaction has gone well. The decrease in the melting point value is caused by the breaking of hydrogen bonds between molecules. The characterization results using an Infrared spectrometer (Fig. 2), show the absorption of the C=O ester group at 1728.22 cm^{-1}, while the absorption at 1635 cm^{-1} and 1497 cm^{-1} indicate the presence of aromatic C=C bonds. The absorption of the OH group that is not visible in the 3400 cm^{-1} area, is an indication that the entire CMPCR raw material has become CMPCROC.

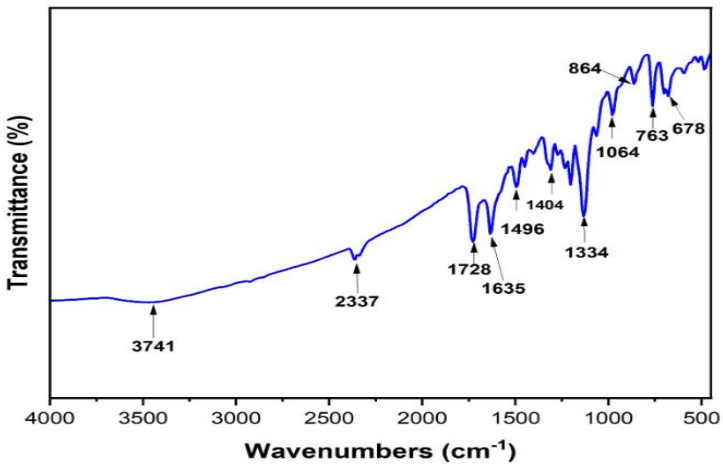

Fig. 2. Infrared Spectrum of CMPCROC.

Further characterization using ^{1}H-NMR spectrum spectrometer (Fig. 3), showed that methoxy protons and calix[4]resorcinarene bridge protons appeared at 6.5 ppm. Benzene ring protons appeared at chemical shifts of 7.6 and 7.7 ppm, while cinnamoyl group protons appeared at 8.4 ppm.

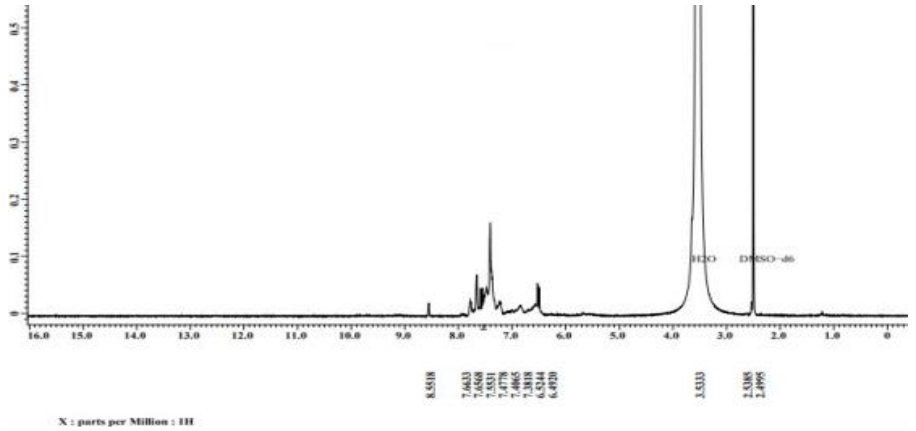

Fig. 3. ^{1}H-NMR spectrum of CMPCROC.

The mass spectrum shows that the synthesized compound is a single compound with a molecular weight of 755 g/mol. The base peak (M+) appears at 652 m/z, which is caused by the release of the cinnamoyl group. The release of the cinnamoyl group is very possible because the ester bond is a bond that is easily broken by the high temperature in the operation of the mass spectrometer equipment.

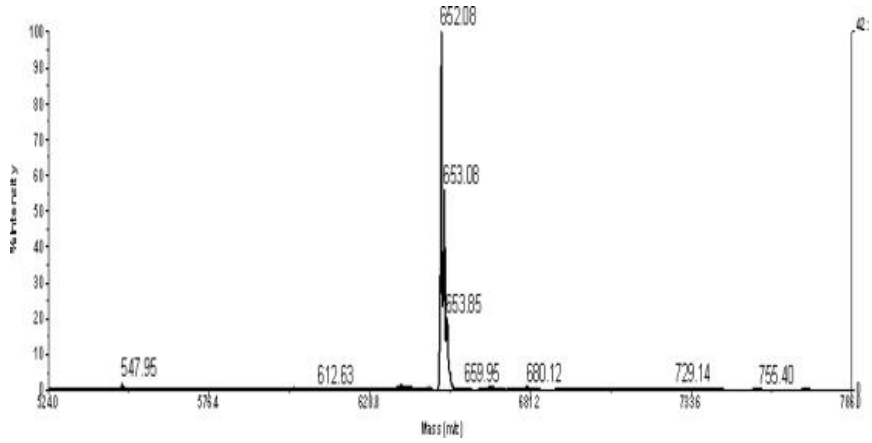

Fig. 4. Mass spectrum of C-Methoxy phenylcalix[4]resorcinaryl octacinnamates (CMPCROC).

Based on the melting point determination and structural characterization using IR spectroscopy, ^{1}H-NMR, and mass spectrometry, it can be concluded that the target compound, CMPCROC, was successfully synthesized. The reaction mechanism for the formation of CMPCROC is illustrated in Fig. 5.

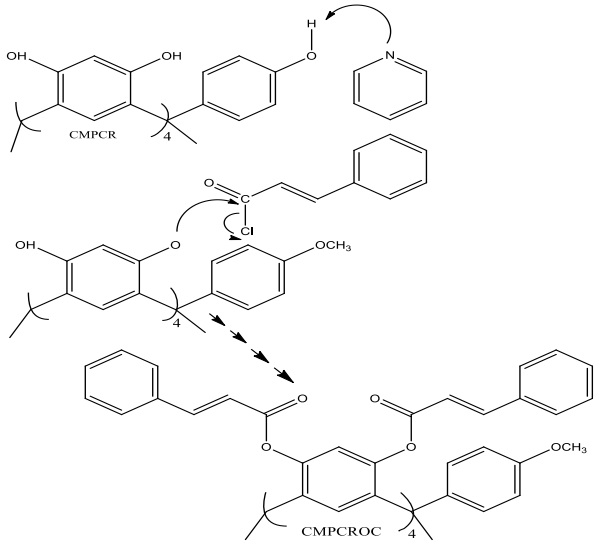

Fig. 5. Reaction mechanism for the synthesis of CMPCROC

The reaction mechanism begins with the binding of hydrogen atoms to basic nitrogen atoms, then the phenoxy ion attacks the carbonyl group of cinnamoyl chloride, followed by the release of the chlorine atom as a good leaving group.

3.2. *Antibacterial activity test of CMPCROC*

The antibacterial activity test was conducted to evaluate the potential of the synthesized compound as a bactericide. The results of the antibacterial activity of CMPCROC against *Escherechia coli* and *Staphylococcus aureus* are summarized in Tables 1 and 2.

Table 1. Inhibition zone diameter of *Escherechia coli* treated with CMPCROC.

No	Treatment (ppm)	Diameter of Inhibitory Zone (mm)				
		I	II	III	Total	Average
1	10	10	10.4	10.5	30.9	10.3
2	30	11.5	11.9	11.9	35.3	11.8
3	50	12.2	12.6	12.5	37.3	12.4
4	70	13	13.3	13.3	39.6	13.2
5	90	14.1	14.4	14.6	43.3	14.4
6	K+	15.6	15.2	15.2	46	15.3
7	K-	0	0	0	0	0

K+ = positive control, K- = negative control

The data presented in Table 1 demonstrate that CMPCROC effectively inhibited the growth of *Escherechia coli* across all tested concentrations. A direct correlation was observed between the concentration of the CMPCROC solution and the inhibition zone diameter. As the concentration increased, the inhibition zone grew larger, indicating enhanced bacterial inhibition. This suggests that higher concentrations of CMPCROC release a greater number of calixresorcinarene molecules, which in turn promotes better penetration of the compound into bacterial cells, effectively inhibiting their growth. The strength of antibacterial activity can be seen from the value of the diameter of the inhibition, a diameter of 5 mm or less is classified as weak, 6-10 mm is categorized as medium, 11-19 is categorized as strong and 20 mm or more is categorized as very strong [26]. Based on data in Table 1, it can be said that the sample solution has a strong category for all concentrations. The inhibition is only slightly lower than the positive control of amoxicillin at the same concentration (15.3 mm). Amoxicillin was used as a comparative solution because amoxicillin proved 80 % resistance to *Escherichia coli*. Tests were also carried out on *Staphylococus aureus* bacteria, the results of which are as shown in Table 2.

Table 2. The inhibitory zone diameter of CMPCROC against *Staphylococus aureus*.

No	Treatment (ppm)	Diameter of Inhibitory Zone (mm)				
		I	II	III	Total	Average
1	10	9.3	10	10.3	29.6	9.86
2	30	10.4	10.7	11	32.1	10.7
3	50	11.9	11.5	11.5	34.9	11.63
4	70	12.2	12.6	12.3	37.1	12.3
5	90	13	13.3	13.4	39.7	13.23
6	K+	14.1	14.8	14.6	43.5	14.5
7	K-	0	0	0	0	0

K+ = positive control, K- = negative control

A CMPCROC solution can be quite good in inhibiting bacterial growth compared to the exciting studies and contributes to the development of new compounds with superior antibacterial activity. CMPCROC demonstrated significantly better bacterial inhibition than previously reported studies. For example, [24] reported that methyl cinnamate at a concentration of 10 ppm produced an inhibition zone diameter of 1.1 cm, whereas in this study, CMPCROC exhibited markedly improved performance. Similarly, Septiani *et al.* [27] observed that ethanol extracts of *C. rotundata* yielded inhibition zone diameters of 5.43 cm at comparable concentrations, further highlighting the enhanced efficacy of CMPCROC. These results underscore the potential of CMPCROC as a novel antibacterial agent with strong activity against *Escherecia coli* and *Staphylococcus aureus*, demonstrating a promising avenue for the development of effective antibiotic.

4. Conclusion

The successful synthesis of the target compound, CMPCROC, and its demonstrated effectiveness in inhibiting the growth of *Escherecia coli* and *Staphylococcus aureus* highlight its potential as a powerful antibacterial agent. The results indicate that CMPCROC exhibits significant antibacterial activity, making it a promising candidate for further exploration and development in the field of antibacterial agents. This study lays the groundwork for future research aimed at optimizing the compound's efficacy, broadening its application spectrum, and understanding its mechanism of action. Additionally, the findings contribute to the ongoing search for novel, effective solutions to combat bacterial infections in both agricultural and clinical settings, underscoring the value of CMPCROC in addressing challenges posed by bacterial resistance.

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