Synthesis of 7-Hydroxy-4-methyl coumarin and its Biological activity of coumarin derivative synthesized from substituted from phenols

Submitted in partial fulfilment of the requirements for the award of Master of Science Degree in Chemistry
By:

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DEPARTMENT OF CHEMISTRY

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SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited with Grade "A" by NAAC I 12B Status by UGC I Approved by AICTE

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March – 2021

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **RIKITA BARDHAN (39910017)** who carried out the project entitled "**Synthesis of 7-Hydroxy-4-methyl coumarin and its Biological activity of coumarin derivative synthesized from substituted from phenols**" under my supervision from December 2020 to March 2021.

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DECLARATION

I, RIKITA BARDHAN, hereby declare that the Project Report entitled "**Synthesis of 7-Hydroxy-4-methyl coumarin and its Biological activity of coumarin derivative synthesized from substituted from phenols"** done by me under the guidance of **Dr. T. Krithiga M.Sc., M. Phil, Ph.D.,** Associate Professor, Department of Chemistry, Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Master of Science degree in Chemistry.

DATE:

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RIKITA BARDHAN

ABSTRACT

The key conclusion of this project is to use oxalic acid as a catalyst in Pechmann Condensation to produce 7-Hydroxy-4-methyl coumarin from Resorcinol and Ethyl acetoacetate. It then focuses on determining antibacterial activity of the same substance using the zone of inhibition process. The biological activity of the assembled product is examined by determining its antibacterial activity. The reaction of Resorcinol and Ethyl acetoacetate with oxalic acid as a catalyst in Ethanol under abatement conditions yielded a substituted coumarin derivative. FTIR, Mass, and NMR spectroscopic techniques were used to characterise the final product. Spectral studies revealed the structure and functional group present in the acquired product. The antibacterial activity of the synthesised coumarin derivatives was tested against a gramme negative bacterium and found to be effective against Bacillus Cereus.

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LIST OF ABBREVIATIONS

MCR	-	Multicomponent Reactions
CPT	-	Camptothecin
FTIR	-	Fourier Transform Infrared
NMR	-	Nuclear Magnetic Resonance

CHAPTER 1

INTRODUCTION

1.1 MULTICOMPONENT REACTION

A multi-component reaction (or MCR) is a chemical reaction in which three or more compounds combine to form a single product. It is also known as a "Multi-component Assembly Process" (or MCAP). [Thomas A S et al 1996]. Multicomponent reactions, by definition, are those in which more than two reactants combine in a sequential manner to produce highly selective products that preserve the majority of their constituents. . For over 150 years, multicomponent reactions have been documented. The Strecker synthesis of -amino cyanides, from which -amino acids could be produced, was the first known multicomponent reaction in 1850. There are several different types of MCRs today, with isocyanide-based MCRs being the most well-known. Other MCRs include free-radical-mediated MCRs, organoboron-based MCRs, and metal-catalyzed MCRs. Since the isocyanide is such a unique functional group, isocyanide-based MCRs are commonly used. It is thought that its tetravalent and divalent carbon forms have resonance. The isocyanide group undergoes both electrophilic and nucleophilic reactions at the CII atom, resulting in an exothermic conversion to the CIV form. Isocyanides have become a valuable functional group due to their presence in natural products. The Passerini 3-component reaction, which produces -acyloxy carboxamides, and the Ugi 4-component reaction, which produces -amino carboxamides, are the two most important isocyanide-based multicomponent reactions. [Akul Mehta et al 2009] The precise nature of this form of reaction is often unknown. difficult to evaluate. The interaction of three or more separate molecules at the same time is less likely to result in a slow reaction rate. A sequence of bimolecular reactions is more likely in these reactions. New MCR's are identified by constructing a chemical library from combinatorial chemistry or by combining existing MCR's [Ivar Ugi S et al,.2001]. Combining the Ugi reaction with t produces a 7-component MCR, for example. MCRs are a significant tool in the development of new drugs. For designing new lead structures of active agents, MCRs may also be expanded into combinatorial, solid phase, or flow syntheses [Thomas J. J. Müller S et al, 1996]

1.2 RESORCINOL

Resorcinol is a naturally occurring organic compound with the formula C6H6O2. It's one of three isomeric benzenediols that's white and water soluble. Resorcinol or m-Dihydroxybenzene are other names for it. It's benzenediol's 1,3 isomer. It's a benzenediol that's dihydroxylated at positions 3 and 1 with benzene. It acts as a sensitizer and an inhibitor of erythropoietin. Resorcin is a crystalized protein. Carbon disulfide and chloroform do not dissolve in it. If it is not in its pure form, it turns pink when exposed to light. However, it is difficult to ignite. It's a common ingredient in pharmaceuticals and plastics. m-Dihydroxybenzene can be made in a variety of ways. A few of them are mentioned in this article. 1,3-diisopropyl benzene is generated by dialkylation of benzene with propylene. Rearrangement by Hock As this compound is oxidized, it yields resorcinol and acetone. Resorcinol, also known as m-dihydroxybenzene, is a phenolic compound used to make resins, colourants, plastics, pharmaceuticals, and a variety of other organic chemical compounds. It's made by sulfonizing benzene with fuming sulfuric acid and then fusing caustic soda to the resulting benzenedisulfonic acid in large quantities. It's made by combining KOH (potassium hydroxide) with resins like asafoetida and galbanum, or by distilling Brazilwood extract. It can also be made by reacting nitrous acid (HNO2) with 1,3-diaminobenzene or 3-aminophenol to form benzene-1,3-disulfonic acid, phenol-3-sulfonic acid, and 3-iodophenol in the presence of potassium carbonate (K2CO3). On fusion with KOH, many para and ortho aromatic compounds, such as benzene-para-disulfonic acid and bromophenols, yield resorcinol (potassium hydroxide) Resorcinol is capable of breaking down rough, hardened, or scaly skin. Eczema, acne, psoriasis, corns, seborrhea, calluses, warts, and other skin conditions are treated with it in its topical form.

1.3 MANNICH REACTION

The Mannich reaction is an organic reaction in which formaldehyde and a primary or secondary amine or ammonia amino alkylate an acidic proton positioned next to a carbonyl functional group. A -amino-carbonyl compound, also known as a Mannich base, is the end result. Mann reactions include those involving aldimines and -

methylene carbonyls. Carl Mannich, a chemist, is honoured with the reaction's name. The Mannich reaction is a nucleophilic amine addition to a carbonyl group followed by dehydration to the Schiff base. The Schiff base is an electrophile that interacts with a compound containing an acidic proton in the second step of an electrophilic addition (which is, or had become an enol). The Mannich reaction is also taken into account. a condensation process The Mannich reaction uses primary or secondary amines, as well as ammonia, to activate formaldehyde. The intermediate enamine is formed by tertiary amines that lack a N–H proton. Carbonyl compounds, nitriles, acetylenes, aliphatic nitro compounds, -alkyl-pyridines, and imines are examples of -CH-acidic compounds (nucleophiles). [Mannich et al, 1912] Double Mannich reactions are also very common to set-up Set-ups for double Mannich reactions are also very common.

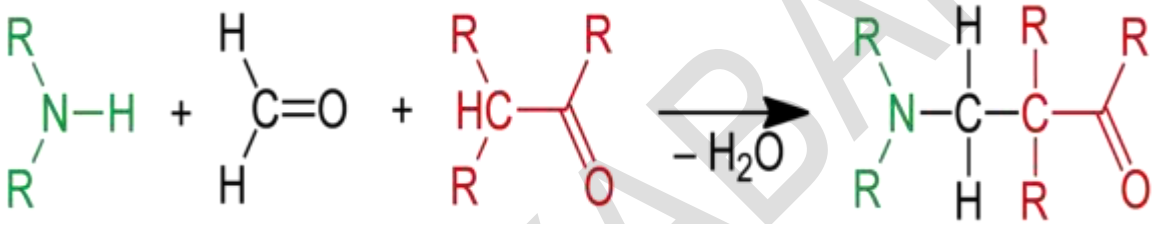

FIG 1.1 MANNIC REACTION OF COUMARIN DERIVATIVES

1.4 PAUSON-KHAND REACTION

The Pauson–Khand reaction (or PKR or PK-type reaction) is a [2+2+1] cycloaddition between an alkyne, an alkene, and carbon monoxide that results in the formation of a,-cyclopentenone. [F.S. Blicke et al, 2011, Pauson et al, 1977]. Ihsan Ullah Khand discovered the reaction while working as a postdoctoral associate with Peter Ludwig Pauson at the University of Strathclyde in Glasgow [Pauson et al, 1977]. Originally, stoichiometric amounts of dicobalt octacarbonyl were used to catalyze this reaction, but newer forms are both more effective and catalytic [Blanco-Urgoiti et al, 2004] Regioselectivity can be a concern with unsymmetrical alkenes or alkynes, but not so much with intramolecular reactions. Regioselectivity can be a concern with unsymmetrical alkenes or alkynes, but not so much with intramolecular reactions. Both terminal and internal alkynes can be used in the reaction, but internal alkynes produce lower yields. Strict cyclic alkene > terminal alkene > disubstituted alkene > trisubstituted alkene is the order of reactivity for the alkene. Tetra substitute unsuitable

alkenes and alkenes with strongly electron withdrawing groups[Gibson et al, 2003]

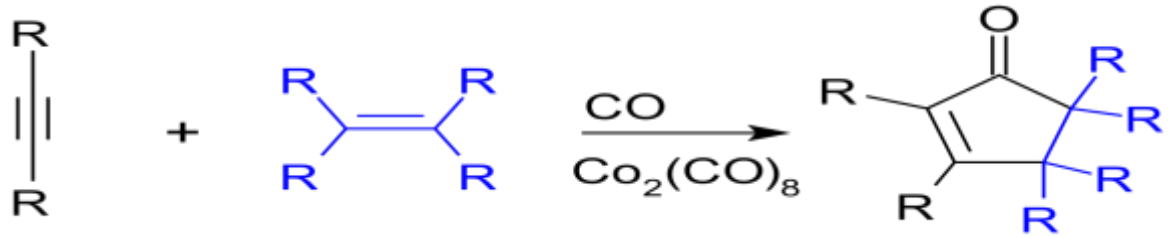

FIG 1.2 PAUSON-KHAND REACTION OF COUMARIN DERIVATIVES

1.5 ANTIBACTERIAL ACTIVITY

Antibacterial and antiviral activity of a molecule is totally correlated with compounds that destroy bacteria and viruses locally or delay their rate of growth without being harmful to surrounding tissues. The most recently found antimicrobial agents are natural molecules that have been chemically engineered, such as b-lactams (penicillins), and carcinogens., carbapenems, or cephalosporin. Pure natural antibiotics, such as aminoglycosides, and synthetic antibiotics, such as sulfonamides, are also commonly used. Antimicrobial agents may be graded as either bactericidal (killing bacteria) or bacteriostatic (slowing the growth of bacteria). Antibacterial agents are crucial in the war against infectious diseases. However, as a result of their widespread usage and violence, bacterial resistance to antibacterial agents has emerged as a major issue for today's pharmaceutical industry. Resistance is most frequently caused by developmental processes that result in inheritable resistance, such as antibiotic therapy [2–6]. Microorganisms are becoming increasingly resistant to antibacterial agents has been the source of major health problems in recent years. The majority of infectious bacteria are immune to at least one of the antibiotics commonly used to treat infections. This problem necessitates the development of novel agents capable of effectively inhibiting the growth of microorganisms. Optical structures, fuel cells, catalysts, biosensors, and other applications for nanoparticles have been consideredDrugs, superconductors, and gene transmission are only a few examples. (7–17) [W.S.et.al, 1912]. Nanomaterials have also been used to improve the physicochemical and therapeutic efficacy of medicines as a novel drug delivery vehicle. Similarly, nanotechnology in pharmaceuticals and microbiological research has shown promising results in combating antibiotic resistance. In recent years, there has been a significant increase in Researchers have investigated a variety of

nanosized antibacterial agents, including carbon-based nanoparticles, metallic and metal oxide nanoparticles, and polymeric chitosan nanoparticles. Silver (Ag), silver oxide (AgO), titanium dioxide (TiO), zinc oxide (ZnO), gold (Au), calcium oxide (CaO), silica (Si), copper oxide (CuO), and ma are examples of metallic and metal oxide nanoparticles.magnesium oxide (MgO) have been found to show both antibacterial and antiviral activity [18–24]

CHAPTER 2

LITERATURE SURVEY

MCRs are convergent reactions in which three or more starting materials react to form a product in which almost all or most of the atoms contribute to the newly formed product. A product is assembled in an MCR using a series of elementary chemical reactions. As a result, there's a web of reaction equilibria that eventually flow through an irreversible phase yield the finished product. The aim is to carry out an MCR in such a way that the network of pre-equilibrated reactions channels into the main product while avoiding side products. The outcome is obviously influenced by the reaction conditions: solvent, temperature, catalyst, concentration, type of starting materials, and functional groups are all variables to consider. Such considerations are particularly important in the development and discovery of novel MCRs. Below are a few works that are related.

Venkata et al. used the click reaction to make a sequence of novel 3-(1-(substitutedphenyl)-1H-1,2,3-triazol-4-yl)methoxyimino)ethyl)-2H- chromen-2-one derivatives 41. (E) 1-((prop-2-yn-1-yloxy)imino)ethyl)-3-(1-((prop-2-yn-1-yloxy)imino)ethyl)-3-(1-((prop-2-yn-1 In THF:H2O, -2H-chromen-2-one 40 and aryl azide 6 were synthesised in the presence of sodium ascorbate and CuSO4-5H2O. The majority of the synthesised compounds were neuroprotective and toxin-free.H_2O_2-induced $PC1_2$ cell lines [Venkataramaiah et al, 2018]

Ablajan et al. synthesized coumarin-containing dihydropyrano[2,3-c]pyrazoles 123.The four-component reaction of -dicarbonyl compound 86, phenylhydrazine 20, aromatic aldehydes 119, and malononitrile 122 in EtOH catalysed by L-proline under ultrasonic irradiation was used by This technique has a number of benefits, including a quick workup procedure and a faster reaction time [M. Seydimemet S.et.al 2016].

Yalcn et al. synthesised a large series of fluorescence coumarin–pyrazole–triazine–based chemosensors (CPT) bearing 5-hydroxypyrazole 65 as a receptoric component. In addition, compound 86 was made. Through the reaction of compound 61 with 6-hydrazinyl-N2,N2,N4,N4-tetramethyl-1,3,5-triazine-2,4-diamine 90, Under reflux conditions, 4-(diethylamino)-2-hydroxybenzaldehyde 84 is cycloadditioned with dimethyl 3- oxopentanedioate 85 in the presence of catalytic amounts of piperidine in EtOH [Yalcn et al., 2018].

Chen et al. used a reaction of 3-(1-(2-bromoacetyl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one 94 and flavone or amine at 40–50 °C to make pyrazoline–coumarin derivatives 95. The condensation of 3-cinnamoyl-2H-chromen-2-one compound 92 with hydrazine 20 yielded compound 94 After cyclization with 2-bromoacetic acid 93 in EtOH at 40–60 °C, Initial testing revealed that some derivatives had higher TNF- and IL-6 inhibitory activity than others [L.Z. Chen S.et.al 2017].

Yana et al created novel 6-pyrazolinylcoumarins 94. 5-Acetoxy-7-methyl coumarins derivatives 117 were synthesised from 5-hydroxy-7-methyl coumarins 116 in Ac2O under reflux conditions with catalytic amounts of pyridine. The reaction of 5-acetoxy-7-methyl coumarins 117 with AlCl3 under reflux conditions yielded 6-acetyl-5-hydroxy-7-methyl coumarins 118 In the presence of pyrrolidine, Claisen–Schmidt condensation of 118 with aromatic aldehydes 119 provided 2-aryl-5-methyl-2,3-dihydropyrano-[2,3-f]chromen-4,8-diones 120. Finally, 6-[5-aryl-4,5-dihydropyrazol-3-yl]pyrazol-3-ylpyrazol-3-ylpyrazol-3-ylpyrazol-3-ylpyra Hydrazine 20 was combined with 2-aryl-5-methyl-2,3-dihydropyrano[2,3-f]chromen-4,8-diones 120 in EtOH to produce -5-hydroxy-7-methyl coumarins 121. [Garazd et al, 2016].

CHAPTER 3

AIM AND SCOPE

3.1 AIM

The main aim of this project is to Synthesis of 7-Hydroxy-4-methyl coumarin from Resorcinol and Ethyl Acetoacetate using using oxalic acid as catalyst. The spectral analysis of this product should be determined by studying FTIR, NMR and Mass Spectroscopy derivatives and the biological activity of product by determining its antibacterial activity.

3.2 SCOPE

- Preparation of Coumarin derivative from Resorcinol and Ethyl Acetoacetate by Pechmann reaction.
- Product is confirmed by characterization studies (FTIR and ^{1}H-NMR).
- Testing should be done for antibacterial activity of the obtained product with Escherichia coli.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS

The materials used in this reaction are Resorcinol, Ethyl acetoacetate, Oxalic acid, and ethanol, which were purchased from Merck chemicals and used as it is.

4.2 CHARACTERIZATION TECHNIQUES

4.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR (Fourier-transform infrared spectroscopy) is a technique for obtaining an infrared spectrum of absorption or emission of a solid, liquid, or gas [Griffiths,P.S.et.al 2007]. An FTIR spectrometer gathers high-resolution spectral data over a large spectral spectrum at the same time. This gives it a big advantage over a dispersive spectrometer, which only measures strength over a small range of wavelengths at at time. The term "Fourier-transform infrared spectroscopy" comes from the fact that raw data must be converted into a spectrum using a Fourier transform (a mathematical process). The use of Fourier-transform spectroscopy to obtain the same knowledge is less intuitive. Rather than using a monochromatic light source (a beam composed of only a single wavelength) This method shines a beam containing several frequencies of light at the sample and tests how much of it is absorbed by the sample. The beam is then modified to contain a different frequency combination, yielding a second data point. This process is replicated several times in a short period of time. After that, a computer takes all of this information and works backwards to figure out what each wavelength's absorption is Starting with a broadband light source—one that contains the entire spectrum of wavelengths to be measured—the beam mentioned above is formed. The light shines through a Michelson interferometer, which is a collection of mirrors with one of them being driven by a motor. Each wavelength of light in the beam is periodically blocked and transmitted as this mirror moves Wave interference is caused by the interferometer. Different wavelengths are modulated at different

speeds, resulting in a different spectrum for each moment or mirror location in the interferometer.

Fig. 4.1 FTIR Spectrometer

4.2.2 Nulear Magnetic Resonance Spectroscopy (NMR)

The study of molecules by recording the interaction of radiofrequency (Rf) electromagnetic radiations with the nuclei of molecules put in a strong magnetic field is known as nuclear magnetic resonance (NMR) spectroscopy. NMR spectroscopy, also known as magnetic resonance spectroscopy, is a form of nuclear magnetic resonance spectroscopy. The spectroscopic technique of magnetic resonance spectroscopy (MRS) is used to observe local magnetic fields around atomic nuclei. The NMR signal is provided by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is detected with sensitive radio receivers, and the sample is placed in a magnetic field. The resonance of a molecule is changed by the intramolecular magnetic field around an atom This allows access to the electronic structure of a molecule as well as its individual functional groups. NMR spectroscopy is the definitive method for identifying monomolecular organic compounds in modern

organic chemistry practise since the fields are special or highly characteristic to individual compounds. NMR spectrometers are relatively expensive; however, they are commonly used in universities However, they are less popular in private businesses. An NMR spectrometer cost between 500,000 and 5 million dollars between 2000 and 2015. Since resolution is directly proportional to magnetic field strength, modern NMR spectrometers have a strong, massive, and expensive liquid helium-cooled superconducting magnet. Permanent magnet machines with lower resolution are also available at a lower cost For certain applications, such as reaction control and sample checking, the output is still adequate. There are also nuclear magnetic resonance spectrometers that can be used on a benchtop. In magnetic fields smaller than a millitesla, NMR can be observed. Low-resolution NMR generates larger peaks that can easily overlap, making it difficult to resolve complex structures. The use of higher-intensity magnetic fields produces strong peak resolution and is the industry norm. [S.et.al., Marc S. Reisch 2015]

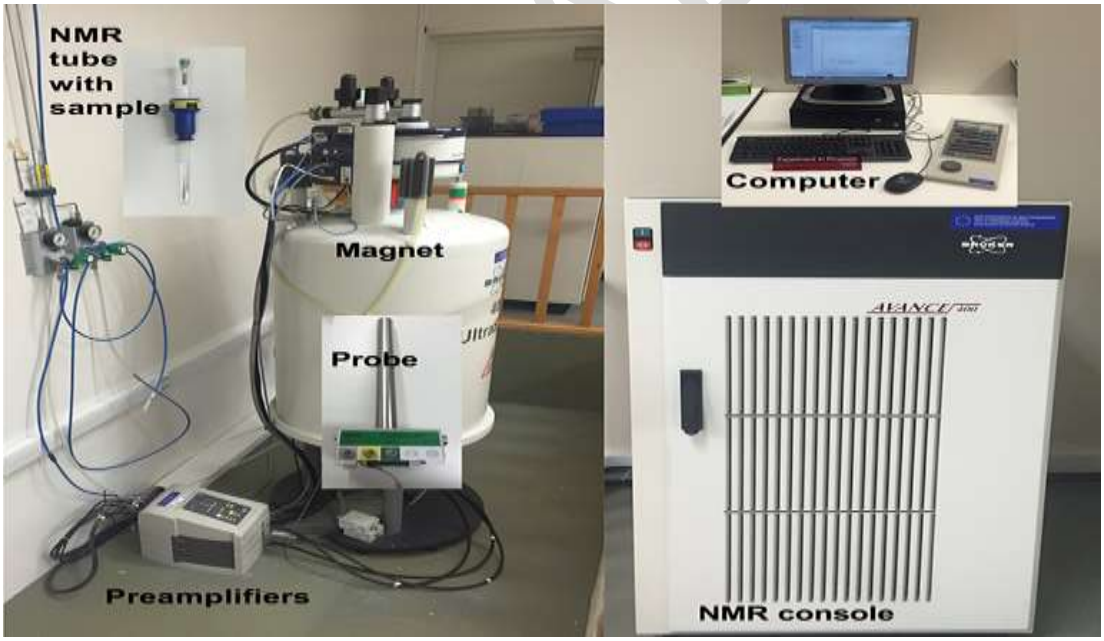

Fig. 4.2 Nuclear Magnetic Resonance Spectrometer

4.2.3 Mass Spectroscopy

The mass-to-charge ratio of ions is measured using mass spectrometry (MS), an analytical technique. A mass continuum, or a plot of intensity as a function of mass-to-charge ratio, is a common way to view the data. Mass spectrometry is used in a

variety of fields and can be used on both pure samples and complex mixtures. A mass spectrum is a graph of the ion signal as a function of the mass of the ions. The mass-to-charge ratio is a measure of how much mass there is in a given These spectra are used to figure out a sample's elemental or isotopic signature, particle and molecule weights, and the chemical identity or structure of molecules and other chemical compounds. A sample, which can be solid, liquid, or gaseous, is ionised in a standard MS procedure by bombarding it with a beam of electrons, for example. Any of the sample's molecules can break up into positively charged fragments or become positively charged without fragmenting as a result of this. These ions (fragments) are then divided based on their mass-to-charge ratio, such as by accelerating them and exposing them to an electric or magnetic field: ions with the same mass-to-charge ratio would go through the same process. The sum of deflection is the same. A mechanism capable of detecting charged particles, such as an electron multiplier, detects the ions. The results are shown as spectra of detected ion signal strength as a function of mass-to-charge ratio. The atoms or molecules in the sample may be detected by comparing known masses (e.g., the mass of an entire molecule) to the identified masses, or by using a mass spectrometer fragmentation pattern.

Fig. 4.3 Mass spectrometer

4.3 ONE POT SYNTHESIS

4.3.1 Synthesis of substituted chromene derivative

In a round bottom flask, a mixture of Resorcinol (10 mmol), Ethyl acetoacetate (10 mmol), and Oxalic acid (1 gramme) (Catalyst) was refluxed at 70oC for around 6 hours with Ethanol (10 mL) as a solvent. After the reaction was completed, the solvent was allowed to evaporate at room temperature, yielding the crude product. The crude was recrystallized to obtain the pure product.with hot ethanol.

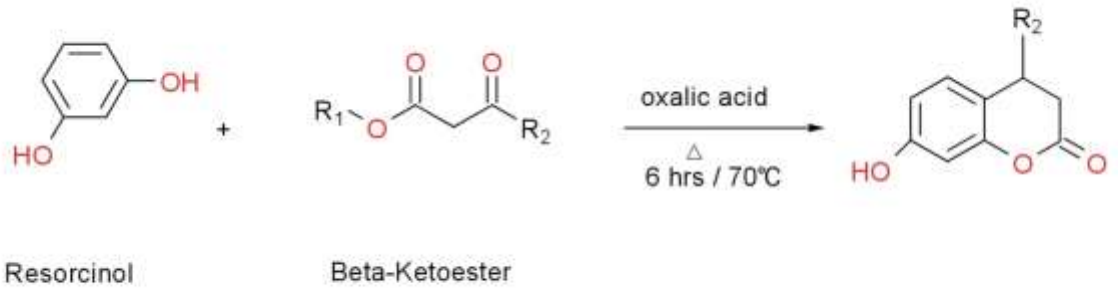

Fig. 4.4 Reaction scheme of formation of substituted Chromene derivative

CHAPTER 5

RESULT AND DISCUSSION

5.1 SYNTHESIS OF 7-HYDROXY-4-METHYL COUMARIN DERIVATIVE

Using oxalic acid as a catalyst, the standard Pechmann condensation reaction is carried out by reacting Resorcinol and Ethyl acetoacetate in a 1:1 ratio. Ethanol is used as a solvent, and the reaction takes about 6 hours to complete. To determine the functional group and structural formula, the obtained product is subjected to spectral studies (FTIR, NMR Spectroscopy).

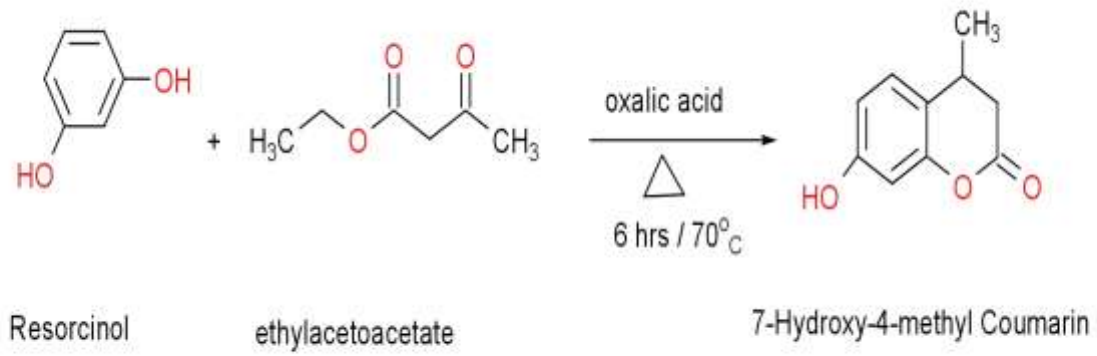

Fig. 5.1 Reaction scheme of formation of 7-Hydroxy-4-methyl Coumarin

5.1.1 Spectral data of 7-Hydroxy-4-methyl Coumarin Derivative

FTIR Data: The collected product's FTIR Spectral data is depicted in Fig. 5.2 below. The 3468 cm-1, 3112 cm-1, and 2921 cm-1 product peaks correspond to OH, Sp^2-CH Stretch, and Sp^3 C=O stretch, respectively

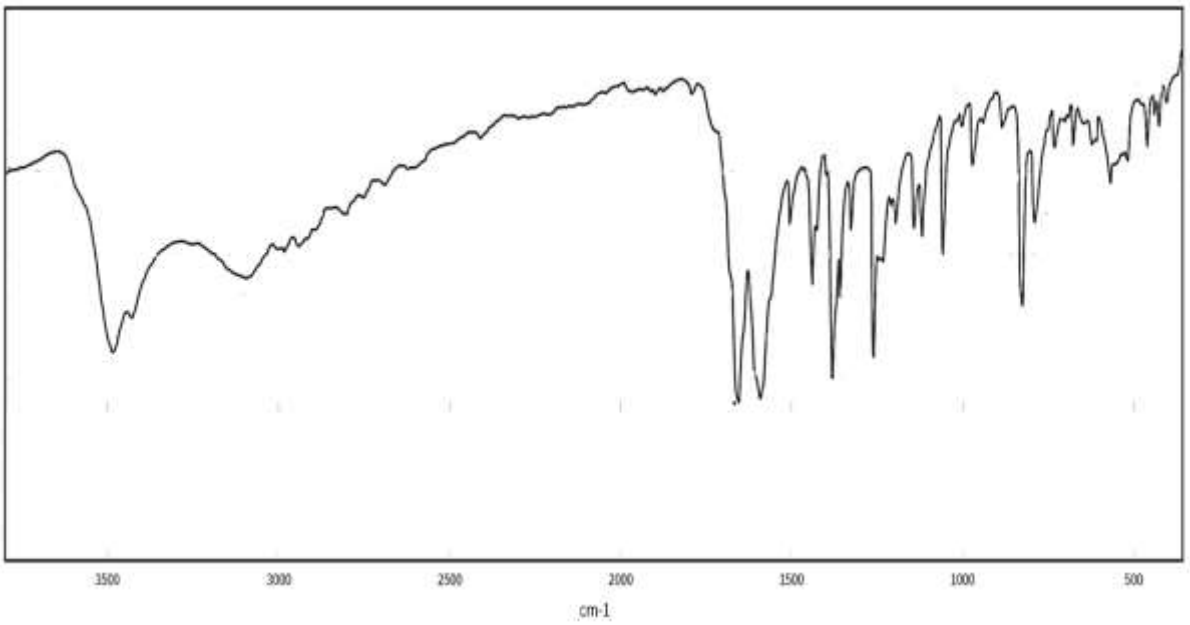

Fig. 5.2 FTIR Spectra of 7-Hydroxy-4-methyl Coumarin Derivative

Table 5.1 FTIR Vibrational frequencies of 7-Hydroxy-4-methyl Coumarin Derivative by FTIR spectra

Vibrational Frequency	Functional group identification
3468	OH Stretching
3112	C-H Stretching (Sp^3)
2921	C-H Stretching($Sp^{3)}$
1668	C=O Stretching
1604	C=C Alkene
1452	C=C Aromatic
1076	C-0-C Ester

NMR Data: The 1H NMR spectra of 7-Hydroxy-4-methyl Coumarin, which confirms its structure. Table 5.2 shows the chemical change of structural units in 7-Hydroxy-4-methyl Coumarin based on the 1H NMR spectrum shows the Figure 5.3

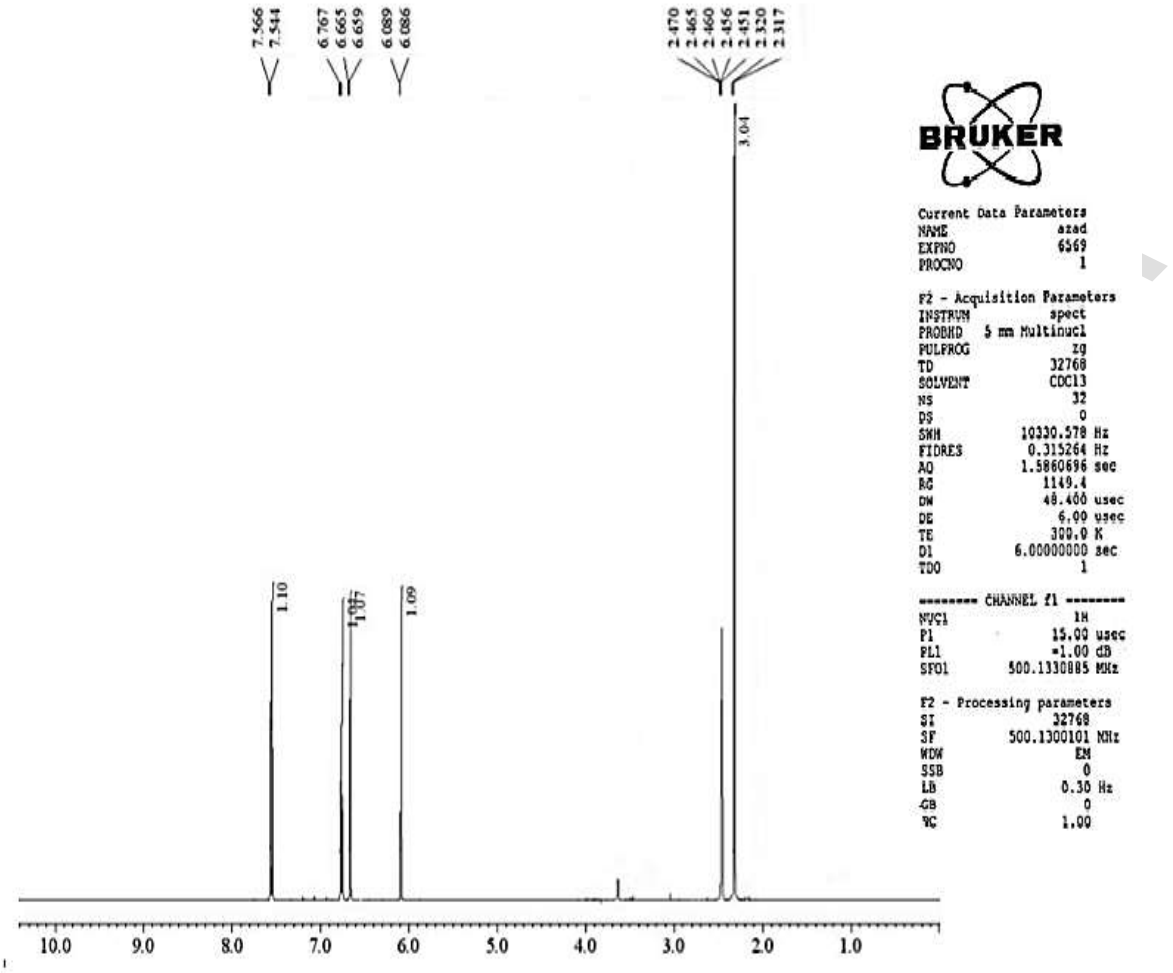

Fig. 5.3 NMR Spectra of 7-Hydroxy-4-methyl Coumarin Derivative

Table 5.2 Chemical shift of structural units in 7-Hydrox-4-methyl Coumarin derivative from ^{1}H NMRSpectrum

Chemical shift (δ)	**Structural unit**
3.04	RO-CH_3
6.086	Vinylic Proton
6.6-6.7	Aromatic Proton
7.5-7.5	Aromatic Proton

5.2 MECHANISM OF FORMATION OF 7-HYDROXY-4-METHYL COUMARIN DERIVATIVE

In this reaction, a strong Bronsted acid, such as oxalic acid, is used. The acid catalyses both the transesterification and keto-enol tautomerization reactions. When the Coumarin skeleton is subjected to a Michael Addition, the next step is established. Rearomatization occurs after this point. The compound is then produced by the removal of water caused by the acid.

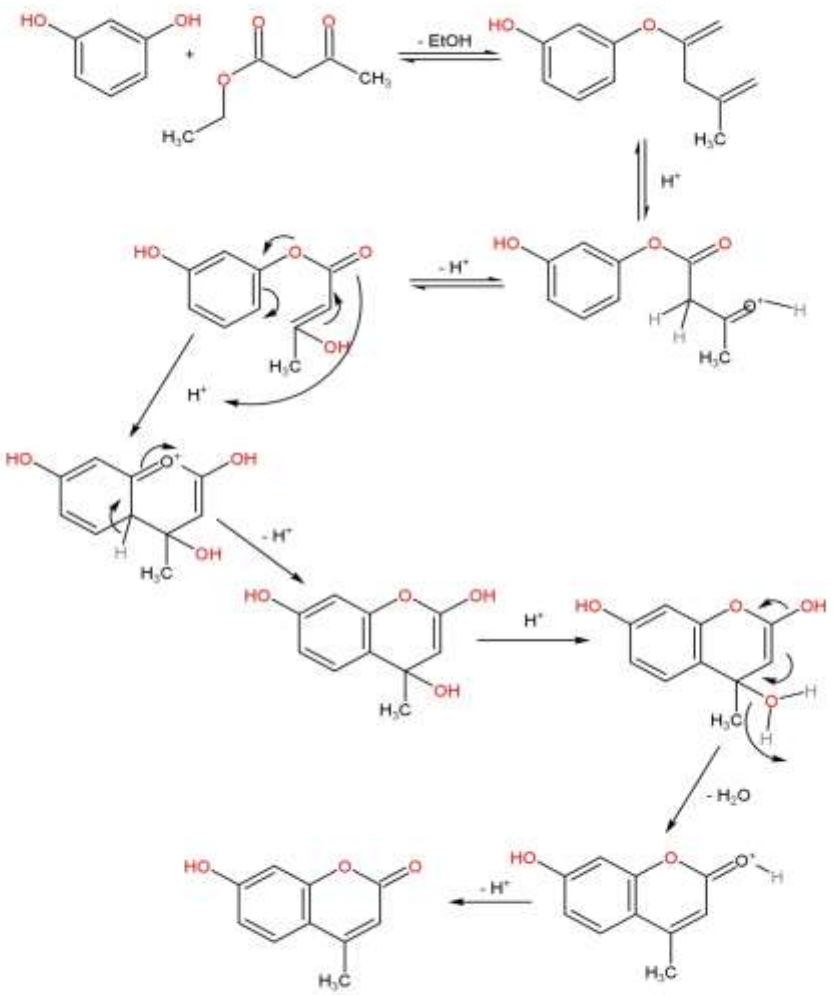

Fig. 5.4 Mechanism of formation of 7–Hydroxy- 4 - Methyl Coumarin Derivative

5.3 ANTIBACTERIAL STUDIES

- Antibacterial research is carried out using the agar well diffusion process.
- It employs antibiotic discs to determine the degree to which antibiotics damage bacteria.
- The region around the sample where the bacteria haven't evolved to the point where they can be seen. This is referred to as a zone inhibition.
- Antibacterial activity has been discovered against Bacillus cereus

Table 5.3 zone of inhibition

MICRO-ORGANISM	ZONE OF INHIBITION IN mm
Bacillus cereus	22

5.3.1 Antibacterial Activity Result

- It is tested for antibacterial activity against this microorganisms: Bacillus cereus was isolated using the well diffusion process.

- ***RESULT***: It has a strong antibacterial effect.

FIG. 5.5Zone of Inhibition of Bacilus cereus

CHAPTER 6

CONCLUSION

The present study reports the synthesis of substituted coumarin by Pechmann reaction. The reaction of the Resicinol with ethyl acetoacetate at ambient condition results in the successful formation of 7-hydroxy-4-methyl coumarin by Pechmann reaction using MCR method. The mechanism of the formation of reaction product through one pot synthesis is proposed. The functional groups of the synthesized product were confirmed by FTIR spectroscopy. The structure of 7-hydroxy-4-methyl coumarin was confirmed by the ^{1}H-NMR spectra. The biological activity of the synthesized 7-hydroxy-4-methyl coumarin was tested against Bacillus Cereus. The synthesized 7-hydroxy-4-methyl coumarin show good antibacterial activity against Bacillus Cereus.

REFERENCES

1. Robert W. Armstrong, Andrew P. Combs, Paul A. Tempest, S. David Brown, and Thomas A. Multiple-Component Condensation Strategies for Combinatorial Library Synthesis Acc. Chem. Res., 1996, Vol 29 pp 123–131

2. Akul Mehta Presentation on Multicomponent Reactions, 2009 pp 263

3. Ivar Ugi Pure Appl. Recent progress in the chemistry of multicomponent reactions Chem., 2001, Vol. 73, No. 1, pp. 187-191

4. Alexander Dömling the discovery of new isocyanide-based multi-component reactions Current Opinion in Chemical Biology 2000,4, 318-323

5. Thomas J. J. Müller Multicomponent reactions (Editor) Thematic Series in the Open Access Beilstein Journal of Organic Chemistry Vol 73 pp 175

6. Carl Mannich; Krösche, "Ueber ein Kondensationsprodukt aus Formaldehyd, Ammoniak und Antipyrin". .Vol 250 pp 647–667.

7. Blicke, F. F. "The Mannich Reaction". Organic Reactions. Vol 1 pp 303–341.

8. Pauson,P.L.; Khand,. "Uses of Cobalt-Carbonyl Acetylene Complexes in Organic Synthesis".Vol 295 pp 2–14.

9. Blanco-Urgoiti, Jaime; Añorbe, Loreto; Pérez-Serrano, Leticia; Domínguez, Gema; Pérez-Castells, Javier. "The Pauson–Khand reaction, a powerful synthetic tool for the synthesis of complex molecules". Chem. Soc. Vol 33 pp 32–42

10. Werner, Helmut "Obituary: Peter Ludwig Pauson Angew. Chem. Int. Ed. Vol 53 pp 3309.

11. Gibson, Susan E.; Stevenazzi, Andrea . "The Pauson–Khand Reaction: The Catalytic Age Is Here!". Vol 42 pp 1800–1810.

12. Jeong, Nakcheol; Hwang, Sung Hee; Lee, Youngshin; Chung, Young Keun "Catalytic version of the Intramolecular Pauson-Khand Reaction". Journal of the American Chemical Society. VOL 116 pp 3159

13. László Kürti,Barbara Czakó Strategic applications of named reactions in organic synthesis: background and details mechanisms Vol 89 pp 72

14. Vivek P. Chavda. Nanotherapeutics and Nanobiotechnology vol 1 Pages 1-

15. Amit K. Goyal, Gautam Rath,Basant Malik Application and Perspective of pH-Responsive Nano Drug Delivery Systems Vol 73 Pages 133

16. M.A. Kumari, C.V. Rao, S. Triloknadh, N. Harikrishna, S. Venkataramaiah, N. Rajendra, C. Trinath, W.D.Y. Suneetha, Res. Chem. Intermed. Vol 44 pp 1989

17. Griffiths, P.; de Hasseth, J. A. Fourier Transform Infrared Spectrometry Wiley-Blackwell. pp 76

18. Marc S. Reisch "NMR Instrument Price Hikes Spook" Vol 132 pp 75-102

19. Khan, A. Khan, S.A. Halim, A. Saeed, S. Mehsud, R. Csuk, A. Al-Harrasi, A. Ibrar, Int. J. Biol. Macromol. Pp 142, 345 (2020)

20. Paudler, William Nuclear Magnetic Resonance pp. 9–11.

21. M.A. Kumari, C.V. Rao, S. Triloknadh, N. Harikrishna, S. Venkataramaiah, N. Rajendra, C. Trinath, W.D.Y. Suneetha, Res. Chem. Intermed. Vol 44, 1989 (2018) pp 17-23

22. Ishikawa, H.; Suzuki, T.; Hayashi, Y. "one-pot" operations" Vol 48 pp 1304–1307

23. Yanina Moglie, Evangelina Mascaró, Victoria Gutierrez, Francisco Alonso, and Gabriel . The Journal of Organic Chemistry 2016, Vol *81* pp 1813-1818.

24. Jia Yang, Tieqiao Chen, Yongbo Zhou, Shuang-Feng Yin, and Li-Biao Han . Mechanistic Studies on the Palladium-Catalyzed Cross Dehydrogenative Coupling of P(O)–H Compounds with Terminal Alkynes: Stereochemistry and Reactive Intermediates. Vol *34* (20) pp 5095-5098.\

25. Kévin Jouvin, Alexis Coste, Alexandre Bayle, Frédéric Legrand, Ganesan Karthikeyan, Krishnaji Tadiparthi, and Gwilherm Evano . Copper-Mediated Selective Cross-Coupling of 1,1-Dibromo-1-alkenes and Heteronucleophiles: Vol *31* pp 7933-7947.

26. Cameron, M.; Hoerrner, R. S.; McNamara, J. M.; Figus, M.; Thomas, S. . "One-Pot Preparation of 7-Hydroxyquinoline". Vol 10 : pp149.