SYNTHESIS,CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF BENZALACETOPHENONE

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Abstract : The reaction between Grignard reagents and **Benzalacetophenone** is an important transformation in organic chemistry. Benzalacetophenone is an α, β-unsaturated ketone belonging to the chalcone family and contains both a carbonyl (C=O) group and a conjugated carbon–carbon double bond (C=C), which makes it highly reactive. Grignard reagents (RMgX) are strong nucleophiles that readily attack electrophilic centers in such molecules. When they react with benzalacetophenone, nucleophilic addition occurs through two possible pathways. In the 1,2-addition pathway, the Grignard reagent attacks the carbonyl carbon, leading to the formation of an allylic alcohol after hydrolysis. In the 1,4-addition pathway, also known as conjugate addition, the nucleophile attacks the β-carbon of the double bond, resulting in a saturated ketone after protonation. Generally, Grignard reagents favor 1,2addition because of their high reactivity, although the presence of copper catalysts can promote 1,4-addition. The outcome of the reaction depends on factors such as solvent, temperature, and catalyst. This reaction is widely used for the formation of carbon–carbon bonds and plays a key role in the synthesis of complex organic molecules. Furthermore, chalcone derivatives obtained from such reactions exhibit important biological activities, including antimicrobial and antioxidant properties. Therefore, this reaction holds significant value in both synthetic and medicinal chemistry, providing a versatile approach for modifying chalcone structures.

INTRODUCTION

Grignard reagents have been one of the most commonly used organometallic compounds in chemistry over the last century.[1] They are usually prepared in ether solvents, such as diethyl ether or tetrahydrofuran (THF), by reacting an organic halide with magnesium metal.[2]

GENERAL REACTION

$$RX + Mg \rightarrow RMgX \text{ (where X = Cl, Br, or I)}$$

These reagents are generally stable when dissolved in ether, but they must be kept away from moisture and oxygen because they react easily with them. Overall, they are highly reactive and very useful in chemical synthesis.[3]

Grignard reagents were discovered in 1900 by Victor Grignard at the University of Lyon. Due to their importance and wide applications in organic chemistry, he was awarded the Nobel Prize in Chemistry in 1912.[4]

In solution, these reagents exist in equilibrium with other magnesium compounds

$$2RMgX \rightleftharpoons R_2Mg + MgX_2.$$

The exact composition depends on the type of organic halide used in the reaction.[5]

An example is ethylmagnesium bromide, which can form a structure coordinated with ether molecules. Its structure was determined in 1964 using X-ray diffraction[1]. Seyferth D. The grignard reagents. Organometallics.[6]

SYNTHESIS OF BENZALACETOPHENONE

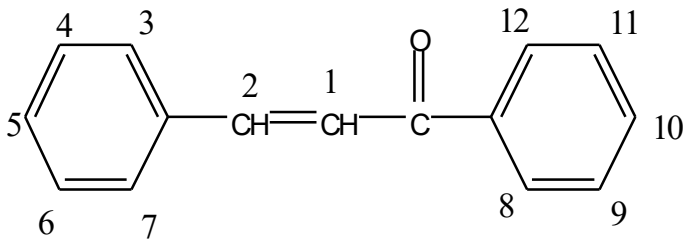
The preparation of benzalacetophenone is achieved through the Claisen–Schmidt condensation reaction involving acetophenone and benzaldehyde as the starting materials.[6] Initially, a clean and dry round-bottom flask is selected, and equimolar amounts of acetophenone and benzaldehyde are accurately measured and introduced into the flask. These reactants are dissolved in an appropriate quantity of ethanol to form a clear and uniform solution with continuous stirring. Separa1tely, a freshly prepared

aqueous sodium hydroxide solution (10–20%) is made and then added gradually to the reaction mixture while stirring constantly to ensure proper mixing and to avoid localized concentration of the base.[7]

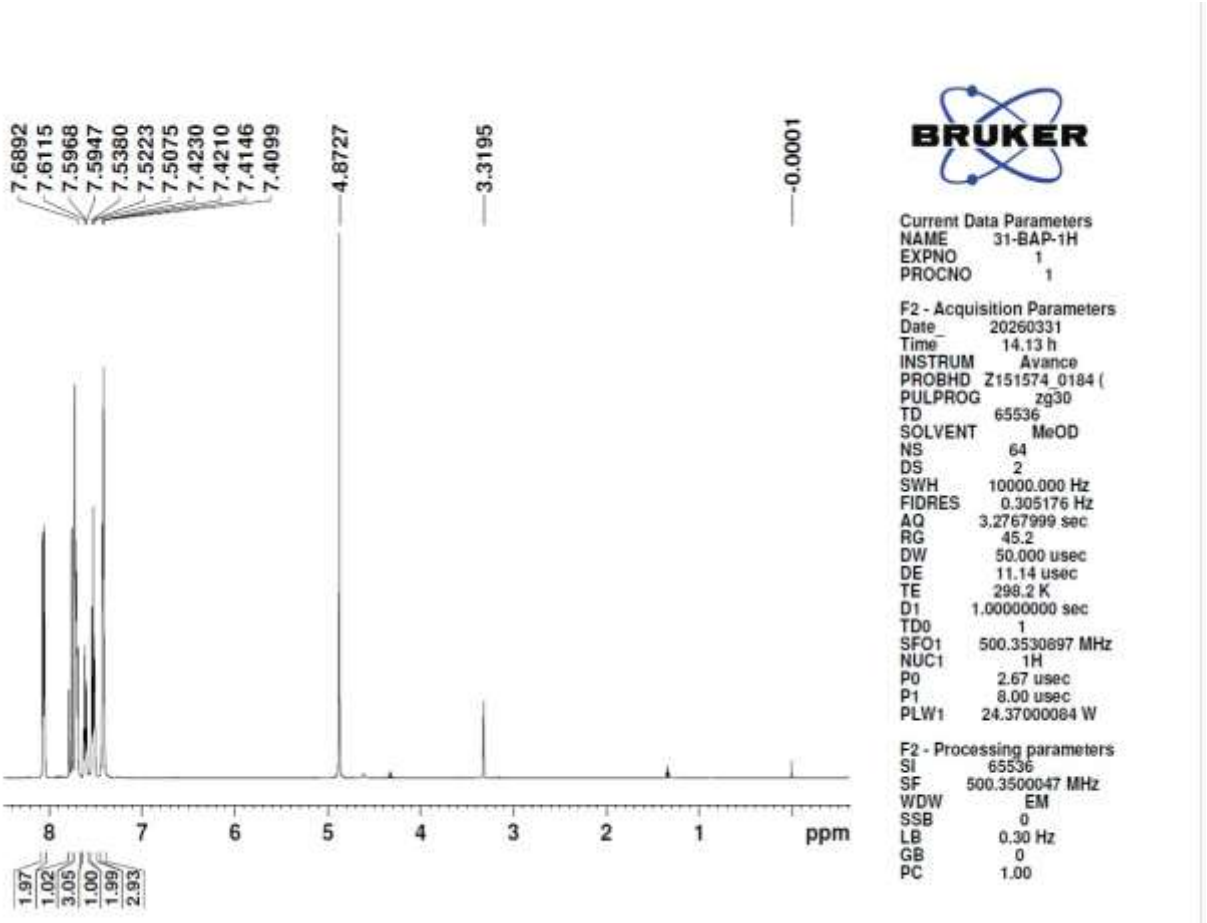
The reaction mixture is maintained at room temperature and stirred continuously for about 2 to 6 hours. During this time, sodium hydroxide abstracts the α-hydrogen from acetophenone, generating an enolate ion, which subsequently attacks the carbonyl carbon of benzaldehyde to form a β-hydroxy ketone intermediate. This intermediate then undergoes dehydration to yield benzalacetophenone, which is an α, β-unsaturated ketone.[8]

As the reaction progresses, a yellow precipitate begins to form, indicating the formation of the desired product. Upon completion, the reaction mixture is poured into cold water to facilitate complete precipitation. The solid product is allowed to settle and is then collected by filtration. The crude product is washed repeatedly with cold water to eliminate any residual alkali and impurities, followed by drying at room temperature or in a desiccator. Finally, the dried product is purified by recrystallization from ethanol, resulting in pure benzalacetophenone obtained as yellow crystalline solid.[9]

RESULTS AND DISCUSSION

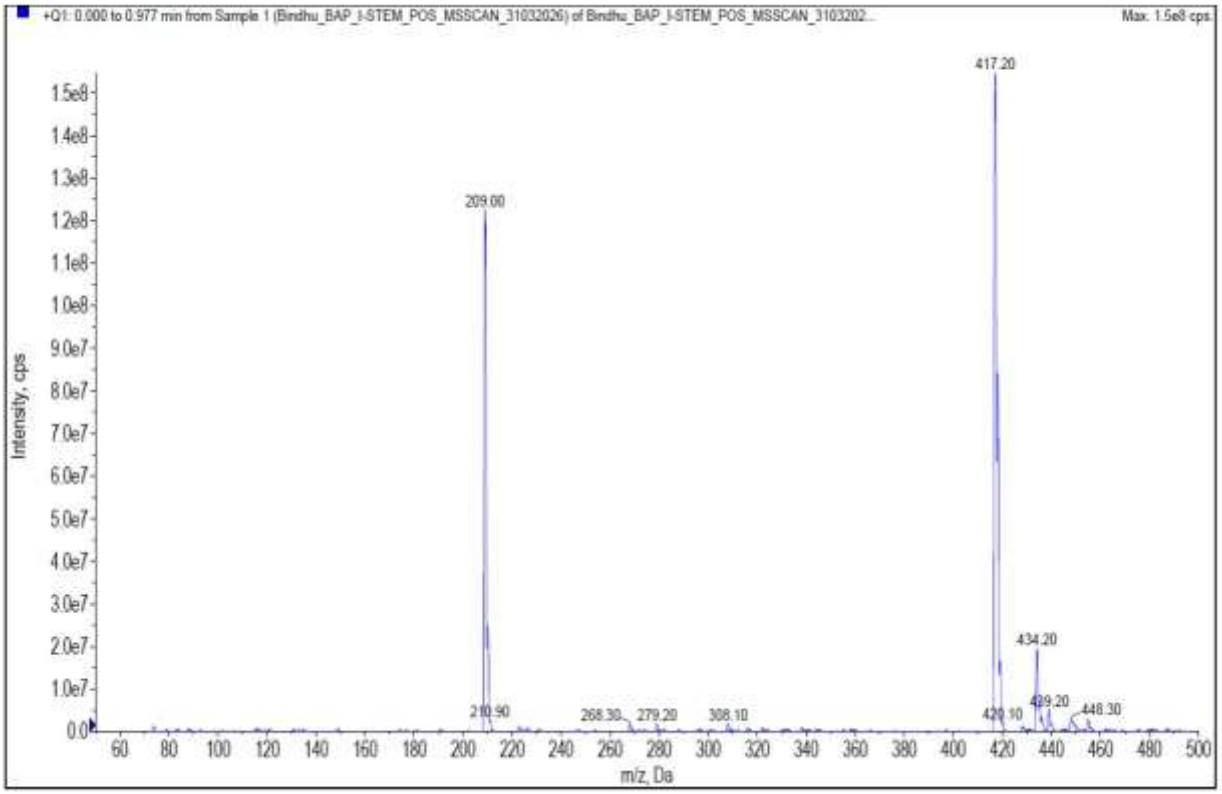


Benzalacetophenone



^{1}H – NMR OF BENZALACETOPHENONE

Compound Data Summary:
• Mol. formula: $C_{15}H_{14}O$
• Mol. weight: 208.26
• Melting point: 56°C
• Percentage yield: 72.03%
Spectral data:^{1}H NMR (CH_3OD, δ ppm):δ 7.68 (2H (Ar, i.e. 3,7)), δ 7.61 – 7.59 (1H(1)), δ 7.60 (3H (Ar,i.e., 4, 5, 6)), δ 7.53 – 7.42 (1H(2)), δ 7.42 – 7.41 (2H (Ar, i.e. 8,12)), δ 7.40 (3H (Ar i.e. 9, 10, 11)).



MASS SPECTRA OF BENZALACETOPHENONE

• ESI MS (m/z): 209.00 (M+1)

ANTIBACTERIAL ACTIVITY OF CHALCONE:

Mueller–Hinton agar was used as the culture medium for microbial growth. About 36 g of the dehydrated medium was dissolved in 100 mL of distilled water according to the manufacturer's instructions. The prepared solution was transferred into a clean conical flask and sterilized in an autoclave at 121 °C for 15 minutes. After sterilization, it was poured into sterile petri dishes and allowed to cool and solidify. A stock solution of each compound (200 µg/mL) was prepared by dissolving 0.002 g of the compound in 10 mL of ethanol.[10]

ANTIBACTERIAL ACTIVITY (ZONE OF INHIBITION):

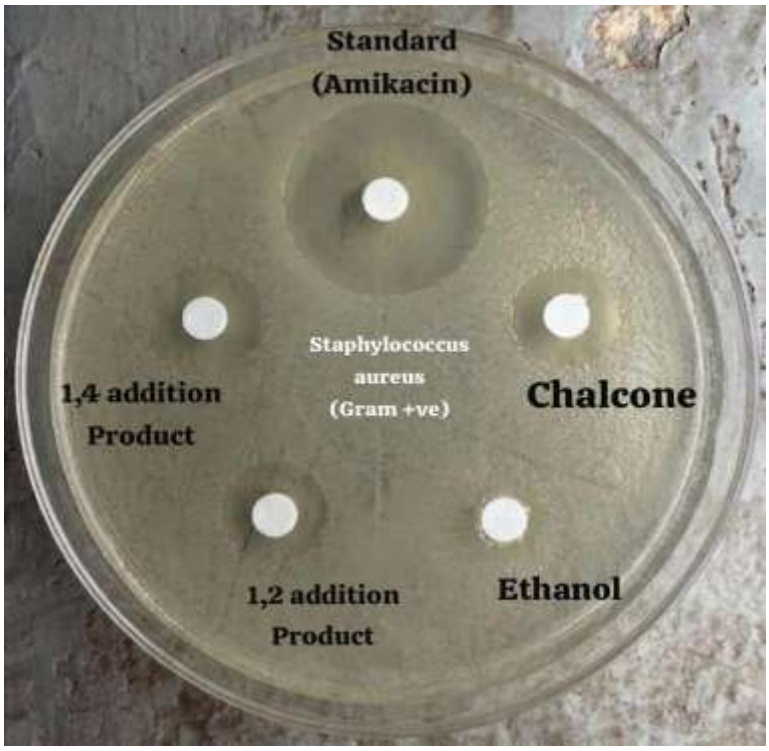
The antibacterial activity was evaluated using the agar diffusion method. The prepared agar plates were inoculated with 0.1 mL of microbial culture and evenly spread using a sterile swab. The plates were then incubated at 37 °C for 30 minutes to allow drying.[11] Wells of 6 mm diameter were made in the agar using a sterile cork borer, and 0.1 mL of the test compound solution was added into each well. The plates were incubated at 37 °C for 24 hours. After incubation, clear zones around the wells were observed and measured in millimeters as an indication of antibacterial activity. Amikacin (10 µg/mL) was used as the standard reference drug. [12]

Minimum Inhibitory Concentration (MIC):

The MIC was determined using the broth dilution method. Mueller–Hinton broth was prepared, dispensed into test tubes, and sterilized at 121 °C for 15 minutes. After cooling, bacterial suspensions were prepared and adjusted to match the turbidity of the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL.[13] Serial dilutions of the compounds were made to obtain concentrations ranging from 200 to 12.5 µg/mL. Each tube was inoculated with 0.1 mL of the bacterial suspension and incubated at 37 °C for 24 hours. The MIC was identified as the lowest concentration showing no visible turbidity.

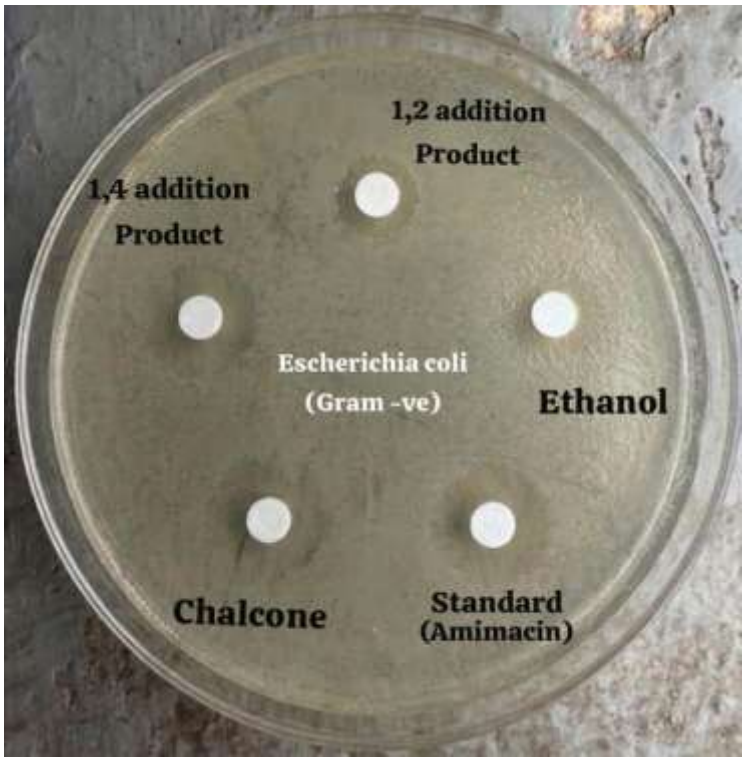
Minimum Bactericidal Concentration (MBC):

The MBC was determined to confirm whether the bacteria were killed or only inhibited. Samples from the MIC test tubes were sub-cultured onto fresh Mueller–Hinton agar plates and incubated at 37 °C for 24 hours. The lowest concentration at which no bacterial colony was observed was recorded as the MBC[3]. Amole KL, Bello IA, Oyewale AO. Synthesis, characterization and antibacterial activities of new fluorinated chalcones. [14]



ANTIBACTERIAL ACTIVITY ON STAPHYLOCOCCUS AUREUS (A GRAM-POSITIVE ORGANISM)

The antibacterial evaluation of the synthesized compounds against Staphylococcus aureus (Gram-positive) showed that the 1,2addition product produced a moderate zone of inhibition measuring approximately 12–14 mm, indicating noticeable activity. The 1,4-addition product exhibited a slightly smaller zone of about 10–12 mm, showing mild to moderate effectiveness.[15] The chalcone derivative demonstrated only weak activity with an inhibition zone of around 8–10 mm. The ethanol control produced no significant zone, confirming the absence of antibacterial activity.[16] As expected, the standard antibiotic amikacin displayed the largest and most distinct zone of inhibition, measuring nearly 22–24 mm, validating its strong effectiveness against Gram-positive bacteria.[17] Overall, the synthesized compounds showed better antibacterial activity toward Gram-positive organisms when compared with their effect on Gram-negative strains.



ANTIBACTERIAL ACTIVITY ON ESCHERICHIA COLI (GRAM-NEGATIVE) ORGANISM

The antibacterial screening of the synthesized compounds against Escherichia coli (Gram-negative) revealed that the 1,2-addition product exhibited a moderate zone of inhibition measuring approximately 10–12 mm, while the 1,4-addition product showed a comparatively smaller zone of about 8–10 mm. The chalcone derivative displayed only weak activity with a zone of 6–8 mm.[18] The ethanol control showed no inhibitory effect, indicating a negligible zone (0–2 mm). In contrast, the standard drug amikacin produced a clear and significantly larger zone of inhibition measuring around 20–22 mm, confirming effective antibacterial activity. These results indicate that the synthesized organic compounds possess only mild to moderate activity against Gram-negative bacteria when compared with the standard a[19]

Overall result on antibacterial activity

The 1,2-addition product, 1,4-addition product, and chalcone derivatives obtained from the reaction of a Grignard reagent with benzalacetophenone generally exhibit higher antibacterial activity against Gram-positive organisms compared to Gram-negative organisms.[20] This is because Gram-positive bacteria possess a simpler cell-wall structure that allows easier penetration of these organic molecules, whereas the outer membrane present in Gram-negative bacteria restricts their entry, resulting in significantly reduced activity.[21]

Conclusion

The synthesis and subsequent reaction of benzalacetophenone with Grignard reagents demonstrate a fundamental and versatile approach to carbon–carbon bond formation in organic chemistry.[22] Through the Claisen–Schmidt condensation of acetophenone and benzaldehyde, benzalacetophenone was successfully produced as a yellow crystalline solid with a significant yield of 72.03%, as confirmed by 1H-NMR and mass spectrometry.[23] The reactivity of this -unsaturated ketone allows for two distinct nucleophilic addition pathways: 1,2-addition at the carbonyl carbon and 1,4-addition at the -carbon. While Grignard reagents typically favor 1,2addition due to their high reactivity, the resulting allylic alcohols and saturated ketones serve as essential precursors for more complex molecular frameworks. Beyond their synthetic utility, the biological evaluation of these derivatives revealed promising medicinal potential. Antibacterial testing via the agar diffusion and broth dilution methods showed that the 1,2-addition and 1,4addition products possess moderate antimicrobial properties. Notably, these compounds exhibited higher efficacy against Grampositive *Staphylococcus aureus* compared to Gram-negative *Escherichia coli*, a difference attributed to the more permeable cell wall structure of Gram-positive bacteria. Although the standard antibiotic amikacin outperformed the synthesized compounds, the measurable zones of inhibition and the determination of MIC and MBC values highlight the potential of chalcone-based derivatives as scaffolds for future drug development.[24] In summary, the Grignard reaction with benzalacetophenone provides a robust method for modifying chalcone structures, yielding compounds with valuable chemical versatility and relevant biological activity in the pursuit of new antimicrobial agents.[25]

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