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Effect of Reaction Conditions on the Claisen-Schmidt Synthesis of Selected 1,3-diphenylprop-2-en-1-one Derivatives

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ABSTRACT

This research aimed to investigate the Claisen-Schmidt condensation reaction for the wet synthesis of chalcones and suggest the ideal reaction conditions for the process. Three chalcones, namely 1,3-diphenylprop-2-en-1-one, 1-(4-nitrophenyl)-3-phenylprop-2-en-1-one and 3-(2,4,6-trimethoxyphenyl)-1-phenylprop-2-en-1-one, were synthesized under varying conditions while closely monitoring the effects on product yield and quality. The temperature was varied between room and ice bath (<10ºC) conditions. Cold aqueous NaOH and KOH were varied as catalysts while absolute ethanol or methanol was used as the solvent in each scenario. Thin layer chromatography (TLC) was employed to monitor the progress of the reaction. The open capillary method was used to determine the melting point of the products and the values obtained were uncorrected. The results obtained showed that a combination of methanol as a solvent and NaOH as a catalyst, under ice-cold condition, was optimum for the wet synthesis of the chalcones. This combination gave the highest yield (98.26%) and purity of products.

Keywords: Catalyst, Chalcones, Claisen-Schmidt, Condensation, Thin layer chromatography

INTRODUCTION

Chalcones are natural products which are found in plants (fruits and vegetables). They have a wide variety of pharmacological importance due to their numerous biological activities (Banoth and Thatikonda, 2020). For many years, man has been using plant sources to solve diverse health problems and cure illnesses (Yuan *et al.,* 2016). Many compounds synthesized or isolated from plants also have many medicinal applications (Ouyang *et al.,* 2021). Herbal remedies are very popular and are used across the world. Some compounds that are active pharmacologically such as steroids, flavonoids, alkaloids, tannins and glycosides are stored in different plant parts ranging from stems, barks, flowers, leaves and roots (Pallab *et al.,* 2013). Chalcones have been found to have significant inhibitory activity on certain bacteria and fungi that are resistant to the common antibacterial and antifungal compounds (Ritter *et al.,* 2015). As a result of its wide application in medicine, chalcones have gained a lot of attention from scientists, as new methods of synthesizing them are being researched and developed. However, the most widely used method is the Claisen-Schmidt condensation method. The method is very efficient and convenient for the synthesis of chalcones.

The crucial interest of scientists in studying these molecules lies in their vast diversity in application. Besides being important as starting material for synthetic purposes, chalcones are being explored as a new class of non-azo dyes (Sharma *et al.,* 2010), as a pharmacological agent exhibiting a large number of activities such as, antifungal, anticancer and anti-inflammatory (Tomar *et al.,* 2007; Bag *et al.,* 2009; Swamy *et al,* 2008), antioxidant (Sivakumar *et al.,* 2011) and anti-depressant (Sui *et al.,* 2012; Ozdemir *et al.,* 2008) activities. In addition, some photo-physical properties of these substances such as non-linear optical properties (Indra *et al.,* 2002; Kiran, 2008) and their use as inflorescent probes (Xu, *et al.,* 2005) have also been investigated.

A series of polyhydroxy chalcones and flavonoids occur in plant tissues naturally, however, most of those that have shown, for example, antioxidant potency, are not commercially available (Agrawal, 2011) and so they need to be prepared synthetically. Chalcones, being plant-based can be isolated from plants but the isolation process is time-consuming, sometimes very complex and very little yield is obtained (Hano and Tungmunnithum, 2020; Tzanova *et al.,* 2020; Satyajit *et al.,* 2005). There is therefore a need for faster and more efficient synthetic routes to be developed to increase the yield and availability of chalcones and chalcone derivatives because they have wide applications and are in high demand. Also, the synthetic methods and research make it possible to synthesize chalcone derivatives which possibly do not exist in nature. The chemical characteristics of chalcones have led to intensive scientific studies throughout the world,

especially those focused on the synthesis and bioactivities of chalcones.

Claisen-Schmidt condensation reaction for the synthesis of chalcones has been carried out under different conditions. However, all the conditions are usually isolated and investigated individually, with very limited research on a side-by-side comparison of the reaction conditions to know the optimum combination of factors (catalyst, solvent and temperature) for the synthesis.

This research seeks to bridge this gap by providing a comparison between different conditions under which this natural product can be synthesized. The study was therefore aimed to investigate the effects of varying the reaction temperature, catalyst, and solvent on the synthesis of the desired chalcone

MATERIALS AND METHODS

Materials

All the chemicals used in this analysis were of analytical grade and were used without further purification. Benzaldehyde (1.044 g/mL, 98.50%), acetophenone (1.03 g/mL, 99.00%) and their derivatives were obtained from BDH Chemicals limited, Poole, England, while ethanol, methanol, n-hexane and ethylacetate where obtained from JHD Chemicals, Guangdong, China.

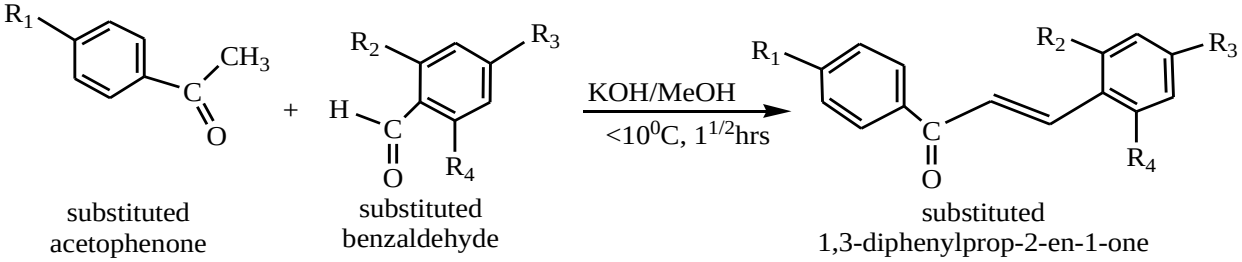
Melting points were determined with a Kofler Electrothermal melting point apparatus CAT No. 1A6304, England. The open capillary method was used and the values obtained were uncorrected. An electronic weighing balance (Model 3002, Golden-Mettler, U.S.A) was used for weighing.

Thin layer Chromatography (TLC)

Thin layer chromatography was carried out on analytical TLC plates pre-coated with silica gel 60 F_{254}, 20 cm x 20 cm (Merck KGaA, Darmstadt, Germany). 20% ethyl acetate in n-hexane (v/v), was used as the mobile phase. Spotted TLC plates were visualized with a UV lamp. Fluorescent compounds were visualized at 366 nm while non-fluorescent compounds were visualized at 254 nm.

Methods

Reaction Scheme



substituted acetophenone

substituted benzaldehyde

substituted 1,3-diphenylprop-2-en-1-one

CA1 $R_1 = R_2 = R_3 = R_4 = H$

CA2 $R_1 = N0_2$, $R_2 = R_3 = R_4 = H$

CA3 $R_1 = H$, $R_2 = R_3 = R_4 = OCH_3$

Scheme 1: Synthesis of the chalcones

A. Synthesis of 1,3-diphenyl prop-2-en-1-one (CA_1)

i At room temperature using NaOH and ethanol

A mixture of benzaldehyde (17 mmol) and acetophenone (17 mmol) was made in a 100 mL conical flask. 10 mL of ethanol (98%) was added to the mixture and it was stirred using a magnetic stirrer set at 100 rpm. As the stirring continued, 2 mL of 20% NaOH was added (in drops) to initiate the reaction. TLC was carried out periodically to monitor the progress of the reaction. The mixture was stirred for 1 hr 30 mins after which the reaction mixture was left to stand in a refrigerator for 12 hours. Thereafter, 50% of glacial acetic acid was added (in drops) until the supernatant was neutral to litmus. The reaction mixture was filtered, the product was dried and the yield was calculated. The purity of the product was ascertained by using TLC and the melting point was also determined.

ii In an ice bath using NaOH and ethanol

A mixture of benzaldehyde (17 mmol) and acetophenone (17 mmol) was made according to the procedure described in *A*(*i)* above and placed in an ice bath while the temperature was kept below 10°C. The ice bath containing the reaction mixture was placed on a magnetic stirrer, set at 100 rpm. The temperature of the ice bath was kept fairly constant by the addition of more ice cubes. The reaction was initiated by the addition of 3 mL of 20% NaOH while stirring. The rest of the reaction was carried out according to the procedure described in section *A(i)* above.

iii In an ice bath using KOH and ethanol

A mixture of benzaldehyde (17 mmol) and acetophenone (17 mmol) was made according to the procedure described in *A(i)* above but the reaction was initiated by the addition of 2 mL of 20% KOH while stirring. The rest of the reaction was carried out according to the procedure described in section *A(i)* above.

iv In an ice bath using NaOH and methanol

A mixture benzaldehyde (17 mmol) and acetophenone (17 mmol) was prepared according to the procedure described in *A(i)* above but methanol was used as the solvent. The reaction was initiated by the addition of 20% NaOH (2 mL), drop-wisely, while stirring. The rest of the reaction was carried out according to the procedure described in section *A(i)* above.

v. In an ice bath using KOH and methanol

A mixture containing benzaldehyde (17 mmol) and acetophenone (17 mmol) was prepared according to the procedure described in *A(ii)* but 10 mL of methanol (solvent) was added to the mixture. The reaction was initiated by the addition of 20% KOH (2 mL) while stirring. The rest of the reaction was carried out according to the procedure described in section *A(i)* above.

B. **Synthesis of 1-(4-nitrophenyl)-3-phenyl prop-2-en-1-one (CA_2)**

i In an ice bath using NaOH and ethanol

A mixture of 4-nitro acetophenone (1 mmol) and benzaldehyde (1 mmol) was made in a 100 mL conical flask. Ethanol (20 mL) was added to the mixture and stirred with a magnetic stirrer until all the crystals of 4-nitro acetophenone dissolved. The reaction mixture was then placed in an ice bath with the temperature monitored and kept below 10°C. 2 mL of 20% NaOH was added (in drops) to the reaction mixture. The mixture was stirred for 1hr 30 mins in the ice bath. The rest of the reaction was carried out according to the procedure described in section *A(i)* above.

ii In an ice bath using KOH and ethanol

Benzaldehyde (1 mmol) and 4-nitro acetophenone (1 mmol) were mixed and reacted according to the procedure in *B(i)*.Then 2 mL of 20% KOH was added (in drops) to the reaction mixture. The rest of the reaction was carried out according to the procedure described in *B(i)* above.

iii In an ice bath using NaOH and methanol

A mixture of 4-nitro acetophenone (1 mmol) and benzaldehyde (1 mmol) was made in a 100 mL conical flask. Methanol (20 mL) was added to the mixture and the rest of the reaction was carried out according to the procedure described in *B(i)* above.

iv In an ice bath using KOH and methanol

Benzaldehyde (1 mmol) and 4-nitro acetophenone (1 mmol) were mixed and dissolved with methanol (20 mL) in a 100 mL conical flask. The mixture was stirred with a magnetic stirrer until all the crystals of 4-nitro acetophenone dissolved. The reaction mixture was then placed in an ice bath with the temperature monitored and kept below 10°C. Then, 2 mL of 20% KOH was added in drops to the reaction mixture. The rest of the reaction was carried out according to the procedure described in section *B(i)* above.

C. **Synthesis of 1-phenyl-3-(2,4,6-phenyl)prop-2-en-1-one (CA_3)**

i In an ice bath using NaOH and ethanol

Acetophenone (7.65 mmol) and 2,4,6-trimethoxy benzaldehyde (7.65 mmol) were mixed in 20 mL of ethanol. The mixture was stirred using a magnetic stirrer (100 rpm) until all the crystals of 2,4,6-trimethoxy benzaldehyde dissolved. The reaction mixture was then placed in an ice bath with the temperature kept below 10°C. Then, 2 mL of 20% NaOH was added (in drops) to initiate the reaction. The rest of the reaction was carried out according to the procedure described in section *A(i)* above.

ii In an ice bath using KOH and ethanol

Acetophenone (7.65 mmol) and 2,4,6-trimethoxy benzaldehyde (7.65 mmol) were mixed and 20 mL of ethanol according to the *C(i)* above. But 2 mL of 20% KOH was added (in drops) to initiate the reaction. The rest of the reaction was carried out according to the procedure described in section *A(i)* above.

iii In an ice bath using NaOH and methanol

Acetophenone (7.65 mmol) and 2,4,6-trimethoxy benzaldehyde (7.65 mmol) were mixed in 20 mL of methanol and stirred with a magnetic stirrer (100 rpm) to dissolve. The rest of the reaction was carried out according to the procedure in section *C(i)*.

iv In ice bath using KOH and methanol

Acetophenone (7.65 mmol) and 2,4,6-trimethoxy benzaldehyde (7.65 mmol) were mixed in 20 mL of methanol. The mixture was stirred using a magnetic stirrer (100 rpm) until all the crystals of 2,4,6-trimethoxy benzaldehyde dissolved. The reaction mixture was then placed in an ice bath with the temperature kept below 10°C. Then, 2 mL of 20% KOH was added (in drops) to initiate the reaction. The rest of the reaction was carried out according to the procedure described in section *A(i)* above.

The structures of the synthesized chalcones have earlier been confirmed in a study done by Aiwonegbe and Iyasele (2022).

RESULTS AND DISCUSSION

This work was carried out to ascertain the best combination of solvent, catalyst and temperature for the synthesis of chalcones by Claisen-Schmidt condensation method.

There are many variations to the conditions under which this condensation can be carried out. Most works claim that the best condition for the preparation of chalcones is using Claisen-Schmidt condensation under ice (below 10°C) as it is believed that Cannizzaro reaction may occur if the reaction is carried out at a higher temperature. There are yet other literature in which chalcones were synthesized at room temperature and in some other works, at higher temperature up to 300°C. All these isolate a specific condition under which Claisen-Schmidt condensation for the synthesis of chalcone can be done. However, limited researches have been focused on a side-by-side comparison of these different reaction conditions.

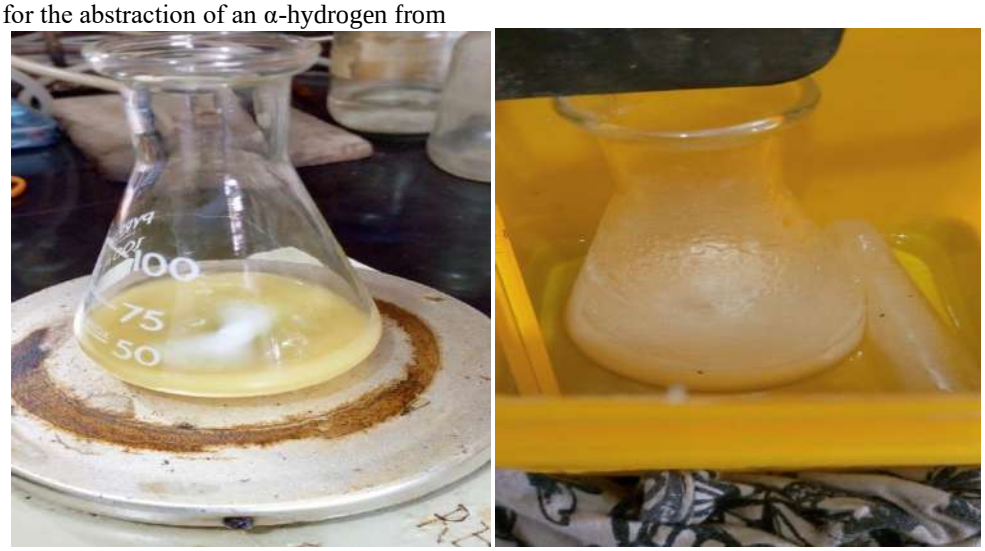
The results for the experimental yield of the chalcones synthesized by the Claisen-Schmidt condensation reaction carried out are shown in Table 1.

Table 1: Percentage yield of the synthesized chalcones at different reaction conditions

Chalcone	Percentage yield			
	Ethanol+NaOH	Ethanol+KOH	Methanol+NaOH	Methanol+KOH
CA_1 (room temperature)	62.24%	ND	ND	ND
CA_1 (ice bath)	36.00%	52.90%	96.15%	ND
CA_2 (ice bath)	88.60%	61.08%	71.05%	42.10%
CA_3 (ice bath)	69.59%	80.44%	98.26%	96.52%

ND= Not determined

The yield was however not calculated (not determined-ND) for reactions that did not progress appreciably. The chalcone, 1-phenyl-3-(2,4,6-phenyl)prop-2-en-1-one (**CA_3**), synthesized under ice-cold conditions, gave the highest yield (98.32%) when methanol was used as the solvent and NaOH as the catalyst precursor. In the wet synthesis of chalcones, the alkali (NaOH or KOH) reacts with the solvent to produce the alkoxide ion necessary for the abstraction of an α-hydrogen from the aryl ketone to generate the nucleophilic centre. Under the same conditions, the rate of attack of the aldehyde by the nucleophile and the stability of the elimination product formed, determine the yield of product formed chalcone. The formation of **CA_3** seems to be more favoured by the nature of the initial nucleophile produced, which is stabilized by the electron-withdrawing effect of the nitro ($-NO_2$) group in the acetophenone.



(a) **(b)**

Plate 1: Synthesis (a) at room temperature and (b) in an ice bath

Table 2: Melting points of the synthesized chalcones at different reaction conditions

Chalcone	Melting point			
	Ethanol+NaOH	Ethanol+KOH	Methanol+NaOH	Methanol+KOH
CA_1 (room temperature)	46°C	ND	ND	ND
CA_1 (ice bath)	46-48°C	38-43°C	47-48°C	ND
CA_2 (ice bath)	91-92°C	102°C	91-92°C	90-98°C
CA_3 (ice bath)	130-134°C	130°C	135-137°C	136°C

ND= Not determined

Table 3: Retention factors of the synthesized Chalcones

Chalcone	Ethanol+NaOH	Ethanol+KOH	Methanol+NaOH	Methanol+KOH
CA_1 (room temperature)	0.65	ND	ND	ND
CA_1 (ice bath)	0.64	0.65	0.64	ND
CA_2 (ice bath)	ND	0.22	ND	ND
CA_3 (ice bath)	0.58	0.54	0.62	0.54

ND = Not determined

Solvent system: n-hexane/ethylacetate (5:1)

Table 4:Thin Layer Chromatographic Analysis of the Synthesized Chalcones

Test	Chalcones	Number of spots	Description
1	**CA_1** (room temperature) using NaOH + ethanol	1	Pure
2	**CA_1** (ice bath) using NaOH + ethanol	3	Impure
3	**CA_1** (ice bath) using using NaOH + methanol	1	Pure
4	**CA_1** (ice bath) using using KOH + ethanol	3	Impure
5	**CA_3** (ice bath) using using KOH + ethanol	1	Pure
6	**CA_2** (ice bath) using using NaOH + methanol	2	Impure
7	**CA_2** (ice bath) using using NaOH + ethanol	1	Pure
8	**CA_2** (ice bath) using KOH + ethanol	2	Impure
9	**CA_2** (ice bath) using KOH + methanol	1	Pure

Table 5: Physical characteristics of the synthesized chalcones

Chalcone	Colour	State
CA_1 (room temperature) using NaOH + ethanol	Light yellow	Crystals
CA_1 (ice bath) using NaOH + ethanol	Light yellow	Crystals
CA_1 (ice bath) using NaOH + methanol	Light yellow	Crystals
CA_1 (ice bath) using KOH + ethanol	Light yellow	Crystals
CA_3(ice bath) using NaOH + methanol	Yellow	Crystals
CA_3 (ice bath) using NaOH + ethanol	Yellow	Crystals
CA_3 (ice bath) using KOH + ethanol	Greenish-yellow	Crystals
CA_3 (ice bath) using KOH + methanol	Yellow	Crystals
CA_2 (ice bath) using NaOH + methanol	Yellow	Crystal-powder
CA_2 (ice bath) using NaOH + ethanol	Yellow	Crystal-powder
CA_2 (ice bath) using KOH + ethanol	Yellow	Crystal-powder
CA_2 (ice bath) using KOH + methanol	Yellow	Crystal-powder

It can also be seen from Table 1 that the combination of solvent and catalyst that gave the highest yield for 1-phenyl-3-(2,4,6-phenyl)prop-2-en-1-one (**CA_3**), also gave very goods yields for the other two chalcones: 96.15% for 1,3-diphenylprop-2-en-1-one (**CA_1**) and 71.05% for 1-(4-nitrophenyl)-3-phenylprop-2-en-1-one (**CA_2**). Furthermore, Table 1 also shows that **CA_1** is the only chalcone that can be produced at room temperature with reasonable yield and the best combination of conditions is NaOH in ethanol. The sharp melting point of this chalcone, as shown in Table 2, indicates that it has a high level of purity. **CA_1** also gave one spot in the TLC analysis as shown in Table 4. The next best results were obtained with ethanol as solvent and KOH as the base in an ice bath. This gave relatively high yields (52.90% - 80.44%). The products obtained from this combination also had high purity as indicated by the sharp melting points in Table 2. The third best combination is ethanol as solvent and NaOH as catalyst. The combination of methanol as a solvent and KOH as the catalyst in an ice bath gave the poorest outcome. The retention factor (R_f) from the TLC spotting of the chalcone is presented in Table 3. The R_f value is a measure of the polarity of the chalcones. Using silica gel as the stationary phase and n-hexane/ethylacetate (5:1) as the mobile phase, a high R_f value equates to high polarity. Table 5 shows the visual characteristics of the synthesized chalcones.

Ritter *et al.,* (2015) reported a yield of 74-94% of products when chalcones were synthesized with glycerine as solvent and NaOH as catalyst. This is a lower yield than obtained in this work using methanol as solvent and NaOH catalyst. Mandge *et al.,* (2007) used ethanol as a solvent and KOH as a catalyst in the synthesis of 1, 3-diphenylprop-2-en-1-one (**CA_1**). This gave a yield of 89% and a melting point of 93°C. In the present study, this combination of reaction conditions for the synthesis of **CA_1** gave a yield of 52.90% and a melting point range of 38- 43°C. However, the melting point of **CA_1** in literature is 55-57°C. Ritter *et al.,* (2015) reported that the differences in melting point chalcones could be a result of the different forms of crystallization used during the purification process. Antonio-Arias *et al.,* (2018) reported that synthesizing chalcones using ethanol as solvent and NaOH as catalyst, in ice, gave

extremely poor yields and in some cases, the reaction did not occur at all. Bui *et al.*, (2016) reported that when chalcones were synthesized with methanol as the solvent and KOH as the catalyst at a high temperature (70°C), the yields were lower than 33.4%. Therefore, every chalcone has a set combination of reaction conditions for optimum yield and high purity of product.

CONCLUSION

Three chalcones were synthesized by Claisen-Schmidt condensation with permutations of reaction conditions (temperature, solvent and catalyst). Chalcone **CA_1** yielded (62.24%) pure product with NaOH and ethanol at room temperature. The chalcone, however, yielded (96.15%) pure product when the synthesis was carried out with NaOH and methanol in an ice bath. **CA_2** gave (88.60%) pure product with NaOH and ethanol in an ice bath. Pure **CA_2** was also obtained with KOH and methanol in an ice bath but the yield was 42.10%. Only one combination of reaction conditions (KOH and ethanol in ice) gave pure **CA_1** with a yield of 80.44%. Therefore, before embarking on the synthesis of a chalcone, a pilot study should be carried out to ascertain the best combination of the factors- temperature, base and catalyst – that will give the best yield and highest purity of the product. However, the overall outcome of this study shows that the best combination of reaction conditions for the synthesis of chalcones are either NaOH and ethanol in an ice bath or KOH and methanol in an ice bath.

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