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Optimization of Reaction Condition for Synthesis of 4-Methoxychalcone from Ethyl *p*-methoxycinnamate

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Abstract: Ethyl *p*-methoxycinnamate (**1**) is the main secondary metabolite found in *Kaempferia galanga* Linn and has various interesting pharmacological activities, including anti-inflammatory, sedative, vasorelaxant, and antiangiogenic activities. This study aimed to optimize the conditions for the conversion of **1** to 4-methoxychalcone (**2**) using a conventional synthetic reaction. The conversion was initiated by the hydrolysis of **1** to *p*-methoxycinnamic acid (**3**) and **3** to 4-methoxybenzaldehyde (**4**), and continued with the Claisen-Schmidt reaction of **4** with acetophenone (**5**). The temperature (room temperature and 45 °C) and ratio of **4** to **5** (1:1 and 1:2) were varied to determine the optimal conditions for the reaction. The results suggested that the reaction of **4** with **5** gave the best yield (42.1 %) when conducted at room temperature in a ratio of 4 to 5 (1:1). The structure of the reaction product was elucidated by spectroscopic analysis and compared with previously published data.

Keywords: Claisen-Schmidt reaction, ethyl p-methoxycinnamate, *Kaempferia galanga*, 4-methoxybenzaldehyde

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1. INTRODUCTION

Ethyl *p*-methoxycinnamate (**1**) is an ester compound with a distinctive aroma that provides the pharmacologic effect of the rhizome of *Kaempferia galanga*. The pharmacological activities of interest include anti-inflammatory (Umar *et al.*, 2014), vasorelaxant (Othman *et al.*, 2006), antiangiogenic (He *et al.*, 2012), and sedative (Huang, Yagura and Chen, 2008). Ethyl *p*-methoxycinnamate (**1**) is the main compound contained in the rhizome of *K. galanga* and can be easily isolated only through the re-crystallization technique from the extract. Previously, we had synthesized derivatives of ethyl *p*-methoxycinnamate (**1**). These derivatives were used to study the structure-activity relationships of some activities. Some compounds have been successfully synthesized from ethyl *p*-methoxycinnamate (**1**) including esters of *p-methoxycinnamates* (Komala *et al.*, 2018), *p*-methoxycinnamamides (Komala *et al.*, 2017; Komala, Supandi and Hardiansyah, 2018), *p*-β-methoxynitrosyrenes (Komala *et al.*, 2017), and *p*-methoxystyryl ketones (Komala *et al.*, 2021).

4-Methoxychalcone (**2**) has a *p*-methoxystyryl ketone moiety. This compound has the potential to be developed for the treatment of metabolic syndromes, diabetes (Han *et al.*, 2017), lung cancer (Lim *et al.*, 2013), and has antiamoebic activity (Wani *et al.*, 2012). Various methods have been used to synthesize 4-methoxychalcone **(2)**. Most of these methods were conducted through the Claisen-Schmidt reaction between *p*-methoxybenzaldehyde and acetophenone under various modification conditions (Montes-Avila *et al.*, 2009; Wani *et al.*, 2012; Díaz-Carrillo *et al.*, 2018; Awala *et al.*, 2019).

Previously, we converted ethyl *p*-methoxycinnamate (**1**) to 4-methoxychalcone **(2)** using a microwave-assisted reaction and obtained very good yields (62,2%) in a short time (7 minutes) (Komala *et al.*, 2021). There are many advantages if the reaction is

carried out using microwaves, including a short reaction time and a a high yield. However, there are some drawbacks to using a microwave synthesis reaction, including expensive equipment and scaling up of the process. Therefore, in this study, we attempted to synthesize 4-methoxychalcone **(2**) using a conventional reaction. The reaction was initiated by converting **1** to *p*-methoxycinnamic acid **(3**) and converting **3** to *p*-methoxybenzaldehyde (**4**). Subsequently, through the Claisen-Schmidt reaction, compound **4** was reacted with acetophenone (**5**) at various temperatures and varying the ratio of **4** to **5**. The structure of the synthesized product was elucidated using spectroscopic data and compared with previously published data.

2. MATERIAL AND METHODS

2.1 Instruments and Chemicals

Instruments: GC/MS-MSD 7890A/5975C (Agilent Technologies) HP-5MS capillary column. NMR Jeol-500 MHz (^{1}H; 500 MHz) and melting point apparatus (Stuart SMP10) without correction. *Chemicals:* Acetophenone, ethyl acetate (Merck), H_2SO4 (Smartlab), Na_2SO_4 (Merck), NaOH (Merck), glacial acetic acid (Merck), silica gel (Merck), and a TLC plate 60 F254 (Merck).

2.2 Preparation of ethyl p-methoxycinnamate (1) and p-methoxycinnamate (3)

Ethyl *p*-methoxycinnamate (**1**) was purified from the rhizome of *K. galanga* and further hydrolyzed to produce *p*-methoxycinnamic acid (**3**), as previously reported (Komala *et al.*, 2017).

***p*-methoxycinnamic acid (3)**, white crystal, 172-176ºC (Komala *et al.*, 2017). $C_{10}H_{10}O_3$, GCMS: 178 $[M]^+$, 161,133, 117, 89,77 dan 63. ^{1}H-NMR data is following the previously reported (Komala *et al.*, 2017)

2.3 Preparation of *p*-methoxybenzaldehyde (4).

p-Methoxybenzaldehyde (**4**) was obtained as the product of cleavage oxidation of **3** when reacted with $Ca(NO_3)_2$. This method was performed using a previously reported method (Komala *et al.*, 2021).

***p*-Methoxybenzaldehyde (4)**, yellow oil, $C_8H_8O_2$, GCMS:136 $[M]^+$, 107, 92, 77, 63. The ^{1}H-NMR data were in agreement with previously reported data (Islam, Sohrab and Jabbar, 2012).

2.4 Synthesis of 4-methoxychalcone (2)

2.4.1 Reaction in the ratio of 4 and acetophenone (5) (1:1)

In the Erlenmeyer flask, *p*-methoxybenzaldehyde (**4**) (19 mg, 0.14 mmol) was mixed with NaOH 5% (5 ml), and then acetophenone (**5**) (16.2 µl, 0.14 mmol) was added. The mixture was stirred at room temperature (24 h) or at 45 °C (for 6 h). Thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GCMS) were used to monitor the reactions. The reaction product was partitioned using distilled water and ethyl acetate (EtOAc). Purification of the ethyl acetate extract using chromatography yielded a white crystal of 4-methoxychalcone (**2**).

2.4.2 Reaction in the ratio of 3 and acetophenone (5) (1:2)

p-Methoxybenzaldehyde (**4**) (30 mg, 0.2 mmol) and NaOH 5% (5 ml) were mixed in the Erlenmeyer flask, and then acetophenone (**5**) (51.2 µl, 0.4 mmol) was added. The mixture was stirred at room temperature (24 h) or at 45 °C (for 6 h). Monitoring of the reaction and purification of 4-methoxychalcone (**2**) followed the above procedure to obtain a white crystal of **2.**

4-methoxychalcone (2), white crystal, 71-72°C, $C_{16}H_{14}O_2$, GCMS: 238$[M]^+$, 207, 161, 133, 108, 77 dan 51. ^{1}H-NMR (500-MHZ, CD3Cl); 3.85 (s, 3H OCH_3), 6.94 (d, *J* = 9, 2H, 2CH-Ar), 7.42 (d, *J* = 16, 1H,

=CH), 7.50 (t, *J* = 7, 2H, 2CH-Ar), 7.56 (tt, J_1=7, J_2=2, 1H, CH-Ar), 7.60 (d, *J*= 9, 2H, 2CH-Ar), 7.78 (d, *J* = 16, 1H, =CH), 8.0 (d, *J*= 7; 2H, 2CH-Ar) (Cabrera *et al.*, 2007; Komala *et al.*, 2021).

3. RESULTS AND DISCUSSION

4-Methoxychalcone (**2**) is a chalcone that has a *p*-methoxystyryl moiety. The synthesis of this compound mostly occurs through the crossed aldol reaction, which is referred to as the Claisen–Schmidt reaction. Claisen–Schmidt reaction is a type of aldol condensation reaction which involves an aldehyde/ketone and a carbonyl molecule that lacks an alpha-hydrogen. This reaction is a well-known method for the formation of C–C bonds between two carbonyl derivatives, and is widely applied in the synthesis of chalcones (Tiecco, Germani and Cardellini, 2016). In continuation of our previous research on the production of derivatives of ethyl *p*-methoxycinnamate, In this study we used ethyl *p*-methoxycinnamate as the starting material.

The reaction was initiated by the conversion of ethyl *p*-methoxycinnamate (**1**) into *p*-methoxybenzaldehyde (**4***)* using a previously reported ((Komala *et al.*, 2021). The reaction was initiated by the hydrolysis of **1** to **3**, and **3** was further reacted with $Ca(NO_3)_2$ to produce the cleavage oxidation product *p*-methoxybenzaldehyde (**4***)*. As *p*-methoxybenzaldehyde (**4***)* was formed, the Claisen-Schmidt reaction began by reacting *p*-methoxybenzaldehyde (**4***)* with acetophenon (**5**).

Synthesis of 4-methoxychalcone (**2**) by stirring *p-methoxybenzaldehyde* (**4**) and acetophenone (**5**) was carried out at various temperatures and ratios of **4** and **5**. Variations in the temperature and ratio were applied to optimize the reaction conditions. Previously, synthesis of 4 has been conducted in temperature room (Suzana *et al.*, 2013) and 40-50^{o}C (Sadeghi, Mirjalili and Hashemi, 2008) in ratio of 4:5 (1:1) in different reaction condition. In this study we used the variation temperature reaction in room temperature and 45 ^{o}C and ration of 4 and 5 in 1:1 and 1:2. The reaction scheme is illustrated in Fig. 1.

As shown in Table 1, the reaction *of p*-methoxybenzaldehyde (**4**) and acetophenone (**5**) gave the best yield when the condensation of **4** and acetophenone (**5**) was conducted at room temperature in a ratio of 1:1; increasing the amount of acetophenone (ratio 1:2) caused a decrease in the yield. It is predicted that an increase in the amount of acetophenone causes an increase in self-condensation

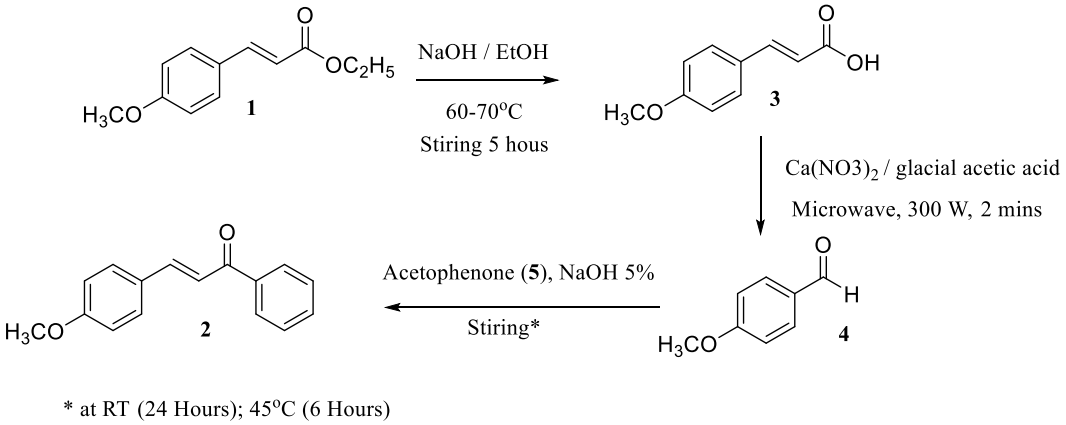
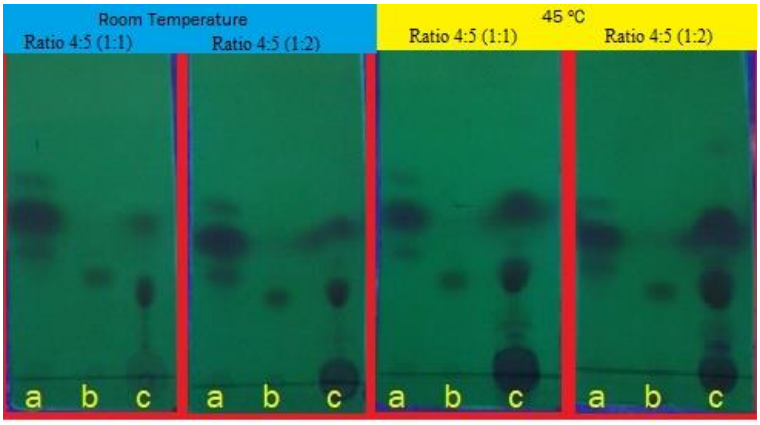


Figure 1. Scheme of Reactions

because acetophenone also contains hydrogen atoms in C-alpha, allowing them to react with each other.

Table 1. Optimization of reaction

No of treatment	Molar Ratio 4:5	Temperature	Time (Hours)	Product (yield %)
1	1:1	RT	24	42.1
2	1:2	RT	24	36.7
3	1:1	45°C	24	11.4
4	1:2	45°C	24	22.8



Note : a: *p*-methoxybenzaldehyde (**4**), b: acetophenone (**5**) c: reaction product

Figure 2. TLC monitoring of the reaction

A comparison of treatments 1 and 3 indicated that increasing the temperature caused a decrease in the yield. As shown in the TLC monitoring in Figure 2, the reaction at room temperature yielded compound **2** as the major product, and increasing the temperature to 45 °C yielded a mixture of some compounds. When the reaction was conducted at 45 °C, an increase in the amount of acetophenone increased the production of compound **2**.

Compound (**2**) was revealed as a white crystal with a melting point of 71-72 ^{o}C. MS data indicate that compound 2 has molecular ion at m/z m/z 238 $[M]^{+}$. The loss of C_6H_5 from the molecule resulted in an ion peak at m/z 161, which indicated the presence of a methoxycinnamic cation. Furthermore, the loss of CO_2 resulted in an ion peak at m/z 133, which is associated with the phenylethylene cation.

The ^{1}H-NMR spectrum showed the presence of ortho-coupled signals, each for two protons at 6.94 and 7.50 ppm assigned as di-substituted benzene, two trans-coupled protons at 7.42 (1H) and 7.78 (1H) ppm assigned for vinylic protons, a singlet signal for 3 protons at 3.85 ppm was assigned for OCH_3. These pattern signals suggested the presence of a *p*-methoxycinnamate moiety. The remaining five protons at 7.56 (1H), 7.60 (2H) and 8.0 (2H) suggest the presence of a phenyl group. Hence, after comparison with a previously published study, this compound was assigned as 4-mthoxychalcone (Cabrera *et al.*, 2007; Komala *et al.*, 2021).

4. CONCLUSION

The conventional reaction in the synthesis of 4-methoxychalcone (2) was successfully conducted by the reaction of 4-methoxybenzaldehyde (4). And acetophenone (5). The reactions at room temperature in ratios of 4 to 5 (1:1) and (1:2) afforded 4 in 42,1 and 35,7 % yields, respectively. The reaction at 45 °C in ratios of 4 to 5 (1:1) and (1:2) afforded 2 in 11.4 and

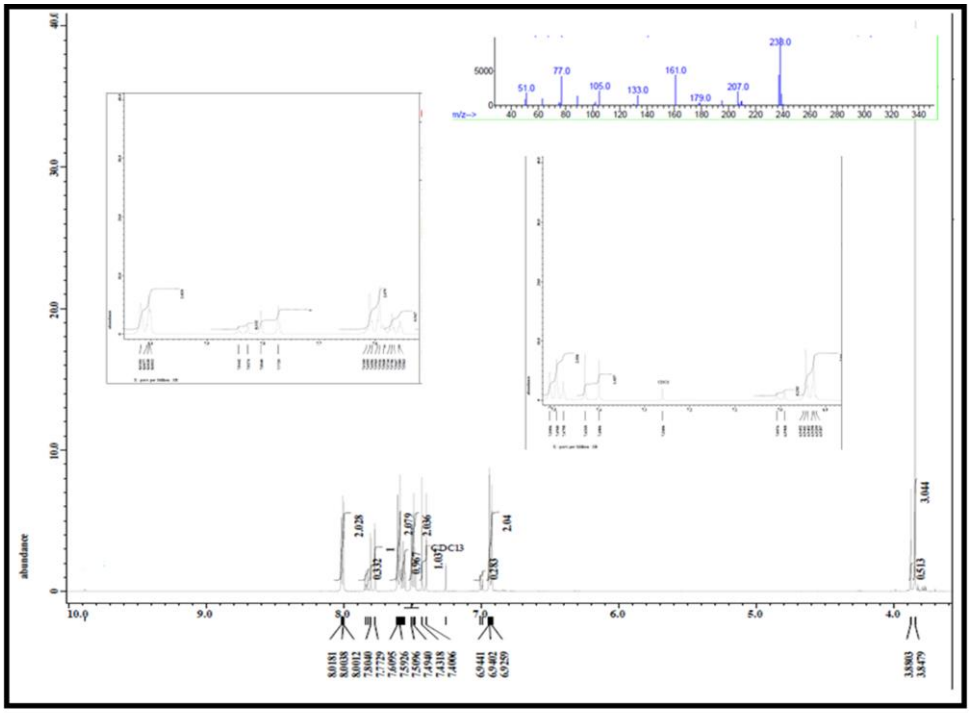


Figure 3. MS and ^{1}H-NMR Spectrum of **2**

22.8 % yields, respectively. This suggested that the reaction occurred in the room temperature with the ratio of 1:1 gave the best yield (42,1%).

5. ACKNOWLEDGMENT

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