Synthetic efforts towards the development of a microwave synthesis of ethyl benzoate.

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ABSTRACT

Green chemistry is a new and rising field working towards reducing the negative impacts of chemical production on the planet and on humans. This is a critical new direction in chemistry because the chemical industry has large impacts on economies, research, and chemical waste production. In this study, we investigated a microwave synthesis of esters using a single mode microwave reactor. Esters served as target compounds for the study due to their relevance for the chemical industry and numerous applications in food flavoring and cosmetics. Traditional ester synthesis employs the conventional heating of the reaction mixtures and, therefore, does not transfer energy efficiently. Our research goal was to collect a proof of concept, to demonstrate that carrying out reaction as a microwave synthesis in a single mode reactor could be a valuable alternative method allowing for shorter reaction times, minimizing heat loss, producing esters with reasonable yields and purity, and, most appealing reducing energetic waste. The preliminary study was conducted using ethyl benzoate and methyl benzoate as target compounds on a gram-scale. The production of ethyl benzoate was successful at this point while synthesis of methyl benzoate was not. Here in we report preliminary results, highlight advantages of the current method, and outline potential limitations to be overcome in the future research attempts. The results of this study will work as a seed project for future generation of students in organic laboratory at UWL and a research community at-large targeting the development of more environmentally friendly synthesis of small organic molecules useful for industrial applications.

Manuscript Narrative

INTRODUCTION

With the pandemic in full force, the amount of greenhouse emissions have reduced by 6%, but the amount of single use plastics created for takeout food, disposable masks, and personal protection equipment has increased microplastics in the environment dramatically (Pence 2021). These changes in our environment have inspired necessary conversations about sustainability around the world as well as in the chemical community. The American Chemical Society as a whole has emphasized the need for chemists to band together to find ways to reduce the footprints left by chemical industry on the environment (Pence 2021). Individual groups, such as *Schwarzman et al.* also emphasize the need for green chemistry because in the past the US chemical policies have failed to emphasize sustainability, but rather focus on product function, price, and performance (Schwarzman 2009).

The intent of Green Chemistry is to "promote sustainability thinking and reasoning" (Timmer 2018). There are 12 key principles that need to be considered such as prevention, less hazardous chemical synthesis, safer solvents, design for degradation, and safer chemistry for accident prevention (Timmer 2018). In this research study we highlight the principles of safer solvents, design for energy efficiency, and less hazardous chemical syntheses.

This need for change in the practices used in production of industrial chemicals was the inspiration for this research study. An additional benefit of the study is incorporation of research experience in the classroom. The American Chemical Community is also emphasizing the need to start off undergraduate students with sustainable practices to facilitate development of next generation of young scientists to contribute in the field of Green Chemistry (Pence 2021). We as a group of students in Organic Chemistry Laboratory course CHM 305 were challenged to investigate a possibility to reduce the energy waste created while producing esters used in flavoring and cosmetics by means of microwave reactions. The two esters we selected to test were methyl benzoate and ethyl benzoate.

For this research project, we selected to produce flavor esters. These esters are used in products like shampoos, body lotions, beverages, soap, lipstick (Alvarez 2019). Their popularity in the cosmetic and food industry are rising rapidly with an increase of their production by 6.4 % since 2015 (Alvarez 2019). They are also popular because many of them are sold as natural products isolated from sustainable sources, e.g., plants. However, the purity of compounds isolated from natural sources could be highly dependent on the origin of the source, growth, and harvest conditions, and can also be affected by the transportation and storage of products. To achieve the highest purity, especially for cosmetics applications the majority of esters are produced synthetically, for example, ethyl benzoate as well as methyl benzoate are common ingredients in perfumes and preservatives (Cosmetics INFO).

Traditionally, esters used in flavoring and cosmetics are created through a synthesis involving conventional heating the reaction can be carried out in distillation apparatus using a round-bottom flask equipped with a water condenser and supplementary components (Figure 1).

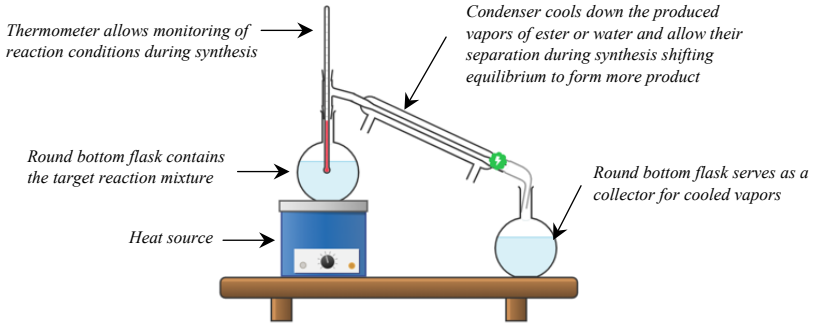


Figure 1. Basic schematics for synthesis of esters using the conventional heating setup.

In general, esterification is the process in which an ester is formed. There are many variations of this reaction based on the exact starting materials used. The combination that utilizes a carboxylic acid and alcohol in presence of

acid catalyst is known as Fischer esterification. To develop a microwave synthesis, it is important to understand the limitations of the process. Therefore, the mechanism of the reaction needs to be considered (Figure 2). Prior the reaction between an acid and an alcohol can begin the protonation occurs (Step 1, Figure 2). After the activation occurs the nucleophile can attack and the skeleton of the ester product is formed. The sequence of proton transfer steps followed by water limitation results in a protonated ester (Step 5, Figure 2). Final proton transfer releases the acid catalyst and activates next equivalents of acid and alcohol to react. Overall, Fischer esterification is an equilibrium process that limits the formation of ester due to the reversibility of the reaction at every step of the process. This can have a drastic negative impact on the reaction yields, careful monitoring of reaction conditions, clever use of reagent quantities, and targeted product isolation should all be considered. Although it is not possible to avoid achieving an equilibrium state in Fischer esterification (Figure 2) according to Le Chatelier's principle, the endothermic equilibrium can be shifted in favor of the products by heating the reaction. Based on computational modeling data we acquired earlier in the semester the enthalpy of the overall reaction was calculated to be positive rendering reaction as an overall endothermic process. Therefore, we hypothesized that increasing the temperature of the reaction for synthesis of methyl and ethyl esters of benzoic acid will allow us to isolate products in appreciable yields in microwave reaction. In addition, increase in energy inside the vial increases the number of particles that have higher kinetic energy and will collide more frequently with higher energy which can result in surpassing the activation energy of the reaction and in the end favor the ester product formation.

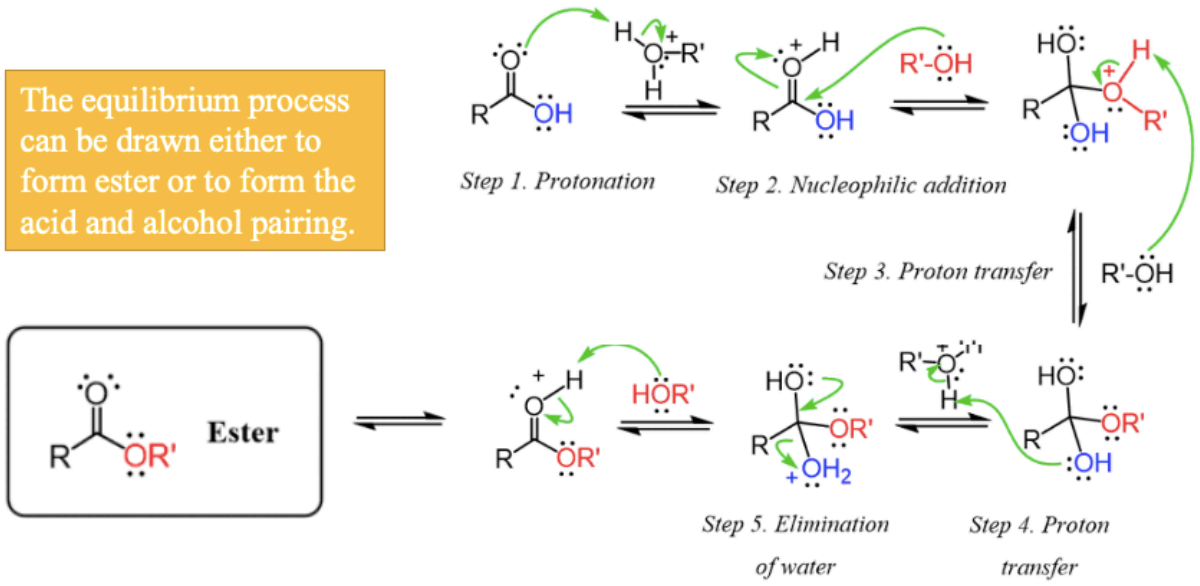


Figure 2. Fischer esterification mechanism adopted from www.chemistrysteps.com, accessed on May 18th 2021.

Elimination of water illustrated on step 5 of Fischer esterification mechanism leads to the formation of stoichiometric amounts of water during synthesis and halt the equilibrium. Physical removal of water from the reaction mixture can allow one to increase the yield of desired ester. Use of distillation apparatus shown on Figure 1 can be helpful in removal of water, however a more advance apparatus was developed. The glassware is known as a

Dean-Stark apparatus and is illustrated on Figure 3. The setup consists of round bottom flask that here serves as both the reaction flask and the product collection flask (see Figure 1 for comparison). The water-cooled condenser is connected by a special tube that allows not only collection of water but also estimation of its volume during the synthesis. However, the apparatus is very useful and practical for the laboratory experiment the translation of this approach to the large scale industrial synthesis does not seem to be practical.

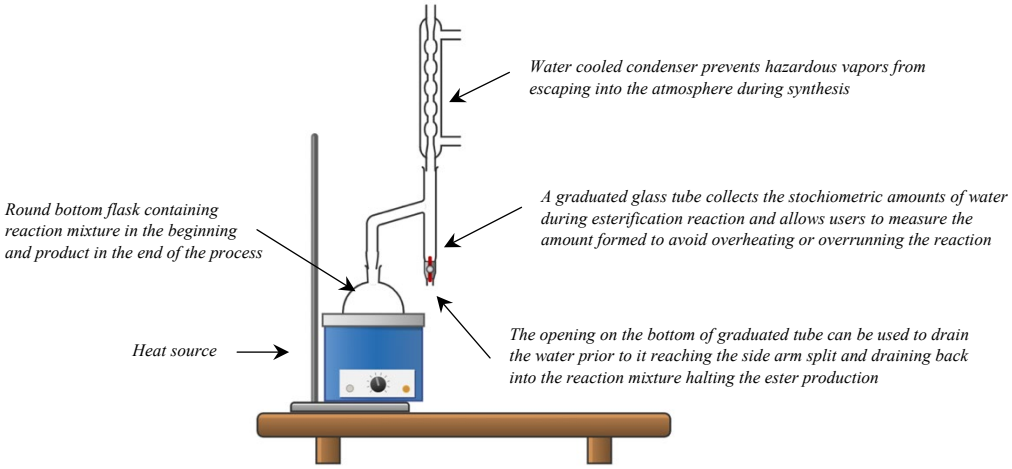
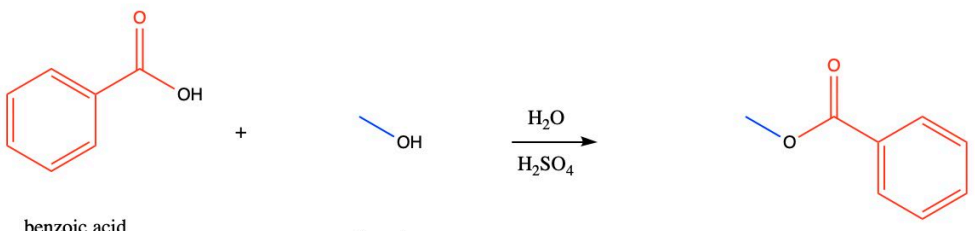


Figure 3. Dean-Stark apparatus for removal of water using conventional heating source.

Microwave synthesis uses electromagnetic waves to deliver heat straight to the molecules of reacting compound instead of heat being absorbed by walls of reaction container. This can produce a large amount of energetic waste due to long heating times with extremely high temperatures. A research study by Eric Martin and Cynthia Yuen previously demonstrated effective use of microwave assisted organic synthesis to perform a simple, greener Wittig reaction in organic laboratory (Yuen & Martin 2007). Microwave synthesis is faster, cleaner, and more economical from multiple venues. In this study we focused our efforts on investigating applicability of microwave synthesis using a single mode Biotage® Initiator+ instrument towards synthesis of two ester products. Biotage® Initiator^{+} is a single-mode microwave synthesizer that allows to run one reaction at a time with a monitoring of pressure, temperature, and stirring. Since reactions are run in sealed vials it is possible to heat compounds above their boiling points, providing a maximum increase of the temperature and energy that is not achievable during the conventional synthesis. In summary, the microwave synthesis minimizes loss of heat during reaction, and can allow to shift equilibrium to maximize the product formation at a shorter reaction time (Patel 2011). The overarching goal of our research project was to provide proof of concept for applicability of Biotage® Initiator+ towards a more environmentally friendly alternative synthesis of common esters in organic laboratory. In particular, we studied translation of the adaptation and limitation of procedure for ethyl benzoate and methyl benzoate. The reactions studied are illustrated on Figure 4.

a)



benzoic acid

b)

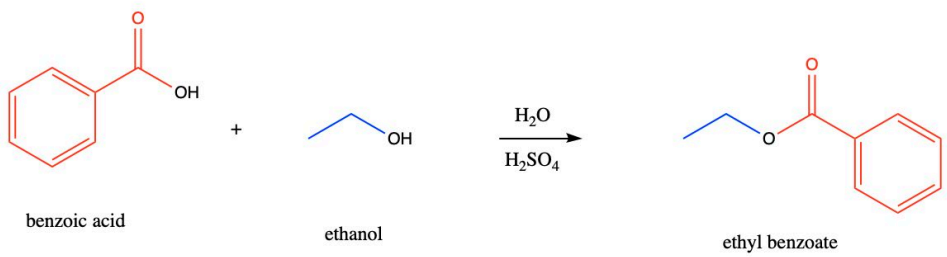


Figure 4. Target reactions: a) synthesis of methyl benzoate and b) synthesis of ethyl benzoate.

The difference between the two reactions is the use of alcohol while the carboxylic acid and the acid catalyst are kept identical between the two reactions (see Figure 4). For simplicity the steps of the mechanism are not shown but the final products are. The attachment of the pieces in the final products and their corresponding origins in starting materials are illustrated using different colors.

RESULTS AND DISCUSSION

As our research question focused on finding an efficient way of synthesizing ethyl benzoate and methyl benzoate using microwave synthesis using Biotage® Initiator+ synthesizer we were limited in the reaction volume (2.5 to 3.5 mL) we could use based on the size of the microwave vial (5 mL) according to the guidelines of the manufacturer. This subsequently imposed a limitation on the quantities of starting materials we were able to use. Considering the benzoic acid is a solid compound while both alcohols are liquid, we determined that we would use an excess amount of alcohol in our reaction and our benzoic acid would be the limiting reagent. Once reaction achieves an equilibrium the remaining excess of starting alcohol would serve as a driving force towards product formation as per Le Chatelier's principle. Based on the instrument's design we were able to modify temperature and time of our reactions. The temperature is monitored by the infra-red sensor build in the instrument and can be set to the 0.1 °C precision, in addition, the temperature is monitored and recorded during the synthesis to ensure the quality and reproducibility of synthetic parameters. Although, we could easily heat reaction beyond the boiling point of alcohols we had to take into the account the pressure accumulation that could be reached. Maintaining the prolonged heating beyond the boiling point forces liquid molecules of alcohol to enter the gaseous phase. The instrument monitors the pressure using the build-in pressure sensor and will abort the experiment if pressure will reach unsafe levels, however, if the pressure were to become too high, it will cause the vial to burst. Considering the

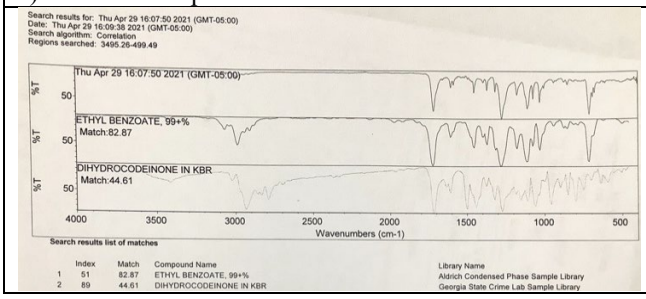
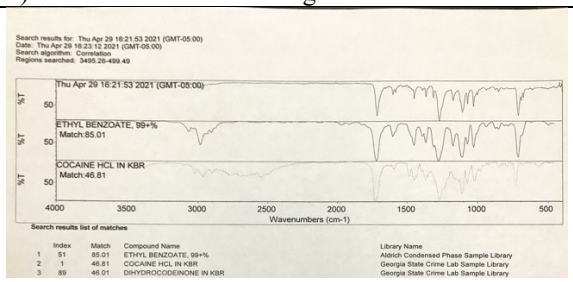
above-described limitations, we have designed and implemented several variations of experimental procedures using different starting material ratios and temperatures with the results summarized in Table 1.

Table 1. Yield of ethyl benzoate from varying amounts of benzoic acid starting material.

Entry	Benzoic acid : Ethanol ratio (mmol)	Theoretical yield (mmol)	Experimental yield (mmol)	Isolated percent yield (%)
1	1:4.28	9.99	9.72	97.3
2	1:3.89	11.0	4.40	40.6
3	1:3.48	12.3	2.70	22.0

Our initial attempts at a microwave synthesis of ethyl benzoate, our hypothesis was that performing reactions at highest possible temperatures within safety measures specified by the manufacturer would provide best product yield. However, we found out that the pressure built up a lot faster than our expectations and we had to maintain temperature low enough so that the microwave vial would be able to sustain the pressure created by the reactants. Using to our advantage the fact that reactions were carried out solvent free we were able to visually inspect the reaction progress as ester and water are not miscible with each other, meaning the ester will appear as a separate layer. Our observations demonstrated that little to no reaction occurred when it was placed in the synthesizer at 150 $^{\circ}$C after 10 minutes, however, a clear layer of an immiscible with water liquid product was obtained when reaction was carried out at 170 $^{\circ}$C after 5 minutes. The purification of reaction mixture was performed using liquid-liquid extractions and collected fractions were analyzed using infrared spectroscopy (IR) initially and followed by nuclear magnetic resonance spectroscopy (NMR). The initial trials at higher temperature produced only small amount of desired product, therefore, we decided to modify starting material ratios in addition to the temperature and time to achieve a higher value and purity sample of ethyl benzoate (Table 2). The optimum ratio for the formation of desired product with highest yield and purity was found to be 1:4.28 of benzoic acid to ethanol (Table 1, entry 1). Decreasing of the ratio even by a small amount had a drastic impact on the product yield and yield.

Table 2. Structure search for collected product indicates ~80% match to the desired compound using all available database search libraries included in the instrument's operating software.

a) Modified temperature conditions	b) Modified ratios of starting materials
	

The combined results of collected NMR spectra containing initial attempts using modification of temperature and time (shown in blue), attempts with manipulated stochiometric ratios of starting materials (shown in red), and

final trials with best combination of parameters (shown in green) for synthesis of ethyl benzoate applied are summarized on Figure 5. All spectra are shown as a full spectrum including regions that are not expected to show any signals to ensure the clear comparison and fair data representation. The expected number of signals for ethyl benzoate is five, three signals are to appear in aromatic region between 6.5 to 8.5 ppm and two signals to appear in aliphatic region between 1 and 4 ppm (Figure 5). Overall, all spectra demonstrate formation of the desired product with green and blue spectra showing the highest purity of the sample. The peak at ~0 ppm should be ignored from consideration as an internal NMR standard of tetramethyl silane as well as residual chloroform peak appearing at 7.37 ppm chemical shift value.

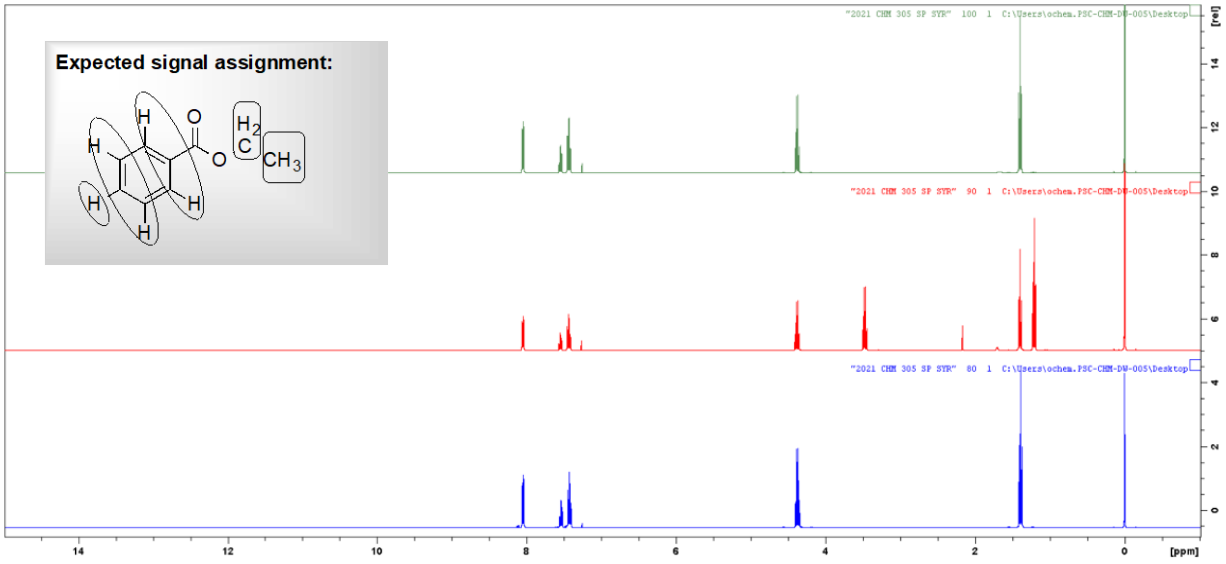
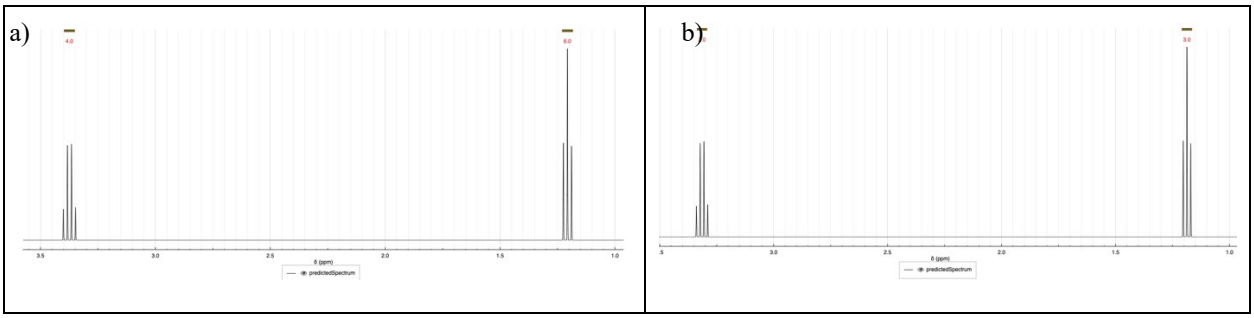


Figure 5. The overlap of NMR data collected for several synthetic attempts with modified conditions.

To facilitate our understanding of the observed impurities (Figure 5, red spectrum) we simulated the spectra of two anticipated impurities: diethyl ether (Table 3a) and ethanol (Table 3b) using NMR predicting software (Aires-de-Sousa 2002)(Banfi 2008)(Castillo 2011). Although the appearance of signals in these two compounds is identical, a quartet for two hydrogens and a triplet for three hydrogen atoms, the position of those signals can be used to clearly identify the impurity.

Table 3. Simulated NMR spectra of two anticipated impurities.



Taking a closer look at the targeted experimental result (Figure 6, signals at 1.2, 2.1, and 3.5 ppm) and predicted spectra (Table 3) we correlated that ethanol is the best match for the observed impurity in the spectrum of ethyl benzoate. In addition, the sample contains a small amount of water (signal at 1.85 ppm) as well indicating that better drying procedure is required for future syntheses.

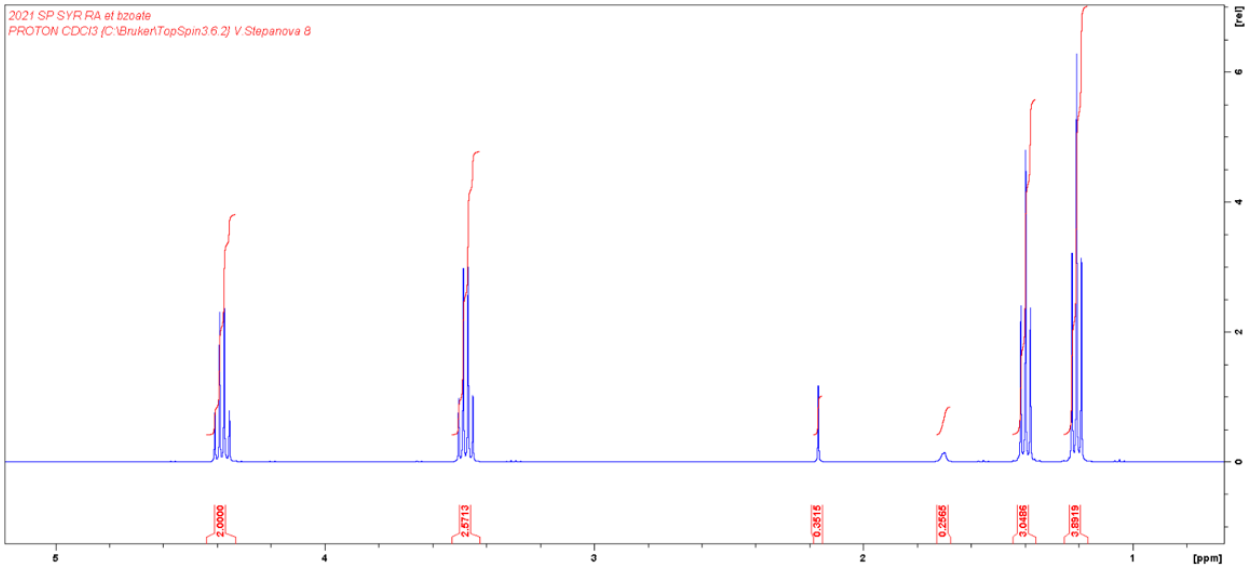


Figure 6. The closer look at impurity's signals in aliphatic region.

Our attempts to translate our microwave synthesis procedure to obtain methyl benzoate (Figure 4a) were not successful. The difference between methanol's boiling point of 65 °C and ethanol's' boiling point of 78 limits the available temperature range for microwave synthesis. In addition, the absorption of microwave energy in methanol is much higher than that for ethanol. This can lead to complications during reactions due to evaporation of methanol prior to it being able to react with benzoic acid. Our results demonstrate that reactions carried out at 150 °C did not provide appreciable amounts of product. In addition, a clear spike in the pressure value was observed on the instrument monitoring software and by visual analysis of the vial. The septum on the vial cap was deformed indicating accumulated pressure during synthesis. Increase of reaction time from 5 minutes to 7 minutes did not have a positive impact on the product's yield and resulted in even higher-pressure accumulation. At this point we were not able to identify best reaction conditions for synthesis of methyl benzoate.

CONCLUSION

The intent of this study was to test possibility to obtain methyl and ethyl esters of benzoic acid using microwave synthesis and single mode Biotage® Initiator+ synthesizer. We were able to design and implement a microwave synthesis of ethyl benzoate and isolate product with 97% yield using temperature of 170 °C and reaction time of 5 minutes. However, our efforts to obtain methyl benzoate were not successful. Accumulation of pressure was too high to support a microwave synthesis of methyl benzoate under previously tested conditions. Further testing of potential improvements to isolation procedure and reaction conditions are required to develop syntheses of esters of benzoic acid with high yields and purities.

MATERIALS AND METHODS

All reactions were carried out using Biotage® Initator+ synthesizer equipped with Robot 8 accessory in original manufacturer recommended 5 mL vials. Chemicals were purchased from Sigma-Aldrich chemical company and used without further purifications. Glassware was provided by instructor. Infrared spectra were collected using Perkin Elmer IR spectrometer using ATR utility with diamond crystal. Proton NMR spectra were collected using Bruker 400 MHz NMR spectrometer.

Microwave synthesis of methyl benzoate

Benzoic acid (0.122 g, 1.000 mmol) was combined with methanol (2.50 mL, 98.6 mmol) and concentrated sulfuric acid catalyst (20 drops) in a microwave vial. Stirring bar was added to the vial, then vial was capped tightly using manufacturer provided crimper tool, transported to and placed in the Biotage® Initiator+ instrument Robot 8 with exact setting of experimental conditions. Upon completion vials were uncapped using a decrimper tool and products were purified using liquid-liquid extraction.

Microwave synthesis of ethyl benzoate

Benzoic acid (0.122g, 1,000 mmol) was combined with ethanol (2.50mL, 42.8 mmol) and sulfuric acid catalyst (20 drops) in a microwave vial. Stirring bar was added to the vial, then vial was capped tightly using manufacturer provided crimper tool, transported to and placed in the Biotage® Initiator+ instrument Robot 8 with exact setting of experimental conditions. Upon completion vials were uncapped using a decrimper tool and products were purified using liquid-liquid extraction.

Liquid-liquid Extraction

The mixture of two immiscible liquid layers was transported from the vial a separatory funnel with 30mL of water. After extraction was completed the two layers were separated and the aqueous layer was drained into a beaker. Next, 20mL of diethyl ether was added to the separatory funnel. After extraction was completed the two layers were separated and the aqueous layer was drained into a beaker. The organic layer was washed twice with 25mL saturated sodium bicarbonate solution. Finally, the organic layer was washed with 20mL of sodium chloride brine solution. The remaining organic layer was drained into an Erlenmeyer flask. Sodium sulfate drying agent was added until dry. A Buchner funnel filtration was used to remove the solid drying agent and concentrate the product.

Characterization of Compounds

To collect an IR spectrum of the product obtained in this reaction, a background spectrum was collected. The purified samples were placed on the crystal of an IR instrument and run to obtain an IR spectrum for each product.

An NMR sample of each product was prepared dissolving the product (0.025 g, 0.166 mmol) in $CDCl_3$. This solution was transferred to an NMR tube and an NMR spectrum was collected.

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