UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Químicas e Ingeniería

TÍTULO: Crossed aldol reactions in water using inexpensive and easily available materials as a tool for reaction optimization teaching in an undergraduate organic chemistry laboratory

Trabajo de integración curricular presentado como requisito para la obtención del título de Químico

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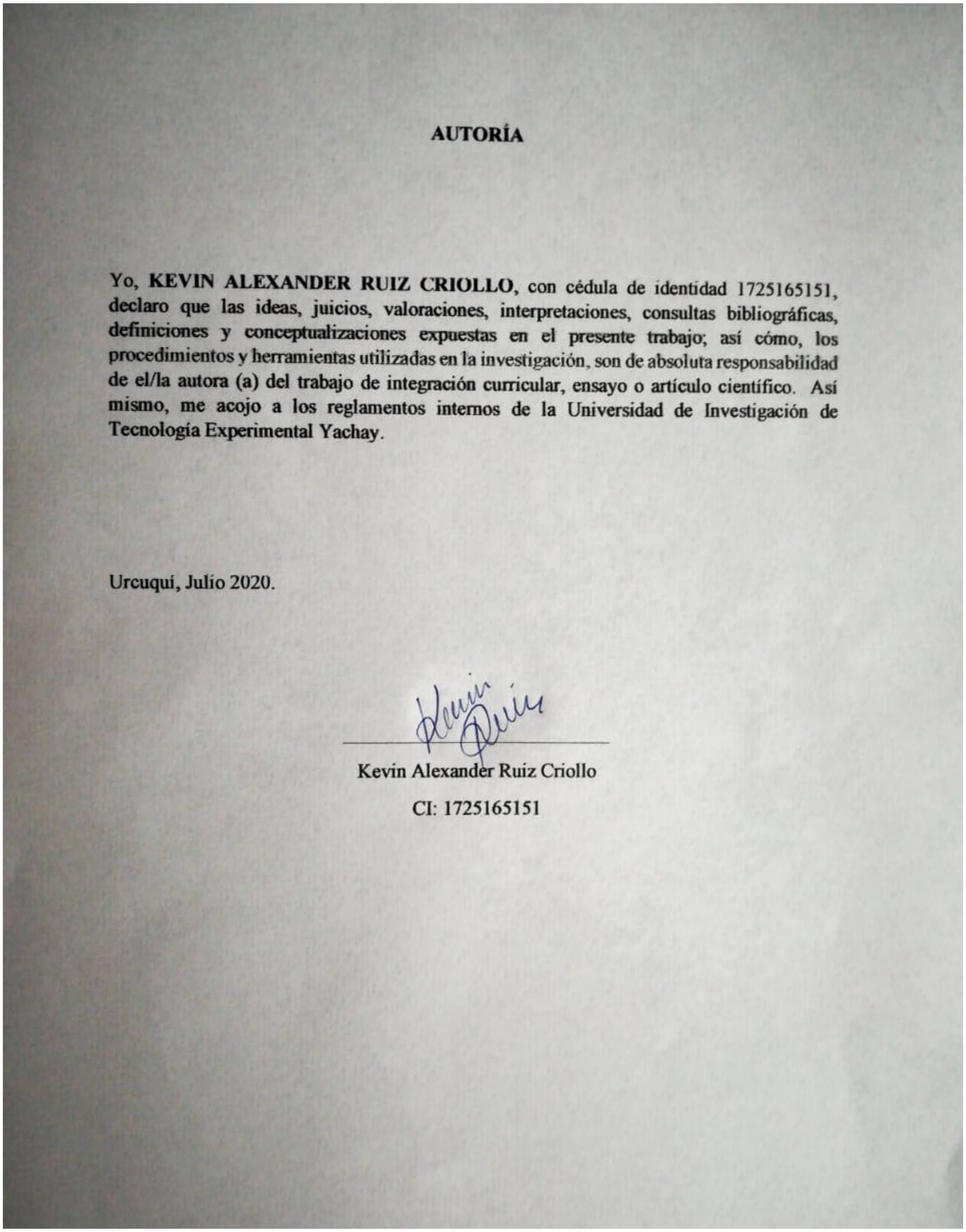

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DEDICATORIA

El presente trabajo de investigación está dedicado a mi madre Maritza por su profundo amor y cariño, además de haber sido siempre mi ejemplo de perseverancia y una motivación para alcanzar mis objetivos. Su apoyo incondicional durante mi formación académica ha sido lo que me permitió superarme y culminar esta etapa con éxito. También quiero dedicar este trabajo a mi hermano por estar presente cuando lo necesito y ser una inspiración para superarme y cumplir mis propósitos. Estoy infinitamente orgulloso de tenerlos como mi familia y sumamente feliz de poder compartir este logro con ustedes.

Kevin Alexander Ruiz Criollo

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Kevin Alexander Ruiz Criollo

RESUMEN

Las reacciones aldólicas cruzadas son reacciones aldólicas en las que intervienen dos tipos diferentes de compuestos carbonílicos, dependiendo de los compuestos involucrados pueden ser útiles o no en síntesis orgánica. Esta reacción puede ser un tema difícil en conferencias y laboratorios para estudiantes de química de pregrado porque se deben considerar varias reglas para predecir el producto. En este trabajo, se presenta un artículo para el *Journal of Chemical Education* con reacciones aldólicas cruzadas acuosas de vainillina y cinamaldehído como experimentos con reactivos de bajo costo y fácilmente disponibles para obtener los productos deshidrozingerona y cinnamilideno acetona respectivamente. En la condensación de vainillina, la reacción de optimización implica el uso de aditivos de LiCl o $NiCl_2$ como catalizadores y su velocidad de reacción es controlada por cromatografía en capa fina. Mientras que en la condensación de cinamaldehído, el uso de materiales económicos y solventes no tóxicos son ventajas para llevar a cabo un procedimiento simple y confiable. Además, el objetivo de este trabajo es lograr los resultados de aprendizaje propuestos para los estudiantes, tales como la toma de decisiones en el laboratorio y las habilidades de gestión del tiempo. Finalmente, el uso de técnicas analíticas para la caracterización de productos finales ayuda a los estudiantes a reforzar el conocimiento en el análisis de compuestos orgánicos.

PALABRAS CLAVE

Reacción Aldólica Cruzada, Vainillina, Cinamaldehído, Cromatografía de Capa Fina, Reacción de Optimización, Resultados de Aprendizaje

ABSTRACT

Crossed aldol reactions are aldol reactions that involve two different types of carbonyl compounds, which depending on the involved compounds, can be useful or not in organic synthesis. This type of reaction can be a difficult topic in lectures and laboratories for undergraduate chemistry students because several rules need to be considered in order to predict the product. In this work, an article for the *Journal of Chemical Education* about aqueous crossed aldol reactions of vanillin and cinnamaldehyde as experiments with inexpensive and easily available reagents to obtain dehydrozingerone and cinnamylidene acetone products respectively is presented. In the vanillin condensation, optimization reaction is implicated with the use of LiCl or $NiCl_2$ additives as catalysts, and its reaction ratio is controlled by thin-layer chromatography. While in the cinnamaldehyde condensation, the use of inexpensive materials and non-toxic solvents are advantages to carry out a reliable and straightforward procedure. Additionally, the goal for this work is to reach the learning outcomes for students, such as in improving the students' lab decision making and time management skills. Finally, the use of analytical techniques for the characterization of final products helps the students to reinforce their knowledge in the analysis of organic compounds.

KEYWORDS

Crossed Aldol Reaction, Vanillin, Cinnamaldehyde, Thin Layer Chromatography, Optimization Reaction, Learning Outcomes

INDEX

CHAPTER 1

1. INTRODUCTION-JUSTIFICATION

1.1. General Introduction

Aldol reaction is a very useful method for C-C bond formation and key step in many complex syntheses of natural products and drugs[1]. Also, it is of essential importance in the chemistry of enols and enolates. Due to this, aldol reactions have been studied intensively in both research and teaching laboratories. In undergraduate organic chemistry lectures and laboratories, after discussing self-condensation, aldol reaction topics move to the more synthetically useful crossed aldol reaction. Crossed aldol reactions involve the attack of an enol or enolate to another carbonyl species, more electrophilic than themselves[2]. For the inexperienced organic chemistry students, it may be challenging to understand all the rules in order to predict the product because they require several things to take into account, such as: which molecule can enolize, which side of the molecule is enolizable, which molecule is more electrophilic and in the case of α, β-unsaturated aldehydes or ketones where the addition occur at carbonyl or β carbon.

Generally, in organic chemistry teaching, it is a good idea to strengthen the theoretical knowledge in a laboratory practice session. The laboratory practices, which will be presented in this research, aim not only to reinforce the concepts of cross aldol reaction but also avoid the issue of chemical supply in Latin American countries, where the access to reagents is very difficult, slow in delivery and has a high cost for the educational institution. The use of inexpensive and readily available starting materials represents a huge benefit for the development of laboratory practices in developing countries and also for the present research.

In this work, vanillin and cinnamaldehyde are presented as natural aldehydes, which can be easily extracted from plants or obtained in drugstores and supermarkets. Both vanillin and cinnamaldehyde, will be used as an electrophile partner in synthesis optimization and two-step semi-synthesis procedures, respectively. While the nucleophilic partner is prepared from acetone in basic media by using sodium hydroxide.

The first reaction discussed in this work is a synthesis optimization procedure, where vanillin is used as starting material to carry out several crossed aldol reactions simultaneously with the use of additives to obtain (E)-4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one, commonly known as dehydrozingerone as final product. By using catalytic amounts of additives (metal halides)[3], the reaction rate will be improved in this synthesis, and its kinetics will be monitored by thin layer chromatography (TLC) to determine the best reaction conditions. In addition to reinforcing the concepts of cross aldol reaction, this practice also improves the student skills in lab decision-making capacities by controlling the reaction ratio. Awad et al. state that it takes (up to) 48 hours for this reaction to take place[4], then this research has as an important goal, the reduction of the reaction time for a 3 hours laboratory session.

The second reaction discussed here, a two-step semi-synthesis procedure, involves the extraction of a natural aldehyde, cinnamaldehyde, and the reported in literature semi-synthesis of cinnamylidene acetone[5] or (3E, 5E)-6-phenylhexa-3,5-dien-2-one. Cinnamaldehyde obtention from cinnamon barks is carried out by steam distillation and liquid-liquid extraction, which is a simple and reliable procedure with the use of non-toxic solvents. The use of a natural product as a source of starting material for this crossed aldol reaction fulfills the purpose of using inexpensive and easily available reagents. In this research, the crossed aldol reaction from cinnamaldehyde is presented as a two steps procedure designed to be carried out in a 3-hour laboratory session in order to reinforce concepts of handling laboratory material and equipment.

After the analysis of both procedures and the final products, their methodologies and results are presented as an article in the thesis, as the present work is, in fact, under revision by the *Journal of Chemical Education*. Hereof, both procedures, synthesis optimization of dehydrozingerone and two-step semi-synthesis of cinnamylidene have the purpose of not only reinforcing knowledge of cross aldol reactions by a reliable and straightforward process but also to let student develop better learning outcomes such as how to approach research problems and to hone their decision-making capacities in the laboratory.

1.2. General and specific objectives

1.2.1. General objective

- Implement the use of inexpensive and easily available materials, vanillin and cinnamaldehyde, for crossed aldol reactions as a tool for reaction optimization in the organic chemistry teaching laboratory.

1.2.2. Specific objectives

- To introduce the role of Lewis acids in organic synthesis.
- To synthesize biologically active dehydrozingerone by crossed aldol protocol under optimized conditions to reduce the reaction time by up to 94% compared with published protocols.
- To extract cinnamaldehyde from a natural source.
- To replicate the cinnamylidene acetone synthesis by crossed aldol reaction reported in literature.
- To characterize dehydrozingerone and cinnamylidene acetone from crossed aldol reaction using spectroscopic technique IR and chromatographic techniques TLC, HPLC, and UPLC-MS.

CHAPTER 2

2. METHODOLOGY AND RESULTS

2.1. Article submitted to the *Journal of Chemical Education*

Crossed aldol reactions in water using inexpensive and easily available materials as a tool for reaction optimization teaching in an undergraduate organic chemistry laboratory

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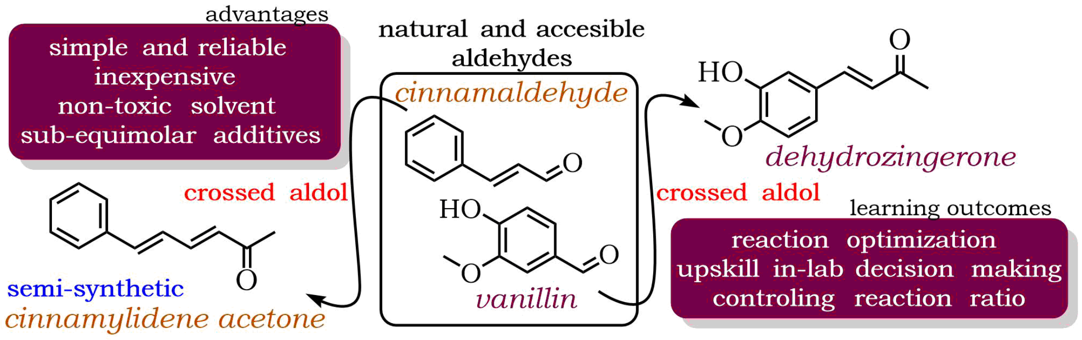
ABSTRACT

We present an experiment designed to hone the decision-making skills of (undergraduate) chemistry students by using aqueous crossed aldol reactions. Here, we present two simple crossed aldol reactions based on inexpensive readily available materials: The condensation of vanillin and cinnamaldehyde with acetone. The procedure uses the variation of reaction conditions (including the use of inexpensive chlorides, namely LiCl or $NiCl_2$, as catalysts and variation between different mild reaction temperatures), as well as different simple preparative and analytical techniques such as filtration, recrystallization, melting point measurements, thin layer chromatography and IR spectroscopy aiming for the students to find the optimal reaction conditions, thus honing their decision making and time management skills. Furthermore, those experiments are designed for a laboratory session of 3 h to 4 h.

GRAPHICAL ABSTRACT



KEYWORDS

Organic Chemistry, Hands-On Learning, Decision Making, Aqueous Solution Chemistry, Synthesis, Thin Layer Chromatography

Aldol condensation is undoubtedly one of the most important reactions for C-C bond formation and has been a very useful tool in the synthesis of complex natural and synthetic products for pharmaceutical purposes[1]. In the classroom, the adventure with aldol reactions starts with auto-condensation and then topics usually move to the synthetically much useful crossed aldol reaction. The study of crossed aldol reactions for the undergraduate organic chemistry student may be difficult. Students need to assimilate many rules and be able to predict product outcomes, whereas many things need to be considered as which molecule can enolize, which side of the molecule is enolizable, which molecule is more electrophilic and, in the case of α, β-unsaturated aldehydes or ketones, where the addition occurs: at a carbonyl or β carbon? These concepts can be strengthened and applied in the laboratory. Many protocols for aldol reactions adapted to organic laboratory teaching have already been published in this journal; however, most of them require either relatively expensive or not readily accessible reagents and catalysts[2–8].

The ideal crossed aldol reaction requires two carbonyl compound partners, one of which is enolizable and the other needs to fulfill two requirements: needs to be unable to enolize and to be much more electrophilic than the other compound. The simplest ketone, very easy to acquire, and an inexpensive, enolizable partner can be acetone, which was our compound of choice. The electrophilic partner, preferable some aldehyde, usually requires acquisition from specialized chemical suppliers. In some developing countries in Africa, Asia, or Latin America, the supply of chemicals for teaching laboratories can be problematic. Cost is a major issue but can be partly minimized by scaling down the reaction and pairing students into larger workgroups per session. Another problem experienced by us, even greater from our perspective, is the delivery time of chemicals, reaching 5-6 months due to the extensive bureaucratic procedures in public procurements.

We have found that cinnamaldehyde and vanillin are suitable aldehydes for our purpose. Cinnamaldehyde can be easily extracted from cinnamon bark by steam distillation, and after liquid-liquid extraction, it can be used without further purification for aldol condensation with acetone to give cinnamylidene acetone (Figure 1a)[9]. This crossed aldol condensation is very fast, after only a few minutes precipitation of the product can be observed. Cinnamaldehyde has the characteristic smell associated with cinnamon and possesses several pharmacological properties, such as anti-microbial anticancer, antioxidative, antiobesity and anti-inflammatory activity[10].

Vanillin can also be obtained from a natural source by extraction from vanilla plant. However, the compound is widely used as a flavoring agent in the food industry and is also available in drugs, perfume & hygiene stores as well as online. The vanillin condenses with acetone in basic aqueous media to generate the product named (*E*)-4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one, it is also known by its more common name dehydrozingerone (DHZ) or vanillidene acetone (Figure 1b). DHZ itself is present in a natural source as rhizomes of ginger, possesses biological activity as antifungal, anticancer, antioxidant activity or anti-Alzheimer's activity, and became of interest as a molecular core for the preparation of other derivatives with improved activity[11–18]. The issue with the reported procedure of that aldol condensation is that vanillin is consumed within 24h hours at room temperature. In a course where organic chemistry laboratory is given once per week, this becomes a problem. The optimization of the reaction by Smith[19] partially solves this problem as with specific conditions the reaction time could be extended to one week. Nonetheless, when time constraints need to be considered, and the laboratory space, as well as material needs to vacate after each session, this solution is suboptimal. Our goal was to reduce the time of the reaction to be suitable for 3h laboratory session. This was achieved after optimization at different temperatures with several additives (metal halides). Hosomi et al. reported successful aldol condensations with improved yields using catalytic amounts of metal chlorides, especially LiCl and $MgCl_2$ in acidic media with quite different systems as α-dimethylsilylesters[20]. In our work, the basic enolization of acetone and condensation with aromatic aldehyde as vanillin with the addition of catalytic amounts of lithium chloride improves the ratio of reaction. During the trials, we have seen that the kinetics of the reaction can be readily altered by the applied conditions and can be easily followed by thin-layer chromatography (TLC). The simple objective of giving a "ready to go" procedure to the student changed to teach the student to determine the best reaction conditions. This experimental approach not only teaches the handling of laboratory material but also introduces the student to approaching research problems and laboratory-related decision-making. Additionally, we have seen that this approach had a better reception by the students as they realize they are not just following a recipe, but applying analytic techniques to solve daily organic chemists' problems.

RESULTS AND DISCUSSIONS

Optimization of synthesis of dehydrozingerone

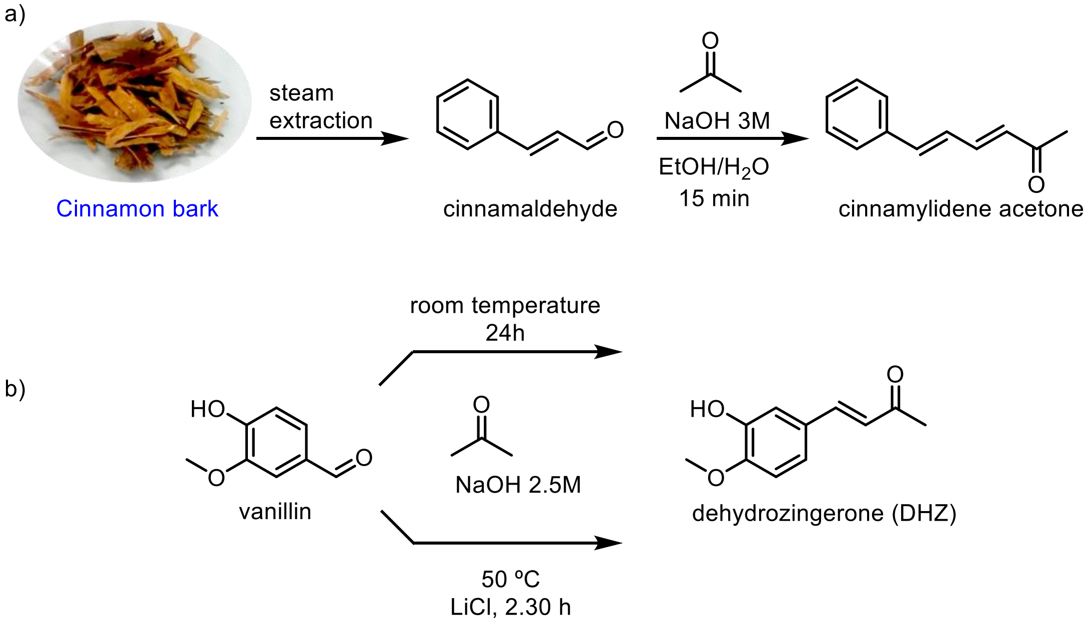


Figure 1. Vanillin and cinnamaldehyde as natural occurring aldehydes used for crossed aldol condensation with acetone.

The optimization of the reaction was carried out using different temperature conditions with LiCl, $NiCl_2{\cdot}6H_2O$, $ZnBr_2$ and $MgCl_2$ as additives. We have found that the best condition is using LiCl (0.1 eq) with $NiCl_2{\cdot}6H_2O$ (0.1 eq) at 40 °C or just LiCl (0.1 eq) at 50 °C where the time of full conversion is shortened from 24 has reported in the literature[16,19] to 2 h and 2.30 h, respectively. Lithium chloride alone as an additive requires 0.5 h longer heating, but the reaction is simpler and greener. The typical reaction condition was carried out on a scale of 0.5 g (3.3 mmol) of vanillin, 5 mL (67.5 mmol) of acetone, 2.6 mL of NaOH 2.5M aqueous solution and additive. The progress of the reaction can be followed by TLC using Toluene:$CHCl_3$ (97/3) and vanillin developer (check SI for details) every 30 minutes, since the product reveals a deep purple spot that can be easily distinguished from the starting material. After the reaction mixture is cooled and acidified, product precipitate and can be isolated and recrystallized. Caring out the process on reflux shortens the reaction time, nevertheless, this way a sticky paste is obtained, which greatly complicates isolation of the product.

Table 1. The proposal of the different conditions and expected results

Additive (Equivalents)	Temperature /°C	Time after vanillin is consumed	Yield (after 3h)
LiCl (0.1 eq)	Room Temperature	8h-10h	40% - 45%
LiCl (0.1 eq)	40	3h00	65-90 %
LiCl (0.1 eq)	50	2h30	
LiCl (0.5 eq)	40	3h30	
LiCl (1.0 eq)	40	3h30	
0.1 LiCl (0.1 eq) + $NiCl_2$ x $6H_2O$ (0.1 eq)	40	2h00	

With the following findings, we propose different reaction conditions (Table 1) for several student groups, which require different times for total consumption of the starting material. Those conditions were chosen as a consensus of appreciable changes in the progress of reaction and quantity of product that can be obtained within the time constraints of a typical laboratory session. Students need to follow the reaction by TLC every 30 min, and asses if the reaction should continue or not, considering the presence of starting material and remaining time of the laboratory session. Then students need to share and collect the information of every group and finally decide which condition is the best taking the progress of the reaction and economic as well as environmental aspects. Possible decision making is shown in Figure 2. We do not recommend to base the final decision on the reaction yield as our experience showed that occasionally yields could be misleading since the quantity of precipitate is sometimes erratic, as it depends on the students' abilities to handle the product as well as the post recrystallization process.

Post-synthesis analysis of the product is a very important topic to be taught, which can be easily executed in a not fully equipped laboratory as DHZ is a solid and can be analyzed simply by comparison of the melting point and retention factor reported in the literature (BP: 127-131 °C and Rf : 0.18, Toluene:AcOEt-9:1). Spectroscopic and chromatographic data for further characterization can be found in supplemental data.

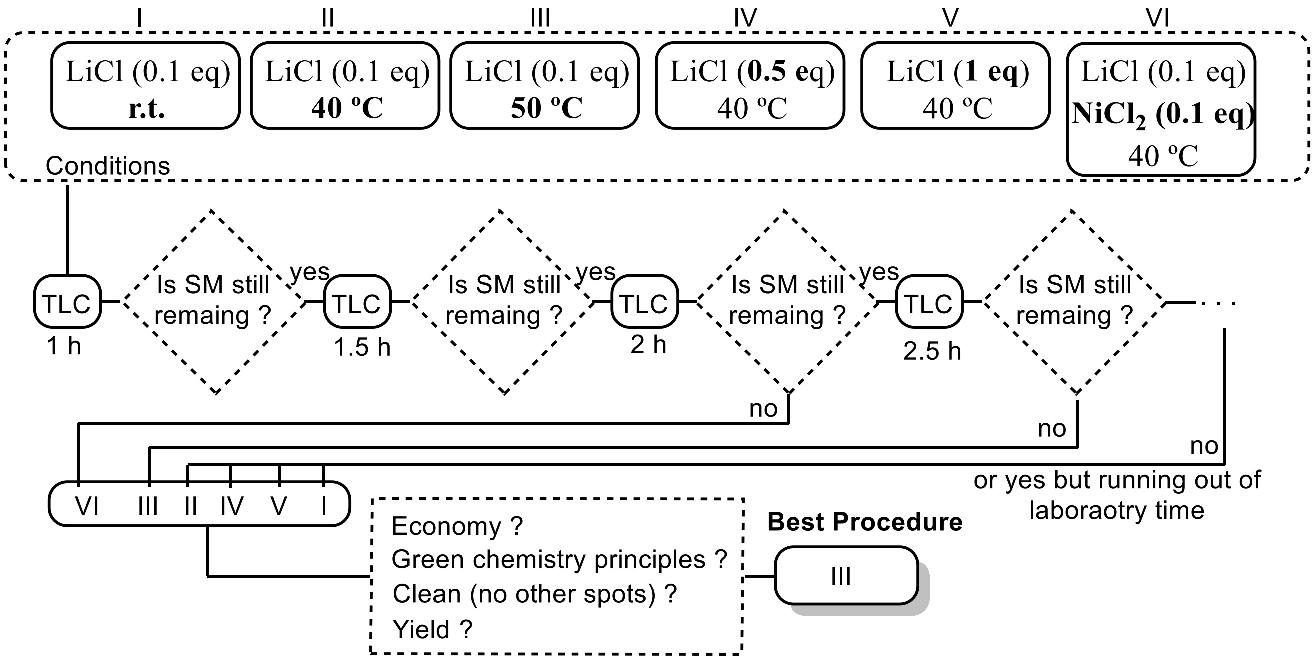


Figure 2. Possible decision-making algorithm to select the best reaction condition

Extraction cinnamaldehyde and synthesis of cinnamylidene acetone

This two-step procedure takes place in two (at least 3h each) laboratory sessions. In the first session, the students extract cinnamaldehyde from cinnamon barks using simple distillation apparatus by steam distillation. For that, distilled water is used and small pieces of cinnamon bark. The distillate is a cloudy suspension of cinnamaldehyde, which can be extracted with chloroform or ethyl acetate. About 30 g of cinnamon bark is suitable for the extraction as with that amount 280-310 mg of cinnamaldehyde can be obtained, which is a comfortable quantity of aldehyde to work with, in the next step. Surprisingly, after HPLC analysis of the crude, we have found that no further purification was needed, as the spectral purity is about 92 % at 254 nm and crude cinnamaldehyde works without any problems in an aldol reaction. The crossed aldol reaction using cinnamaldehyde is much simpler than using vanillin, as condensation with acetone is almost immediate at room temperature. For that, the procedure using acetone and cinnamaldehyde in a 1:1 mole ratio was used in ethanol and an aqueous solution of sodium hydroxide[4].

We think that this simple two-step semi-synthesis of cinnamylidene acetone can be an excellent alternative to the dehydrozingerone synthesis when vanillin is somehow difficult to acquire. This approach for the introduction of the crossed aldol reaction to the students uses the extraction of a natural product, which is then modified. Once again, the product is solid, so it can be analyzed using inexpensive equipment and simple analytical techniques. The melting point of cinnamylidene acetone is 70-74 °C and Rf=0.53 (Toluene:AcOEt-93:7). Chromatographic and spectroscopic data are available in the Supporting information.

TROUBLESHOOTING AND TIPS

- After the reaction of dehydrozingerone is finished and cooled, hydrochloric acid is added for precipitation. A greater quantity of precipitate is obtained when the mixture is cooled in an ice bath and left for at least 10 min. If precipitation does not occur, the product can be extracted with chloroform or dichloromethane 3 times, wash with brine, dried, the solvent removed and the crude recrystallized from hot ethanol.
- For efficient cinnamaldehyde extraction cinnamon bark should be flaked into small pieces. However, it shouldn’t be pulverized as this causes foam buildup when boiled with water and can pass to the collecting flask.

HAZARDS

As in every chemistry laboratory practice, the use of a lab coat, safety eyewear, and protecting gloves are mandatory. The entire procedure must be carried out in the fume hood. Reagents must be handled appropriately; in the case of sodium hydroxide, it is corrosive and may cause burns. Acetone is flammable and may cause drowsiness or dizziness if inhaled. Vanillin and Cinnamaldehyde used in these laboratory experiments are used for food flavoring and, although relatively safe, can produce skin and eye irritation.

CONCLUSIONS

Two non-expensive laboratory experiments introducing crossed aldol reactions were designed and successfully adapted to a typical session of the undergraduate organic chemistry laboratory. The starting material cinnamaldehyde and vanillin are two natural occurring aldehydes that are safe to handle but also easy to acquire, even in countries with limited chemical supplies. Additionally, we took advantage of the flexible reaction kinetic of vanillin condensation with acetone to teach students about the typical optimization process in the organic laboratory. Students were able to use and reinforce the basics of IR spectroscopy and thin layer chromatography to follow the reaction and analyze the results. Besides, the importance of Lewis acid, additives, and catalyst in organic chemistry were discussed and demonstrated in the synthesis of dehydrozingerone. Ready to implement in classroom laboratory guide for student and instructor can be found in Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI:10.1021/acs.jchemed.XXXXXXX.

Laboratory Guides for students together with detailed commentaries and tips for an instructor with HPLC, IR spectra and exact mass are provided separately for each experiment (editable doc).

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2.2. Supporting Material from Article

2.2.1. Vanillin Reaction

Practice

Crossed Aldol Reaction: Synthesis Optimization of Dehydrozingerone

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SUPPORTING MATERIAL

Contents

NOTE: The instructions in RED color are for laboratory instructors only!

1. LIST OF CHEMICALS AND HAZARDS

Chemicals	CAS No.	Labelled as	GHS Hazard Statements[1]	Hazard Pictograms
Vanillin ($C_8H_8O_3$)	121-33-5	Vanillin	H319	
Sodium Hidroxide (NaOH)	1310-73-2	NaOH 2.5 M	H314	
Acetone (C_3H_6O)	67-64-1	Acetone	H225, H319, H336	 
Hydrochloric Acid (HCl)	7647-01-0	HCl 6M	H314, H335	 
Lithium Chloride (LiCl)	7447-41-8	Anhydrous LiCl	H302, H315, H319	

Niquel (II) Chloride Hexahydrate ($NiCl_2$*$6H_2O$)	7791-20-0	$NiCl_2$*$6H_2O$	H301+H331, H315, H317, H334, H341, H350i, H360D, H372, H410	
Toluene (C_7H_8)	108-88-3	Toluene	H225, H304, H315, H336, H361, H373, H402	
Ethanol (C_2H_6OH)	64-17-5	EtOH	H225	
Ethyl Acetate ($C_4H_8O_2$)	141-78-6	EtOAc	H225, H319, H336	
Vanillin TLC Stain*	NA	Vanillin Revelator	H225, H302, H314, H340	
Distilled water (H_2O)	7732-18-5	Distilled H_2O	Not Hazardous	None

[1] The meaning of each Hazard Statement is explained in section 1.1, shown below.

*The Vanillin TLC stain can be prepared in the laboratory previous to the synthesis. The solution contains 0,5 g of Vanillin, 0,5 mL of H_2SO_4 and 9 mL of Ethanol.

1.1. Definition of GHS Hazard Statements

The GHS hazard statements of reagents used in this practice are defined as follow:

Physical Hazards

H225 - Highly flammable liquid and vapor.

Health Hazards

H302 Harmful if swallowed

H304 May be fatal if swallowed and enters airways.

H314 Causes severe skin burns and eye damage.

H315 Causes skin irritation.

H317 May cause an allergic skin reaction.

H319 Causes serious eye irritation.

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

H335 May cause respiratory irritation.

H336 May cause drowsiness or dizziness.

H340 May cause genetic defects.

H341 Suspected of causing genetic defects.

H350i May cause cancer by inhalation.

H360D May damage the unborn child.

H361 Suspected of damaging fertility or the unborn child.

H372 Causes damage to organs through prolonged or repeated exposure.

H373 May cause damage to organs (central nervous system, liver, heart) through prolonged or repeated exposure.

H301+H331 Toxic if swallowed or if inhaled.

Environmental Hazards

H402 Harmful to aquatic life.

H410 Very toxic to aquatic life with long-lasting effects.

2. EQUIPMENT AND MATERIALS

- 50 mL round bottom flask
- Magnetic stirrer
- Hot plate
- Reflux condenser
- Büchner flask
- Filter paper
- Spatula
- Büchner funnel
- Crystallizer
- Glass rod
- Capillaries
- Analytical balance
- TLC silica plate
- Chamber for TLC
- UV-Vis Lamp

2.1. List of quantities of reagents

Reagents	Molecular Formula	Molecular Weight	Quantity

Vanillin	$C_8H_8O_3$	152.15 g/mol	0.5 g
Sodium Hidroxide (2.5M)	NaOH	39.997 g/mol	2.63 mL
Acetone	C_3H_6O	58.08 g/mol	5 mL
Hydrochloric Acid (6M)	HCl	36.46 g/mol	2.5 mL
Lithium Chloride	LiCl	42.394 g/mol	0.013 g – 0.139 g[1]
Nickel (II) Chloride Hexahydrate	$NiCl_2 \times 6H_2O$	129.594 g/mol	0.0425 g
Toluene	C_7H_8	92.14 g/mol	9 mL
Ethanol	C_2H_6OH	46.07 g/mol	5 mL[2]
Ethyl Acetate	$C_4H_8O_2$	88.11 g/mol	1 mL
Distilled water	H_2O	18.018 g/mol	10 mL

[1] The amount of lithium chloride depends on the equivalents of each group, which goes from 0.1 Eq to 1 Eq.

[2] The amount of ethanol used can vary, depending on the crystallization process.

3. STUDENT HANDOUT

3.1. Theoretical Introduction

Aldol Reaction

The Aldol condensation is one of the most important reactions for C-C bond formation and has been a very useful tool in the synthesis of complex natural and synthetic products for pharmaceutical purposes[1].

Carbonyl compounds can enolize in acidic or basic media (see Figure 1). In acidic media, carbonyl compounds can be protonated, and a proton on the alpha carbon can be extracted to produce a double bond. On the other hand, protons on alpha carbons to carbonyl are slightly acidic (pKa ~25), and when a strong base is used that proton can be removed to produce either an enolate as oxoanion or a carbanion with the dual nucleophilic site. When a weak base as NaOH (pKa ~15) is used, only a small portion of enolate is produced, however in the presence of electrophiles, it can react, then equilibrium keto-enolate cause generation of more enolate species. So even the NaOH is not strong enough to remove quantitively the proton from alpha carbon, the reaction is still possible whenever the enolate can find electrophile. The reactivity of nucleophilic sites of enolates is explained in the coursebook – Organic Chemistry by Clayden, Greeves and Warren on page 453 in the second edition[2].

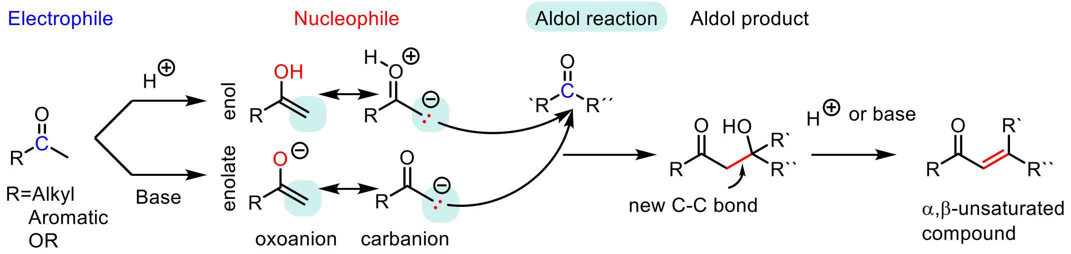


Figure 1. Aldol reaction. Enolization in acidic media to produce enol and in basic media to produce enolate. Both enol and enolate act as nucleophiles and attack another carbonyl to produce a new C-C bond.

An aldol reaction, when the alpha carbon acts as a nucleophile and attacks the carbonyl of the same species, is called auto condensation. When enol or enolate attack another carbonyl species, more electrophilic than themselves, it is called crossed aldol reaction. From a synthetic point of view, the last one is much more useful.

Crossed Aldol Reaction

In order to achieve a successful crossed-aldol reaction, some characteristic must be fulfilled (see Figure 2):

- One of the partners should be enolizable and only one enolate should be possible, in the case of unsymmetrical ketones only one side should be enolizable. In the case of symmetrical ketones, only one enolate is possible.
- The second partner should be more electrophilic than the first pre-enolized partner.

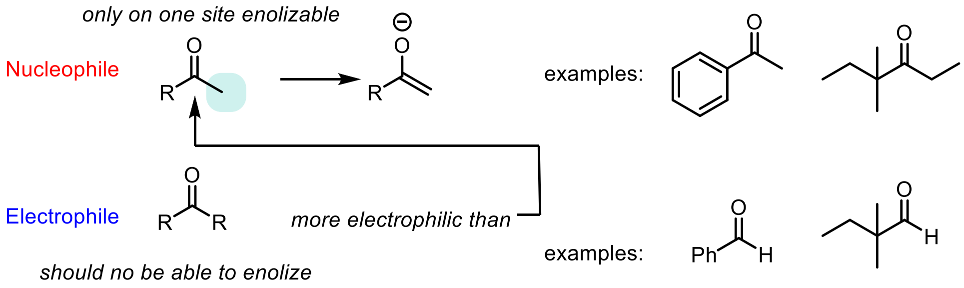


Figure 2. Requirements for nucleophile and electrophile in order to carry out a crossed aldol reaction with a unique product

Vanillin: A natural aldehyde and our electrophile used for the crossed aldol reaction

Vanillin and acetone fulfill the requirements for a crossed aldol condensation. Vanillin can be easily condensed with acetone to give *E*-4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one (also known as dehydrozingerone or vanillideneacetone).

Vanillin can be easily obtained from a natural source by extraction from vanilla plants; however, the compound is also available in drug- and hygiene stores because vanillin is commonly used as a flavoring agent in the food industry. Interestingly, the high demand of the vanillin made it impossible to obtain it in industrial scale by extraction, and other biotechnological protocols are applied to semi-synthesize the vanillin from biomass [3].

Dehydrozingerone or DHZ is a remarkable scaffold comprising of a phenyl ring bearing a methoxy group ortho to the phenolic OH and an α,β-unsaturated carbonyl group with a terminal methyl group. It is present in a natural source as rhizomes of ginger, it also exhibits a wide range of biological activities such as anti-inflammatory, antioxidant, anti-microbial, and cytotoxic activity[4–6].

The synthesis reported in literature has some disadvantages: the reaction is carried out for up to 48h, and the reported yields are poor to moderate, around 50%.[7] In this laboratory practice, you will try to optimize the reaction in terms of time and yield of the reaction. Since using some metal halide salts was previously reported to improve the yield of the aldol reaction[8], you will use lithium chloride and/or nickel chloride (see Figure 3) at different temperatures. The progress will be monitored by TLC. Since acetone is volatile, doesn´t have a chromophore moiety in its structure, and is used in excess, the vanillin consumption will be monitored, and the disappearance of the spot on the TLC will be the sign of the end of the reaction. For that, it is important to separate the starting material (vanillin) and the product (dehydrozingerone). For the procedure to visualize and separate starting material and product, see the experimental section.

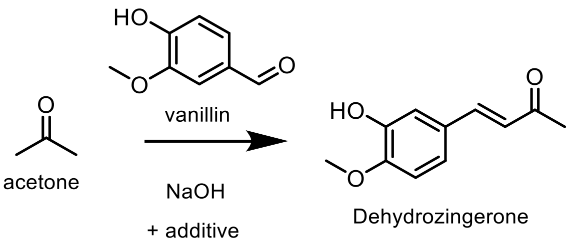


Figure 3. Scheme of reaction using acetone and vanillin.

Each group will be work using a different reaction condition, and the goal at the end of the session is to assess which conditions are the best in terms of reaction ratio, economy, waste production, energetic efficiency and yield.

3.2. Objectives

- Synthesis of biologically active dehydrozingerone by crossed aldol protocol.
- Application of thin layer chromatography in the reaction optimization process.
- Determination of the optimal conditions.
- Introduction of the role of Lewis acids in organic synthesis.

3.3. Experimental Procedure

The instructor first needs to divide the students into six groups, each one working with different conditions (see Table 1). If the laboratory conditions permit, one student per group can be made, and each condition can be studied by two or more groups. In that case, it will be much easier to detect human errors.

Synthesis of Dehydrozingerone

1. In a 50 mL round-bottom flask, add 0.5 g (3.3 mmol) of vanillin then 5 mL (67.5 mmol) of acetone with vigorous stirring. After vanillin has dissolved completely (usually very fast) add 2,63 mL NaOH 2.5 M (the concentration of NaOH is important as using more concentrated NaOH will produce a thick paste mixture which extends reaction time and further complicates the precipitation process). Now add the additive/s (check the table below and workgroup to know which additive to add, perform calculations, and the corresponding time for each reaction). If the reaction is heated, you need to assemble a condenser to the flask to avoid evaporation of acetone.

Table 1. The proposal of different conditions and expected results

Work Group	Additives (Equivalents)	Temperature conditions/°C	Time after vanillin is consumed
1	LiCl (0.1 eq)	Room Temperature	8-10h
2	LiCl (0.1 eq)	40	3h00
3	LiCl (0.1 eq)	50	2h30
4	LiCl (0.5 eq)	40	3h30
5	LiCl (1 eq)	40	3h30
6	LiCl (0.1 eq) + $NiCl_2$ x $6H_2O$ (0.1 eq)	40	2h00

2. In the cases of groups 2 to 6, you need to assemble a water bath with a thermometer to control the temperature. Also, the condenser assembles in a flask to avoid evaporation. (DO NOT forget to control the temperature, if it is too hot, add some ice cubes to quickly achieve the correct temperature). The instructor must demonstrate the assembly of both pieces of equipment.
3. In all reactions, be prepared to do TLC every 30 min (Since none of the reaction finishes before 2h, the first two samples can be done at time 0 and then after 1h or 1h30) in order to control the kinetic progress of the reaction.

   TLC Conditions:

   - (Toluene: AcOEt / 9:1)
   - Visualization in UV-light + Vanillin stain (For vanillin stain visualization, wet the silica plate with the solution, dry and burn it gently by applying heat from hotplate or heat gun.
4. Once the reaction is complete (or if laboratory session is about to finish –consult your instructor) stop it. Is a good idea that all students quench the reaction at least 30 min before the session is finished as is the usual time that they need to precipitate, filter and clean the working area
5. Add drop by drop, 2.5 mL of HCl 6M to the round bottom flask, and cool to room temperature for about 5 min.
6. Assemble a cold bath with ice and place the round bottom flask in it. By using the glass rod, scratch the bottom of the flask to improve the precipitation of the

compound. The precipitate is usually free of starting material, and 5 min is the usual time for the precipitate to start appearing in an ice bath. However, sometimes it needs up to 15 min, in which case the temperature can be lowered using ice and salt to force the precipitation. If there is no precipitation (few cases), in the next session, the student should evaporate acetone and extract the aqueous layer with CH_2Cl_2 or AcOEt.

7. Filter by vacuum and wash with little very cold distilled water several times
8. The yellow crystals will be recrystallized from hot ethanol in a water bath while vigorously stirring. Once precipitated, filter the crystals once again by vacuum.
9. Weight, label, and store the product properly for characterization.

Characterization of Dehydrozingerone

- TLC 9:1 (Toluene/AcOEt).
- Determine the melting point (MP) of the product and compare values reported in the literature (See Entry 5).
- Infrared Spectroscopy (IR). (See Entry 5)
- If available in the teaching laboratory – HPLC, MS spectrometry (see Entry 5) and ^1H NMR

Students should use MP and IR spectroscopy to determine the identity of the product. If available, HPLC/UPLC, mass or NMR spectroscopy can be used to determine the purity of the product. HPLC and FT-IR spectra are attached at the end of this file

3.4. Laboratory Questions

1. Draw the mechanism of the aldol reaction generated in this session.
2. Calculate the reaction yield and compare it with all groups.
3. Collect all the data from all groups. What is your conclusion? Which conditions and additive(s) accomplish the purpose of optimization? The instructor must warn the students that the yield is not the definitive factor to base of the final decision on, as our experience has shown that occasionally yields can be misleading as the quantity

of precipitate is sometimes erratic and depends on students handling abilities as well as the post recrystallization process.

4. How do you think that LiCl or $NiCl_2$ improves the reaction ratio? What is a Lewis acid?
5. Describe the product obtained by IR spectroscopy and compare it with literature (ask the instructor for a copy of IR spectra or check the database: https://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi). What difference can you observe regarding the starting material?

3.5. Laboratory Answers

1. The mechanism is shown as follow:

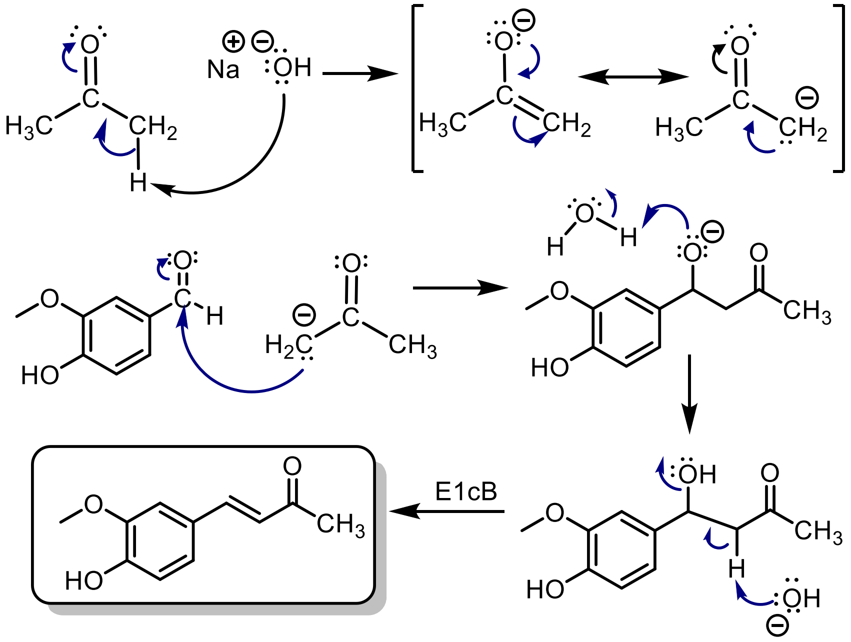


Figure 4. Mechanism of crossed aldol reaction for vanillin.

1. $Reaction\ yield = \frac{Experimental\ mass\ of\ DHZ}{Theoretical\ mass\ of\ DHZ} x\ 100$
2. The best condition is using LiCl (0.1 eq) with $NiCl_2 \cdot 6H2O$ (0.1 eq) at 40 °C or just LiCl (0.1 eq) at 50 °C where the time of full conversion is shortened from 24 h as reported in the literature to 2 h and 2.30 h, respectively. The first mentioned condition requires additional reagents, and even at a 10% molar ratio, it will still

result as waste, so lithium chloride alone as an additive only requires 0.5 h longer heating, but the reaction is greener.

3. The lithium cation has a great affinity to the oxygen. When enolate-ketone is established with a weak base as NaOH only a small portion of enolate can be observed, which can act as a nucleophile. However, the presence of lithium cations probably favors the generation of more enolates, which accelerates the reaction. On the other hand, the presence of metals such as nickel interacts with the carbonyl oxygen increasing the electrophilicity of the aldehyde. In the Lewis theory of acid-base reactions, acids accept pairs of electrons. A Lewis acid is, therefore, any substance, such as the H+ ion, that can accept a pair of nonbonding electrons. In other words, a Lewis acid is an electron-pair acceptor.
4. The students must identify at least the next IR bands values in the spectrum: strong C=O stretching for a conjugated ketone at 1685-1666 cm^{-1}. C=C stretching for an alkene at 1662-1626 cm^{-1}. O-H stretching for hydroxyl (phenolic) at 3550-3200 cm^{-1} with a strong appearance. C=C bending for alkene at 730-665 cm^{-1} with a strong appearance. The differences in spectra for starting material and final product are C=O stretching for ketone at 1685-1666 cm^{-1} and C=O stretching for aldehyde at 1710-1665 cm^{-1} for dehydrozingerone and vanillin, respectively. Also, C-H stretching for aldehyde at 2850-2695 cm^{-1} and C-O stretching for ether at 1000-1300 cm^{-1}; 1070-1150 cm^{-1} appears only in the vanillin IR spectrum.

4. ECONOMIC ANALYSIS OF EXPERIMENTAL CLASS

Chemicals	**Amount per semester**[a]	**Source**	**Sigma Aldrich Code**	**Cost ($)**	**Cost ($) by group**
Vanillin ($C_8H_8O_3$)	3 g	Drugstore-Ibarra	NA	15$ - 300g	0.15 $
Sodium Hidroxide	15.78 mL (2.5M) *	Local Chemical	NA	17$ - 500mL	0.26 $

(NaOH-5M)		SupplierIbarra (M&M)			
Acetone (C_3H_6O)	30 mL	Sigma-Aldrich	8222511000	26$ - 1L	0.78 $
Hydrochloric Acid (HCl)	15 mL	Sigma-Aldrich	320331	81$ - 500mL	2.43 $
Lithium Chloride (LiCl)	0.30 g	Sigma-Aldrich	73036	82$ - 100g	0.24 $
Niquel (II) Chloride Hexahydrate ($NiCl_2*6H_2O$)	0.05 g	Sigma-Aldrich	223387	70$ - 25g	0.14 $
Toluene (C_7H_8)	9 mL	Sigma-Aldrich	1083231000	39.5$ - 1L	0.35 $
Ethanol (96%) (C_2H_6OH)	30 mL	Drugstore-Ibarra	NA	9$ - 1gallon	0.07 $
Ethyl Acetate ($C_4H_8O_2$)	1 mL	Sigma-Aldrich	8222772500	61$ -2.5L	0.024 $
Total cost by semester	4.44$				
Cost by group	0.74 $				

[a] The amount of chemicals is approximated for 6 groups in a laboratory practice over one semester.

* 7.89 mL of 5M solution of NaOH are needed to prepare the 15.78 mL 2.5M of NaOH.

5. CHARACTERIZATION OF STARTING MATERIAL AND FINAL PRODUCT

5.1. Melting point

Reported[9]

Vanillin: 81°C – 83°C

Dehydrozingerone: 127°C – 131°C

Experimental

Vanillin: 81°C

Dehydrozingerone: 129°C-130°C

5.2. Infrared Spectroscopy (IR)

Vanillin

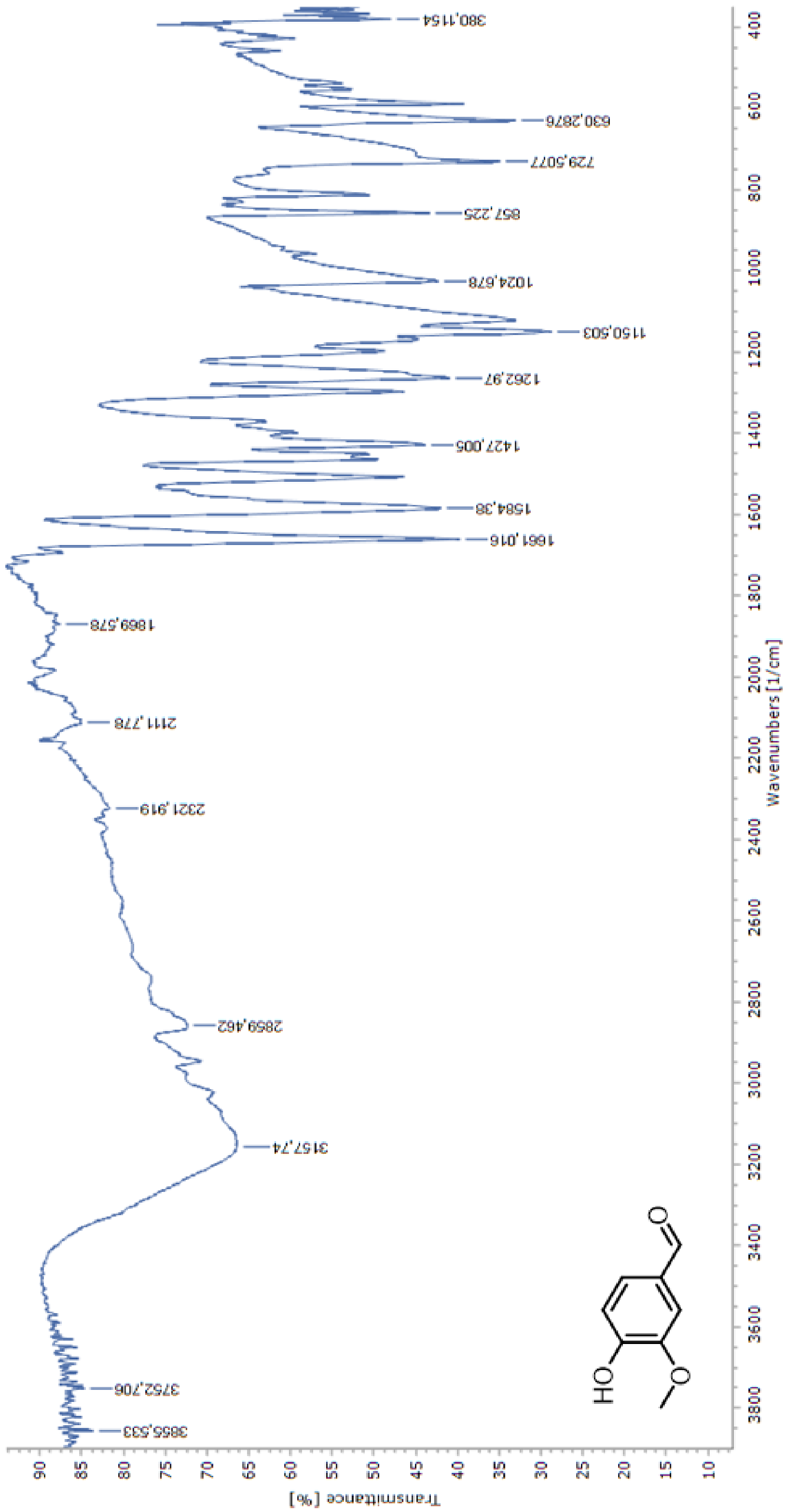


Fig. 4. IR spectrum (ATR sampling technique) of starting material (Vanillin) for crossed aldol reaction.

Dehydrozingerone

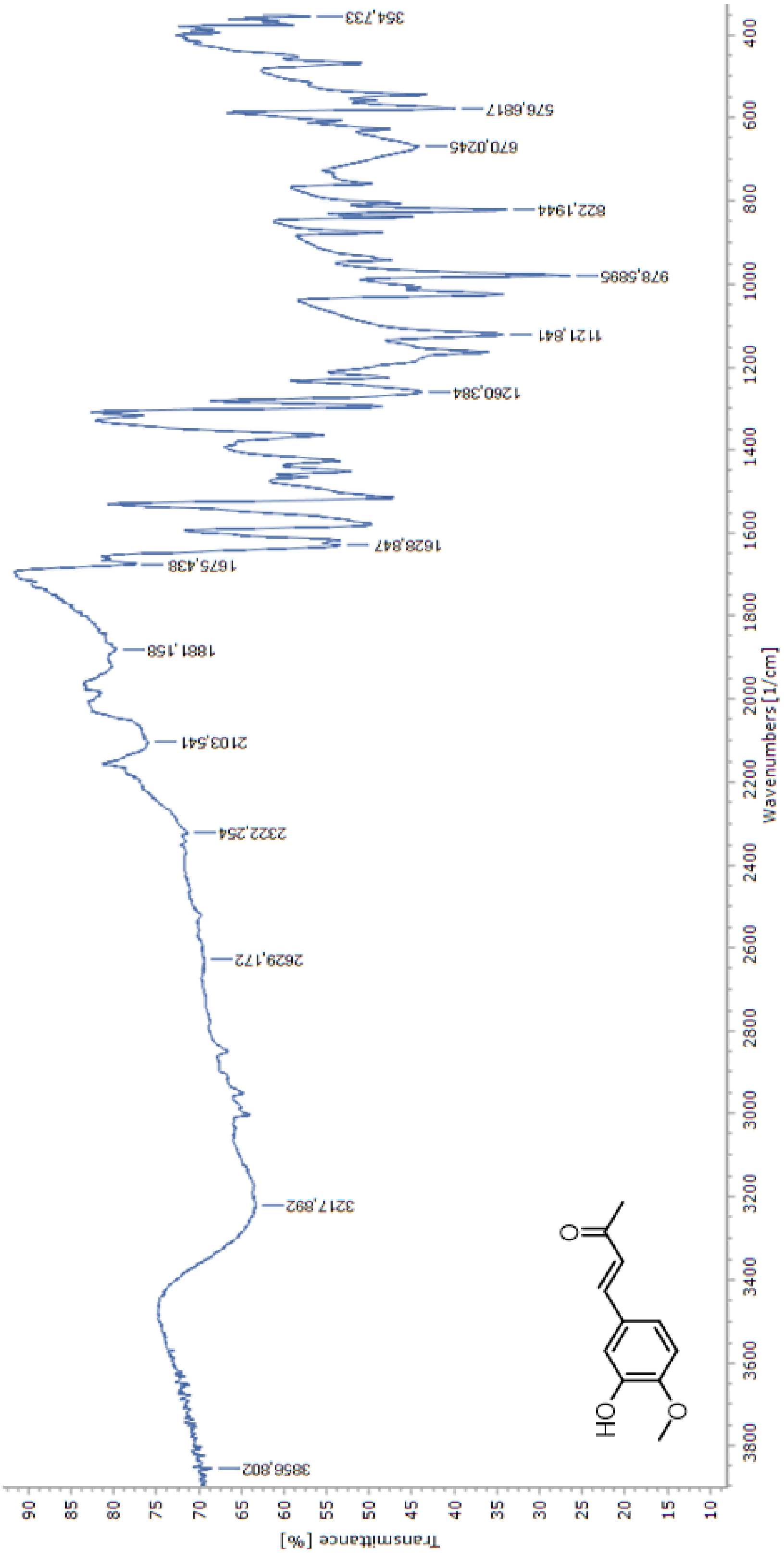
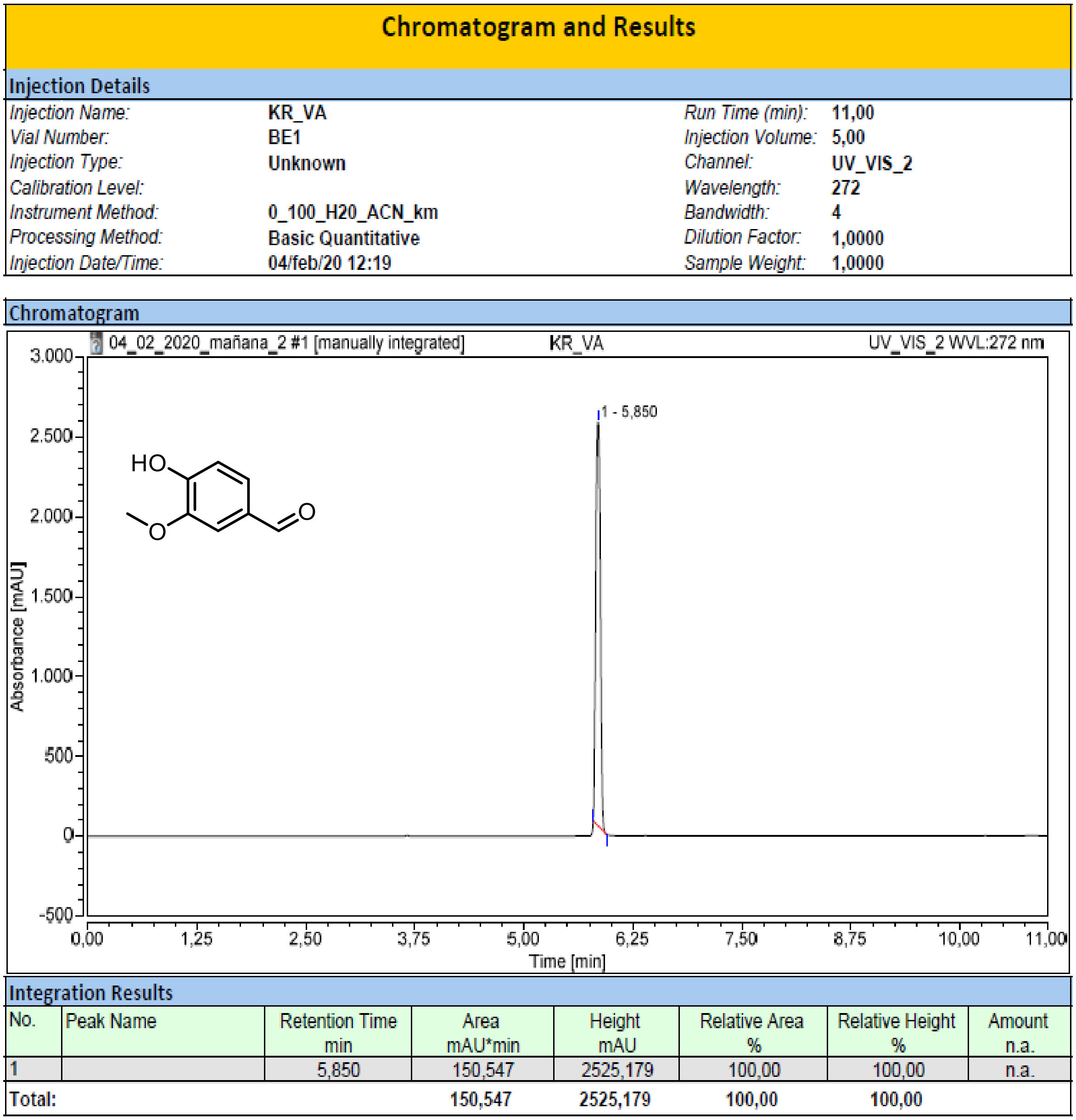


Fig. 5. IR spectrum (ATR sampling technique) of reaction product (Dehydrozingerone) from crossed aldol reaction of vanillin.

5.3. High Performance Liquid Chromatography (HPLC)

The analytical method was developed by using HPLC apparatus UltiMate 3000, C-18 column for HPLC Hypersil GOLD™ (150 mm x 4.6 mm, 5µ particle size). The instrument method used was 0-100 H_2O and Acetonitrile gradient.

Vanillin



Chromatogram and Results

Injection Details

Injection Name:	KR_VA	*Run Time (min):*	11,00
Vial Number:	BE1	*Injection Volume:*	5,00
Injection Type:	Unknown	*Channel:*	UV_VIS_2
Calibration Level:		*Wavelength:*	272
Instrument Method:	0_100_H20_ACN_km	*Bandwidth:*	4
Processing Method:	Basic Quantitative	*Dilution Factor:*	1,0000
Injection Date/Time:	04/feb/20 12:19	*Sample Weight:*	1,0000

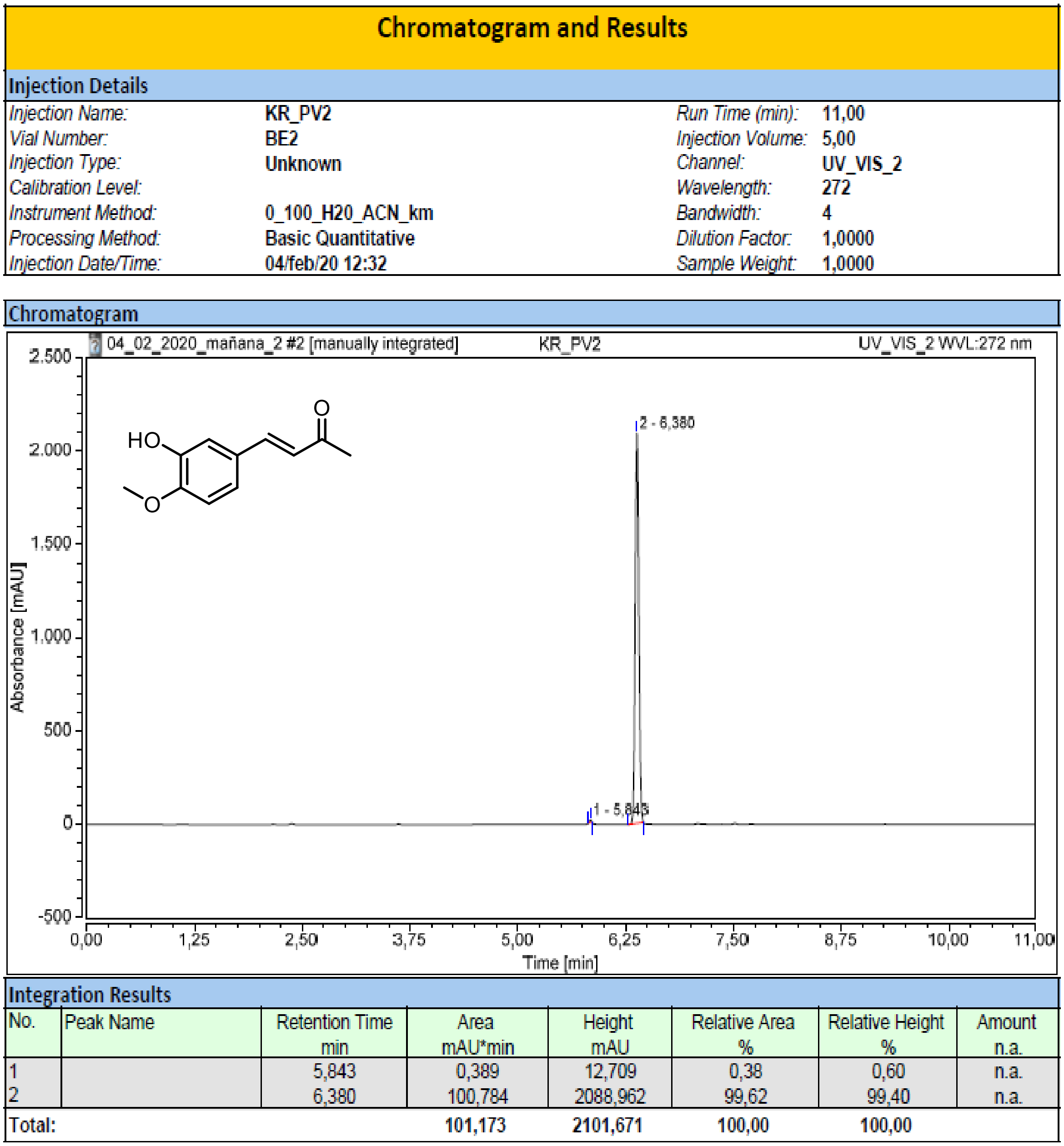
Chromatogram

Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		5,850	150,547	2525,179	100,00	100,00	n.a.
Total:			**150,547**	**2525,179**	**100,00**	**100,00**	

Fig. 6. HPLC Chromatogram starting material (Vanillin) for crossed aldol reaction.

Dehydrozingerone



Chromatogram and Results

Injection Details

Injection Name:	KR_PV2	*Run Time (min):*	11,00
Vial Number:	BE2	*Injection Volume:*	5,00
Injection Type:	Unknown	*Channel:*	UV_VIS_2
Calibration Level:		*Wavelength:*	272
Instrument Method:	0_100_H20_ACN_km	*Bandwidth:*	4
Processing Method:	Basic Quantitative	*Dilution Factor:*	1,0000
Injection Date/Time:	04/feb/20 12:32	*Sample Weight:*	1,0000

Chromatogram

Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		5,843	0,389	12,709	0,38	0,60	n.a.
2		6,380	100,784	2088,962	99,62	99,40	n.a.
Total:			101,173	2101,671	100,00	100,00	

Fig. 7. HPLC Chromatogram reaction product (Dehydrozingerone) from crossed aldol reaction of vanillin.

5.4. Mass Spectrometry (MS)

ESI/MS spectra were obtained using nitrogen as the collision gas within a mass range of m/z 100–800. MS parameters were as follows: the cone and capillary voltages were set at 20 kV and 2,5 kV, respectively, the source temperature was 80°C, and desolvation flow was 600 (L/hr). The analytical method was developed using an Acquity BEH C-18 column (2.1 × 100 mm, 1.7 µm) (Waters).

ESI+ $[M+H]^+$ calculated 193.0786 and found 193.1237

6. REFERENCES

(1) Ferreira, M.; Dias, L.; Leonarczyk, I.; Polo, E.; de Lucca, E. Exploring the Aldol Reaction in the Synthesis of Bioactive Compounds. *Curr. Org. Synth.* **2015**. https://doi.org/10.2174/1570179412051508211249O1.

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2.2.2. Cinnamaldehyde Reaction

Practice

Two Steps Procedure for Crossed Aldol Reaction: Extraction of Cinnamaldehyde and Cinnamylidene Acetone Synthesis

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SUPPORTING MATERIAL

Contents

NOTE: The instructions in RED color are for laboratory instructor only!

1. LIST OF CHEMICALS AND HAZARDS

First Session				
Chemicals	**CAS No.**	**Labelled as**	**GHS Hazard Statements[1]**	**Hazard Pictograms**
Cinnamon Bark	NA	Cinnamon	H316, H319	
Sodium Chloride Solution (NaCl)	7647-14-5	Saturated NaCl Solution	H303, H316	
Chloroform ($CHCl_3$)	67-66-3	Chloroform	H302, H315, H319, H331, H351, H361, H372	  
Magnesium Sulfate ($MgSO_4$)	7487-88-9	Anhydrous $MgSO_4$	H302, H312, H332	
Distilled water (H_2O)	7732-18-5	Distilled H_2O	Not Hazardous	None
Second Session				
Cinnamaldehyde (C_9H_8O)	104-55-2	Extracted C_9H_8O	H312, H315, H317	
Sodium Hidroxide (NaOH)	1310-73-2	NaOH 3 M	H314	

Acetone (C_3H_6O)	67-64-1	Acetone	H225, H319, H336	
Toluene (C_7H_8)	108-88-3	Toluene	H225, H304, H315, H336, H361, H373, H402	
Ethanol (C_2H_6OH)	64-17-5	EtOH	H225	
Ethyl Acetate ($C_4H_8O_2$)	141-78-6	EtOAc	H225, H319, H336	
Distilled water (H_2O)	7732-18-5	Distilled H_2O	Not Hazardous	None

[1]The meaning of each Hazard Statement is explained in the section 1.1, shown below.

1.1. Definition of GHS Hazard Statements

The GHS hazard statements of reagents used in this practice are defined as follow:

Physical Hazards

H225 - Highly flammable liquid and vapor.

Health Hazards

H302 Harmful if swallowed.

H303 May be harmful if swallowed.

H304 May be fatal if swallowed and enters airways.

H312 Harmful in contact with skin.

H314 Causes severe skin burns and eye damage.

H315 Causes skin irritation.

H316 Causes mild skin irritation.

H317 May cause an allergic skin reaction.

H319 Causes serious eye irritation.

H331 Toxic if inhaled.

H332 Harmful if inhaled.

H336 May cause drowsiness or dizziness.

H351 Suspected of causing cancer.

H361 Suspected of damaging fertility or the unborn child.

H372 Causes damage to organs through prolonged or repeated exposure.

H373 May cause damage to organs (central nervous system, liver, heart) through prolonged or repeated exposure.

Environmental Hazards

H402 Harmful to aquatic life.

2. EQUIPMENT AND MATERIALS

- 250 mL and 100 mL round bottom flasks
- 50 mL beaker
- 250 mL separator funnel
- Magnetic stirrer
- Heat plate
- Büchner flask
- Filter paper
- Spatula
- Büchner funnel
- Plastic funnel
- Cinnamon
- Cotton
- Crystallizer
- Glass rod
- Capillaries
- Analytical balance
- Distillation apparatus
- TLC silica plates
- Chamber for TLC
- UV-Vis Lamp
- Heat gun
- Rotary evaporator

2.1. List of quantities of reagents

Reagents	Molecular Formula	Molecular Weight	Quantities

Cinnamon	NA	NA	30 g
Sodium Chloride Saturated Solution	$NaCl$	58.44 g/mol	~30 mL[1]
Magnesium Sulfate anhydrous	$MgSO_4$	120.37 g/mol	1 spoon (~3 g)
Acetone	C_3H_6O	58.08 g/mol	0.1113 g
Sodium Hidroxide (3M)	$NaOH$	40.00 g/mol	4 mL
Cinnamaldehyde	C_9H_8O	132.16 g/mol	0.2647 g
Chloroform	$CHCl_3$	119.38 g/mol	45 mL[1]
Toluene	C_7H_8	92.14 g/mol	9 mL
Ethanol	C_2H_6OH	46.07 g/mol	12 mL[2]
Ethyl Acetate	$C_4H_8O_2$	88.11 g/mol	1 mL
Distilled water	H_2O	18.018 g/mol	150 mL

1 These amounts can vary depending on the amount of organic phase obtained. Students must check this in section 3.3.

2 This amount may vary depending on the crystallization process.

3. STUDENT HANDOUT

3.1. Theoretical Introduction

Steam Distillation

Distillation is a method used in chemistry laboratories mainly to separate mixtures based on differences in their boiling points. For instance, if we have a mixture of water (bp. 100 °C at 1 atm) and acetone (56 °C at 1 atm), the first component which will change the phase will be acetone and first to condensate. In this practice, the distillation equipment will be used for another purpose – steam distillation. In this case, the boiling water produces steam, which will work as a carrier of other volatiles, which will be recollected in a condenser. The non-volatile residue remains in the flask. In the first part of this experiment, we will carry out the steam distillation of cinnamaldehyde from cinnamon barks.

Aldol Reaction

Aldol condensation is one of the most important reactions for C-C bond formation and has been a very useful tool in the synthesis of complex natural and synthetic products for pharmaceutical purpose[1].

Carbonyl compounds can enolize in acidic or basic media (see Figure 1). In acidic media, carbonyl compound can be protonated, and proton on alpha-carbon can be extracted to produce a double bond. On the other hand, protons on alpha carbons to carbonyl are slightly acidic (pKa ~25), and when a strong base is used, those protons can be removed to produce either enolates as oxoanions or carbanions with a dual nucleophilic site. When a weak base as NaOH (pKa ~15) is used, only a small portion of enolate is produced, however in the presence of electrophiles, it can react, then equilibrium keto-enolate cause generation of more enolate species. So even the NaOH is not strong enough to remove quantitively the proton from alpha carbon, the reaction is still possible whenever the enolate can find electrophile. The reactivity of nucleophilic sites of enolates is explained in the course book – Organic Chemistry by Clayden, Greeves, and Warren on page 453 in the second edition[2].

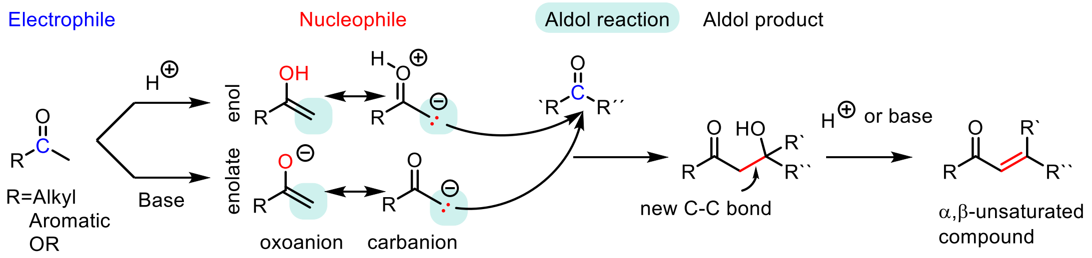


Figure 1. Formation of a new C-C bond: Enolization of carbonyl compound in acidic and basic media to form nucleophiles in order to achieve an aldol product.

An aldol reaction, when the alpha carbon acts as a nucleophile and attacks the carbonyl of the same species, it is called auto condensation. When it attacks a more reactive electrophilic species, it is called crossed aldol reaction. The last one synthetically is much more useful.

Crossed Aldol Reaction

In order to achieve a successful crossed aldol reaction, some requirements must be fulfilled (see Figure 2):

- One of the partners should be enolizable, and only one enolate should be possible. In the case of unsymmetrical ketones only one side should be enolizable. In the case of symmetrical ketones, only one enolate is possible.
- The other reaction partner should be more electrophilic than the first pre-enolized reaction partner.

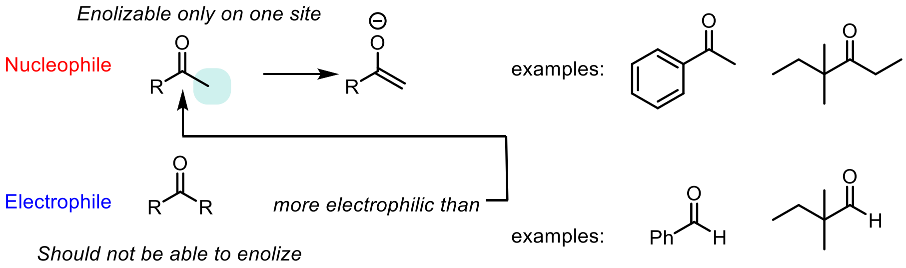


Figure 2. Requirements for nucleophile and electrophile in order to carry out crossed aldol reaction.

Cinnamaldehyde: A natural aldehyde as an electrophile in crossed aldol condensation

Cinnamaldehyde and acetone fulfill both requirements mentioned before for crossed aldol condensation. Cinnamaldehyde can be easily condensed with acetone to give (3E, 5E)-6-phenylhexa-3,5-dien-2-one (also known as cinnamylidene acetone). Cinnamaldehyde is a yellow oil derived from cinnamon trees and other species of the genus Cinnamomum[3]. It can be easily extracted from cinnamon bark by steam distillation and liquid-liquid extraction. It possesses several pharmacological properties as anti-microbial anticancer, antioxidative, antiobesity and anti-inflammatory[4,5] additionally has a nice smell and is very pleasant to work with.

Cinnamylidene acetone can be prepared through a single step base promoted aldol type condensation between acetone and cinnamaldehyde, which were taken in 1:1 mole ratio[6]. In this laboratory session, we will carry out a steam distillation using simple distillation equipment and cinnamon barks to extract cinnamaldehyde. In the following session, we will use the extracted natural aldehyde to reproduce the conditions of a crossed aldol reaction from literature[6] (see Figure 3).

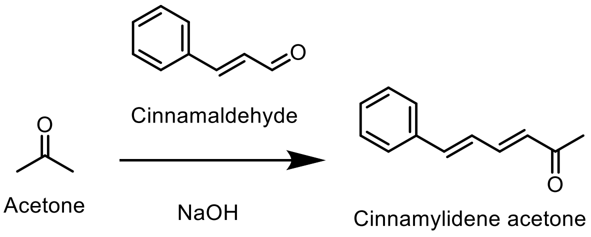


Figure 3. Scheme of reagents and product to achieve crossed aldol reaction for cinnamaldehyde.

3.2. Objectives

- Extract cinnamaldehyde from a natural source with sufficient quantity for the next step.
- Cinnamylidene acetone synthesis by crossed aldol reaction.

3.3. Experimental Procedure

First Session: Extraction of cinnamaldehyde

1. In a 500 mL round bottom flask from distillation apparatus, add 30 g of cinnamon sticks then 150 mL of distilled water. After 2 hours of distillation, make sure that there is not more distilled water in the flask and stop the distillation. (The instructor should recommend that for an efficient cinnamaldehyde extraction, cinnamon should be flaked into small pieces in a mortar. However, it should not pulverize as this makes foam when boiled with water and the mixture can pass to collecting the flask.)
2. The cooled milky mixture should be transposed to the separatory funnel followed by addition of 15 mL of chloroform (AcOEt or CH_2Cl_2 can be used as well) in order to extract the cinnamaldehyde to the organic phase. This process is repeated 3 times.
3. The collected organic phases are washed with a saturated solution of NaCl. (The students need to add an amount of saturated NaCl-solution equivalent to a about the third part of the organic phase total amount)
4. Separate the organic phase. Then add one spoon of $MgSO_4$ to the beaker containing the organic phase.
5. Filter the content of the beaker with cotton into a 100 mL round bottom flask.

6. Evaporate the organic phase under reduced pressure. After approximately 15 min, take a sample of cinnamaldehyde for TLC analysis.
7. Collect the final product (cinnamaldehyde oil) in a vial. Label and store appropriately in the freezer for the next laboratory session.

Second Session: Synthesis of Cinnamylidene Acetone

The instructor should recommend cooling the ethanol as it will be used for washing of the product and it is important that it is cooled.

1. In a 50 mL beaker, add 0.265 g (2.0 mmol) of cinnamaldehyde oil and 4 mL of ethanol followed by 0,111 g (2.0 mmol) of acetone under magnetic stirring.
2. Add 4 mL of NaOH 3M drop by drop, with constant magnetic stirring.
3. After approximately1 min, yellow product precipitates. Let the mixture stir for 20 min to complete the reaction. (Usually, the precipitate appears within 30s to 120s after NaOH has been added.)
4. Prepare a 10 mL mixture of ethanol and water in a 1:2 ratio, and add 5 mL to the flask containing the precipitate. Add the remaining 5 mL of the mixture and stop the reaction. (The students must observe a change in color from red to yellow in the solution)
5. Filter the mixture by vacuum and wash the precipitate with cold ethanol. Then dry and recrystallize the product from hot ethanol.
6. Weight, label, and store the product properly for characterization.

Characterization of Cinnamylidene Acetone

- TLC 97:3 (Toluene/AcOEt).
- Determine the melting point (MP) of the product and compare values reported in the literature (See Entry 5).
- Characterize the product by Infrared Spectroscopy (IR). (See Entry 5)
- If available in the teaching laboratory – HPLC, MS spectrometry (see Entry 5), and ^{1}H NMR is recommended.

Students should use MP and IR spectroscopy to identify the product. If available HPLC/UPLC, mass or NMR spectroscopy can be used to determine the purity of the product. The HPLC and FT-IR spectra are attached at the end of this file.

3.4. Laboratory Questions

1. Draw the mechanism of the aldol reaction carried out in this experiment.
2. Investigate 3 of the biological effects of cinnamaldehyde and describe briefly one of them.
3. Calculate the reaction yield and compare it with your partners.
4. Describe the product obtained by IR spectroscopy and compare it with the literature (ask the instructor for a copy of IR spectra or check the database: https://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi). What difference can you observe regarding starting material?

3.5. Laboratory Answers

1. The mechanism is shown as follow:

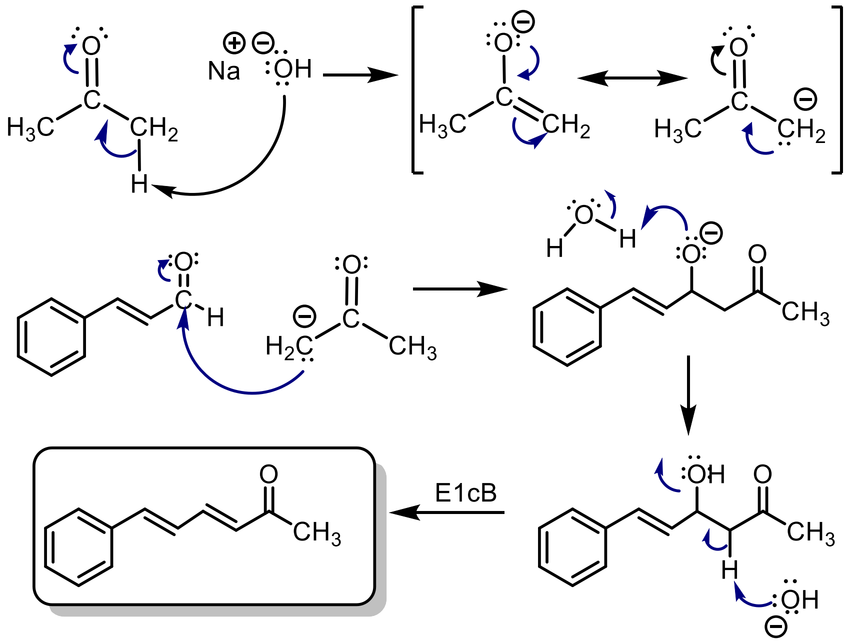


Figure 4. Mechanism of crossed aldol reaction for cinnamaldehyde.

2. Anti-oxidative. Anti-inflammatory. Anti-microbial: cinnamaldehyde prevents oral bacterial growth by more than 50%. This compound is very effective against bacteria

living at the back of the tongue, reducing anaerobic bacteria populations by about 43%[7].

3. $Reaction\ yield = \frac{Experimental\ mass\ of\ Cinnamylidene\ acetone}{Theoretical\ mass\ of\ Cinnamylidene\ acetone} x\ 100$

4. The students must identify at least the next IR bands values in the spectrum: strong C=O stretching signal for a conjugated ketone at 1685-1666 cm^{-1}. C=C stretching for an alkene at 1662-1626 cm^{-1}. C=C bending for the aromatic ring at 1700 – 1500 cm^{-1}. C-H stretching for the aromatic ring at 3100 – 3000 cm^{-}1. The differences in the spectra of starting material and final product are C=O stretching for ketone at 1685-1666 cm^{-1} and C=O stretching for aldehyde at 1710-1665 cm^{-1} for cinnamylidene acetone and cinnamaldehyde, respectively. Besides, C-H stretching for aldehydes at 2830-2695 cm^{-1} appears only in the cinnamaldehyde IR spectrum.

7. ECONOMIC ANALYSIS OF EXPERIMENTAL CLASS

Chemicals* / Materials	**Amount for semester**[a]	**Source**	**Sigma Aldrich Code**	**Cost ($)**	**Cost ($) by group**
Cinnamon bark	180 g	Supermarket	NA	8$ - 300g	4.80 $
Sodium Hidroxide (NaOH-5M)	24 mL[b]	Local Chemical Supplier	NA	17$ - 500mL	0,40 $
Acetone (C_3H_6O)	0,84 mL	Sigma-Aldrich	8222511000	26$ - 1L	0,02 $
Chloroform ($CHCl_3$)	270 mL	Sigma-Aldrich	1024311000	55$ - 1L	14,85 $
Magnesium Sulfate ($MgSO_4$)	3 g	Sigma-Aldrich	MX0075	89$ - 500g	0,53 $
Toluene (C_7H_8)	9 mL	Sigma-Aldrich	1083231000	39.5$ - 1L	0.35 $
Ethanol (C_2H_6OH)	72 mL	Local Drugstore-	NA	9$ - 1gallon	0.07 $
Ethyl Acetate ($C_4H_8O_2$)	1 mL	Sigma-Aldrich	8222772500	61$ -2.5L	0.024 $
Total cost by semester		21,04 $			
Cost by group		3.50 $			

[a]The amount of chemicals is estimated for 6 groups in a laboratory practice over one semester.
*The saturated solution of NaCl is not included in the total cost because it can be prepared in the laboratory with distilled water and table salt (NaCl).
b 14.4 mL of 5M solution of NaOH are needed to prepare the 24 mL 3M of NaOH.

5. CHARACTERIZATION OF STARTING MATERIAL AND FINAL PRODUCT

5.1. Melting point

Literature[8] (Cinnamaldehyde is oil, then students evaluate freezing instead melting point)

Cinnamaldehyde: -8 °

Cinnamylidene Acetone: 68°C – 70°C

Experimental in our hands

Cinnamaldehyde: -7°C

Cinnamylidene Acetone: 68°C

5.2. Infrared Spectroscopy (IR)

Cinnamaldehyde

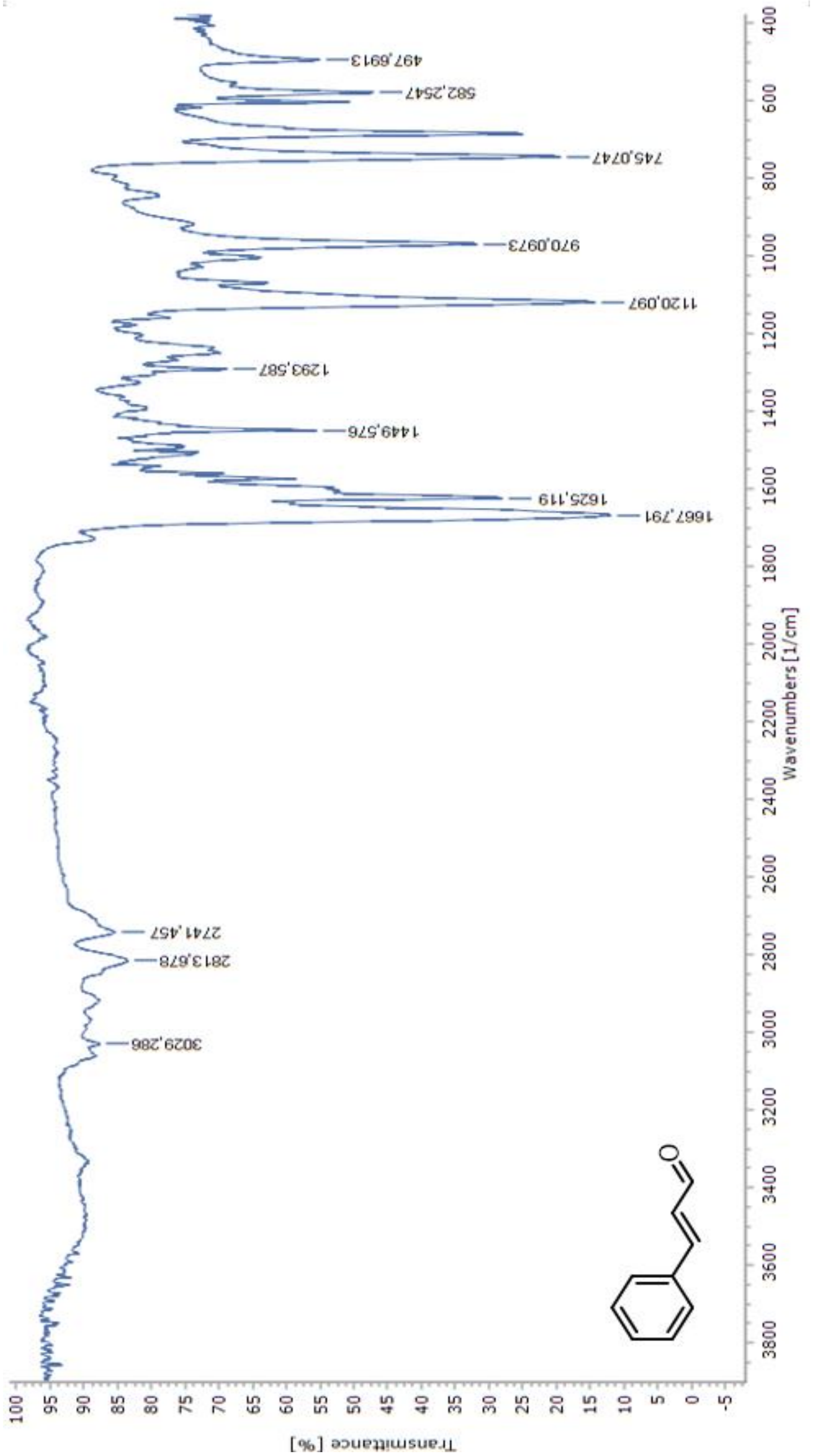


Fig. 4. IR spectrum (ATR sampling technique) of starting material (Cinnamaldehyde) for crossed aldol reaction.

Cinnamylidene acetone

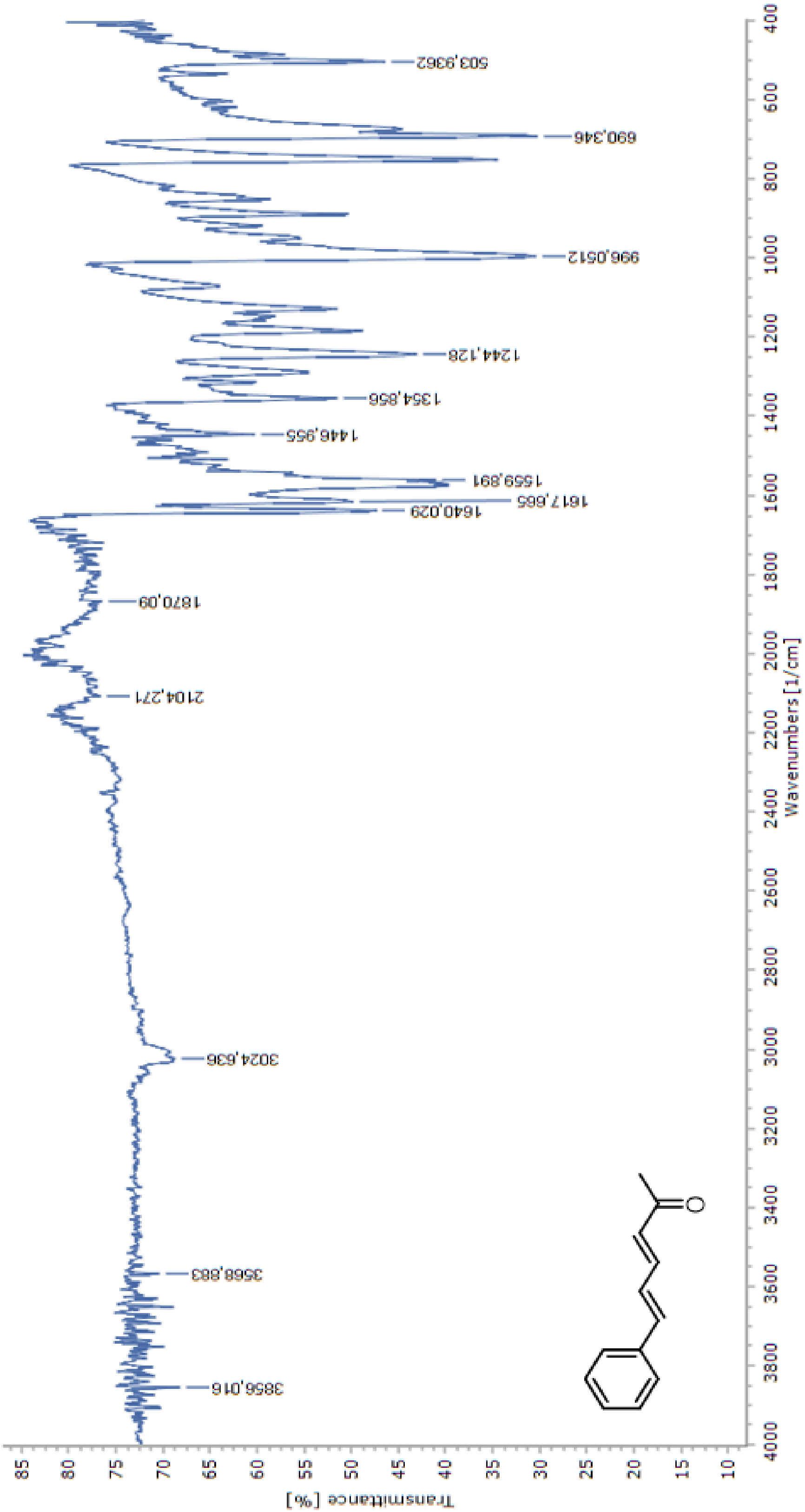
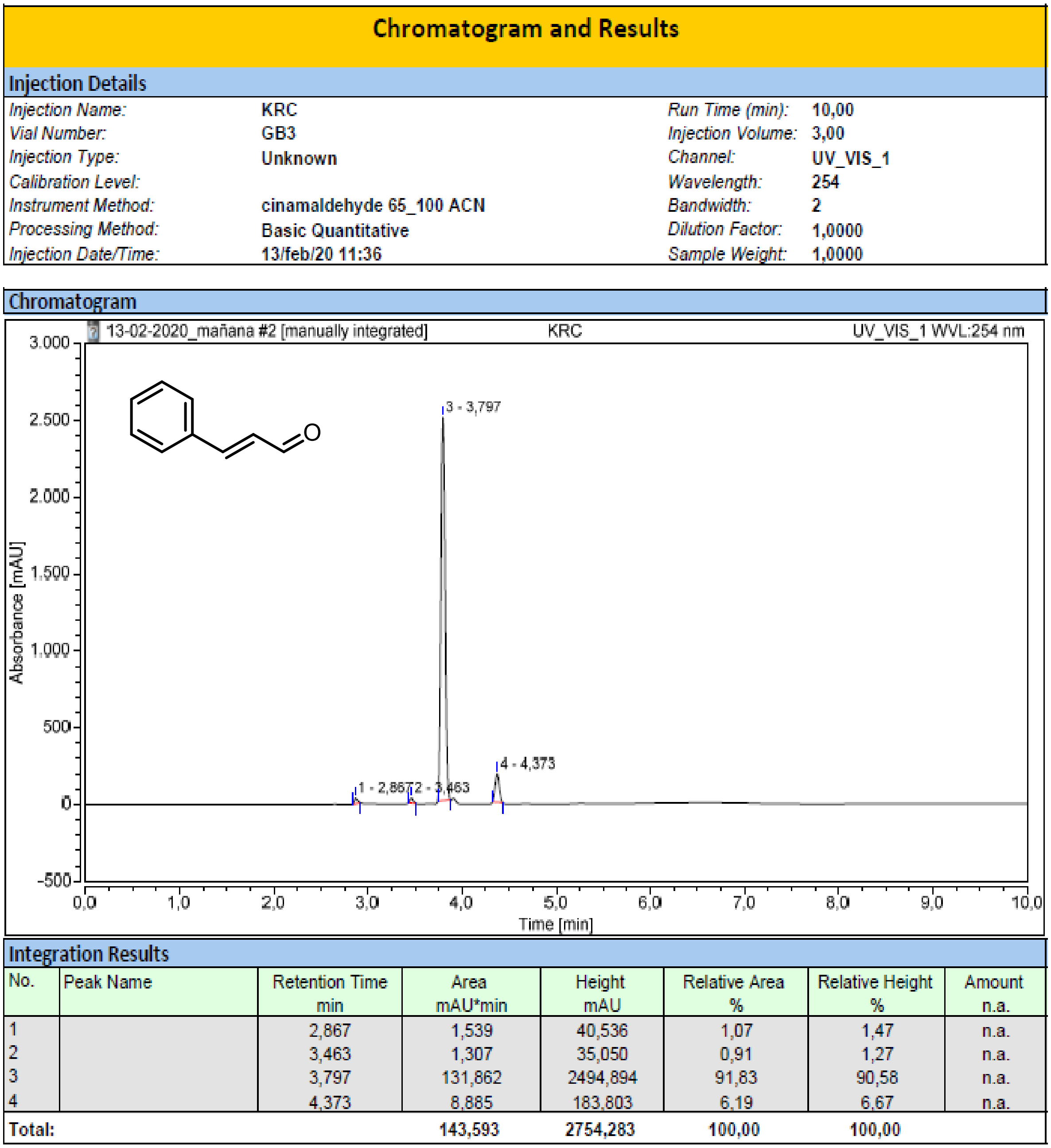


Fig. 5. IR spectrum (ATR sampling technique) of reaction product (Cinnamylidene acetone) from crossed aldol reaction.

5.3. High Performance Liquid Chromatography (HPLC)

HPLC apparatus UltiMate 3000, C-18 column for HPLC Hypersil GOLD™ (150 mm x 4.6 mm, 5µ particle size). The instrument method used was 65-100 H_20 and ACN gradient for cinnamaldehyde and 80-100 H_20 and ACN gradient for cinnamylidene acetone.

Cinnamaldehyde



Chromatogram and Results

Injection Details

Injection Name:	KRC	*Run Time (min):*	10,00
Vial Number:	GB3	*Injection Volume:*	3,00
Injection Type:	Unknown	*Channel:*	UV_VIS_1
Calibration Level:		*Wavelength:*	254
Instrument Method:	cinamaldehyde 65_100 ACN	*Bandwidth:*	2
Processing Method:	Basic Quantitative	*Dilution Factor:*	1,0000
Injection Date/Time:	13/feb/20 11:36	*Sample Weight:*	1,0000

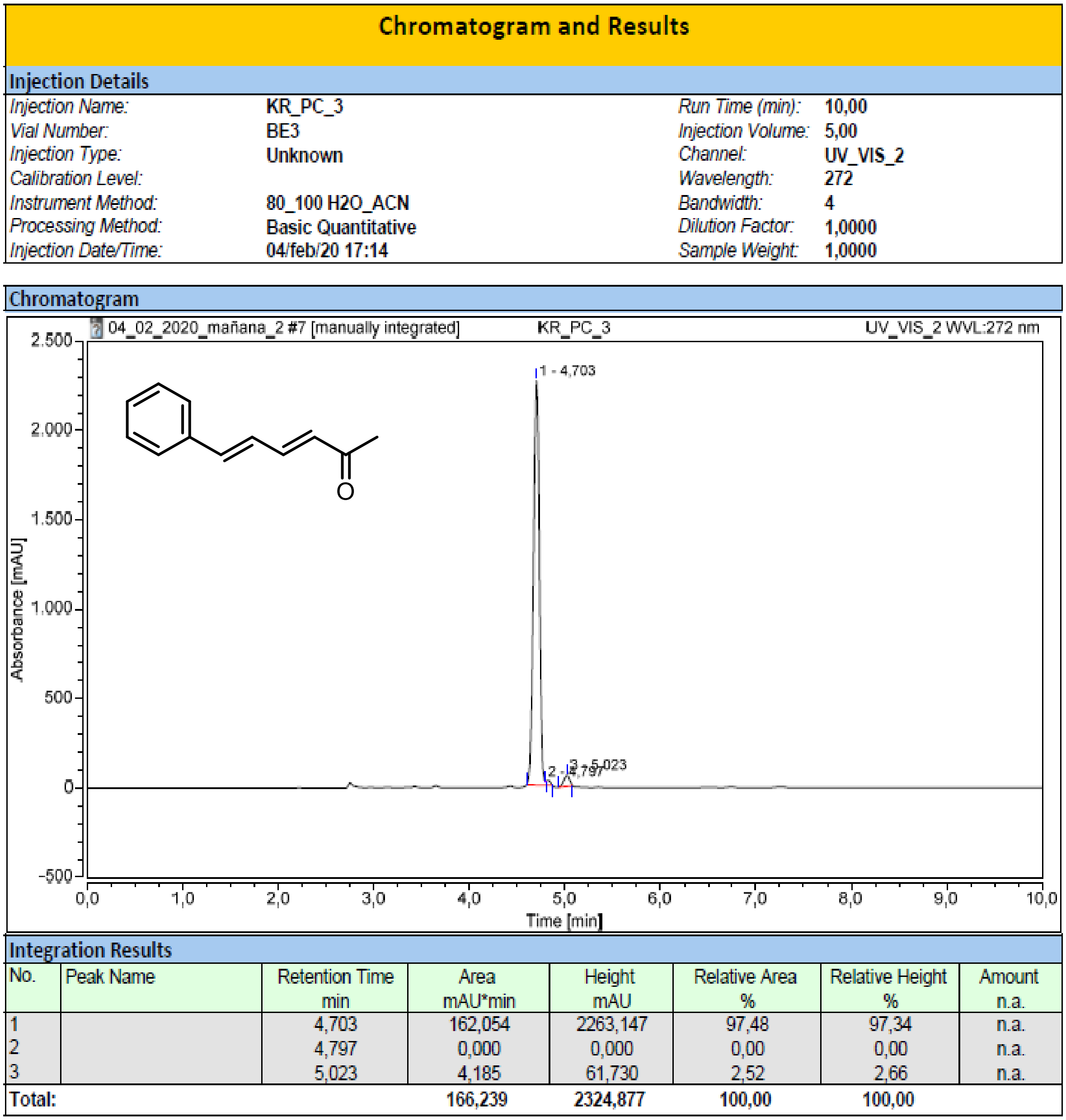
Chromatogram

Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		2,867	1,539	40,536	1,07	1,47	n.a.
2		3,463	1,307	35,050	0,91	1,27	n.a.
3		3,797	131,862	2494,894	91,83	90,58	n.a.
4		4,373	8,885	183,803	6,19	6,67	n.a.
Total:			**143,593**	**2754,283**	**100,00**	**100,00**	

Fig. 6. HPLC Chromatogram starting material (Cinnamaldehyde) for crossed aldol reaction.

Cinnamylidene acetone



Chromatogram and Results

Injection Details

Injection Name:	KR_PC_3	*Run Time (min):*	10,00
Vial Number:	BE3	*Injection Volume:*	5,00
Injection Type:	Unknown	*Channel:*	UV_VIS_2
Calibration Level:		*Wavelength:*	272
Instrument Method:	80_100 H2O_ACN	*Bandwidth:*	4
Processing Method:	Basic Quantitative	*Dilution Factor:*	1,0000
Injection Date/Time:	04/feb/20 17:14	*Sample Weight:*	1,0000

Chromatogram

Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		4,703	162,054	2263,147	97,48	97,34	n.a.
2		4,797	0,000	0,000	0,00	0,00	n.a.
3		5,023	4,185	61,730	2,52	2,66	n.a.
Total:			**166,239**	**2324,877**	**100,00**	**100,00**	

Fig. 7. HPLC Chromatogram reaction product (Cinnamylidene acetone) from crossed aldol reaction.

5.4. Mass Spectrometry

ESI/MS spectra were obtained using nitrogen as the collision gas within a mass range of m/z 100–800. MS parameters were as follows: the cone and capillary voltages were set at 20 kV and 2,5 kV, respectively, the source temperature was 80°C, and desolvation flow was

600 (L/hr). The analytical method was developed using an Acquity BEH C-18 column (2.1 × 100 mm, 1.7 µm) (Waters).

Cinnamaldehyde

ESI+$[M+H]^+$ calculated: 133.0575, found: 133.0608

Cinnamylidene acetone

ESI+$[M+H]^+$ calculated: 173.0888, found: 173.0802

6. REFERENCES

(1) Ferreira, M. A. B.; Dias, L. C.; Leonarczyk, I. A.; Lucca, E. C. P. and E. C. de. Exploring the Aldol Reaction in the Synthesis of Bioactive Compounds. *Current Organic Synthesis*. 2015, pp 547–564. https://doi.org/http://dx.doi.org/10.2174/157017941205150821124901.

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CONCLUSIONS

- The two aqueous crossed aldol reactions carried out in this work using vanillin and cinnamaldehyde were successfully adapted in a typical laboratory session for undergraduate organic chemistry students. The general objective of this work was achieved, because both natural aldehydes fulfilled the requirement of easily available and inexpensive starting materials to avoid the issues tied to the limited chemical supply in our country. The use of non-toxic solvent is a great advantage in both syntheses as well. Also, the fact that both final products, dehydrozingerone and cinnamylidene acetone, were successfully synthesized, makes this practice accessible and reproducible in other teaching laboratories.
- The use of additives (metal halides) in the synthesis of dehydrozingerone from the crossed aldol reaction of vanillin demonstrated the importance of Lewis acids as catalysts in organic synthesis and contributed to the optimization process in the laboratory. The kinetics of the reaction was controlled by TLC in order to determine the optimal conditions in terms of yield and time after vanillin is consumed to complete the reaction. The use of 0.1 Eq of LiCl at 50°C and 0.1 Eq of LiCl + 0.1 Eq of $NiCl_2$ additives spend less than 3 hours to reaction takes place with promising yields above 65%. Then, not only the time of reaction was reduced to less than a tenth from the original reaction time reported in the literature, but the yield is also good considering an organic synthesis.
- The semi-synthetic procedure in the case of crossed aldol reaction of cinnamaldehyde showed that the starting material could be extracted from an easy to acquire source (cinnamon). The extracted cinnamaldehyde showed a surprising spectral purity above 92 % at 254nm. The crude did not present any problem during the synthesis, making this procedure convenient when other aldehydes are difficult to acquire. In terms of time, this crossed aldol reaction is very suitable because the complete precipitation of the final product takes place in less than an hour. Due to the obtention of the starting material as the initial step, the whole procedure, including extraction and synthesis of cinnamylidene acetone takes two sessions to be reproduced in an organic teaching laboratory with 3-hour sessions.

- The use of IR Spectroscopy, HPLC and UPLC Mass Spectrometry contributes to reinforce the basics of the most common analytical techniques as well as to characterize the final products dehydrozingerone and cinnamylidene acetone and the purity of starting material cinnamaldehyde. In addition to this, both procedures show positive learning outcomes for the undergraduate student.
- This experimental approach not only teaches the handling of laboratory material and reinforces theoretical knowledge in analytical techniques, but also introduces the student to approaching and managing research problems and independent decision-making in the laboratory.

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