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 on 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 ºC at 1 atm) and acetone (56 ºC 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 couse generation of more enolate species. So even the NaOH is not strong enough to remove

quantitavly 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 course book – Organic Chemistry by Clayden, Greeves and Warren on page 453 in the second edition[2].

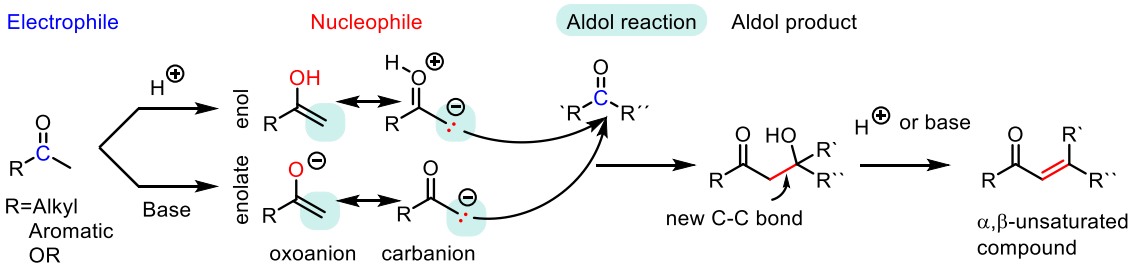


Figure 1. Formation of a new C-C bond: Enolization of carbonyl compound in acidic and basic media to form nucleophiles in order to achieve an aldol product.

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

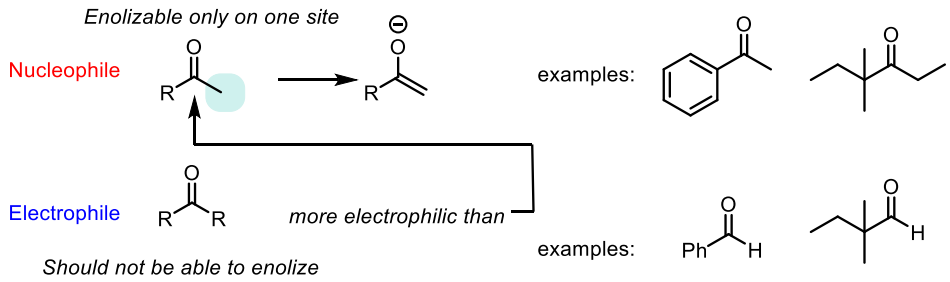


Figure 2. Requirements for nucleophile and electrophile in order to carry out crossed aldol reaction.

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

Cinnamaldehyde and acetone fulfil 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 antimicrobial 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).

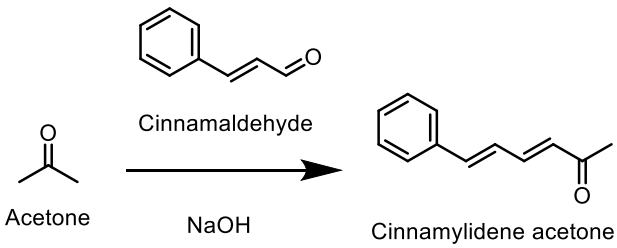


Figure 3. Scheme of reagents and product to achieve crossed aldol reaction for cinnamaldehyde.

Optimization of the reaction in terms of atomic and energetic economy, low waste production and hazard prevention – The Principles of Green Chemistry

One aspect that is sometimes underrated in chemical research and chemical industry is the pursuit to avoid materials that are hazardous to humans and to the environment, as

much as possible. This approach has been called “Green Chemistry” and is defined by 12 fundamental principles[8]:

- Prevention of waste: Try to prevent the production of waste, rather than treat or clean it up after its formation
- Atom Economy: All materials used in the process should be incorporated in the product
- Implementation of less hazardous chemical synthesis routes: Use and generate substances that are as little toxic as possible to human health and the environment
- Design of safer chemicals: Products should be designed to be as harmless as possible while maintaining their desired properties
- Use of safer solvents and auxiliary substances: Chosen solvents and other agents should have minimal toxicity and be recyclable if possible
- Design energy efficient chemical routes: Routes should be carried out at room temperature and pressure to minimize energy demands
- Use renewable materials: Renewable feedstocks should be used, such as chemicals derived from biological sources to avoid substances derived from petrochemicals.
- Use the least reaction steps: Try to avoid the use protecting groups or other derivatives, as these steps require additional reagents and generate waste
- Use efficient catalysts: The use of catalysts enables reactions of higher atom efficiency and the catalyst itself can be reused many times over
- Design degradable products: Where possible, products that are capable to be degraded by UV light, water or bio-degradation should be the goal
- Prevent pollution through constant monitoring: Monitoring in real-time can prevent accidents that cause unwanted reactions and release toxic material
- Design safer chemical procedures: By eliminating the use of hazardous chemicals from (industrial) chemical process, risks are largely minimized in the first place.

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 pulverized 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 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 30 s to 120 s 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 Dehydrozingerone

- TLC 97:3 (Toluene/AcOEt).
- Determine the melting point (MP) of 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 with your partners.
4. Describe the product obtained by IR spectroscopy and compare 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?
5. Describe with your own words, what the pursuit of green chemistry entails.
6. Name at least 6 of the 12 Principles of Green Chemistry

3.5. Laboratory Answers

1. The mechanism is shown as follow:

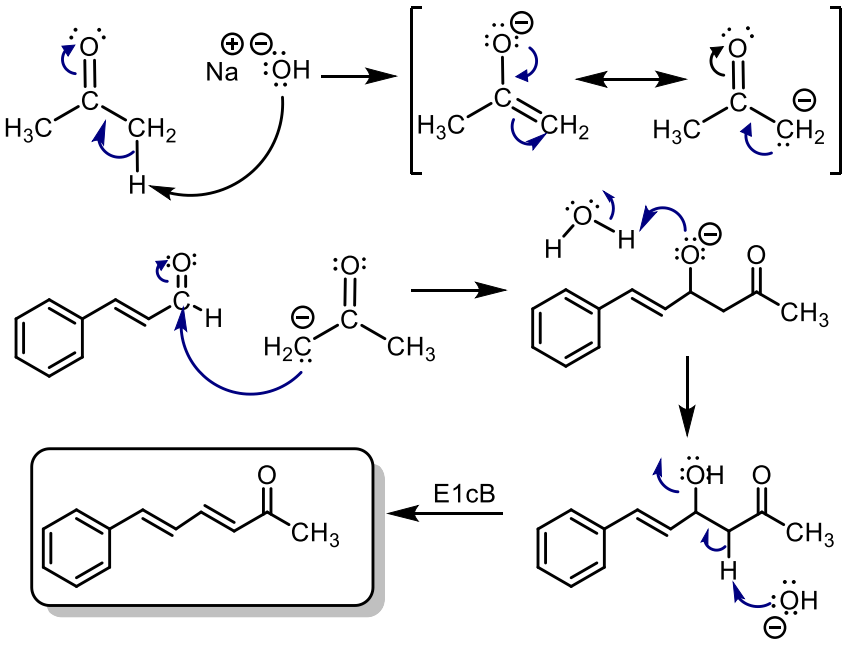


Figure 4. Mechanism of crossed aldol reaction for cinnamaldehyde.

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

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.
5. Green Chemistry aims to reduce negative effects on human health and the environment and maximizing efficiency by implementation of 12 fundamental principles in chemical research and also in chemical industry.
6. The 12 Principles of Green Chemistry are:
    7. - Prevention of waste
    8. - Atom Economy
    9. - Implementation of less hazardous chemical synthesis routes
    10. - Design of safer chemicals
    11. - Use of safer solvents and auxiliary substances
    12. - Design energy efficient chemical routes
    13. - Use renewable materials
    14. - Use the least reaction steps
    15. - Use efficient catalysts
    16. - Design degradable products
    17. - Prevent pollution through constant monitoring
    18. - Design safer chemical procedures

4. 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

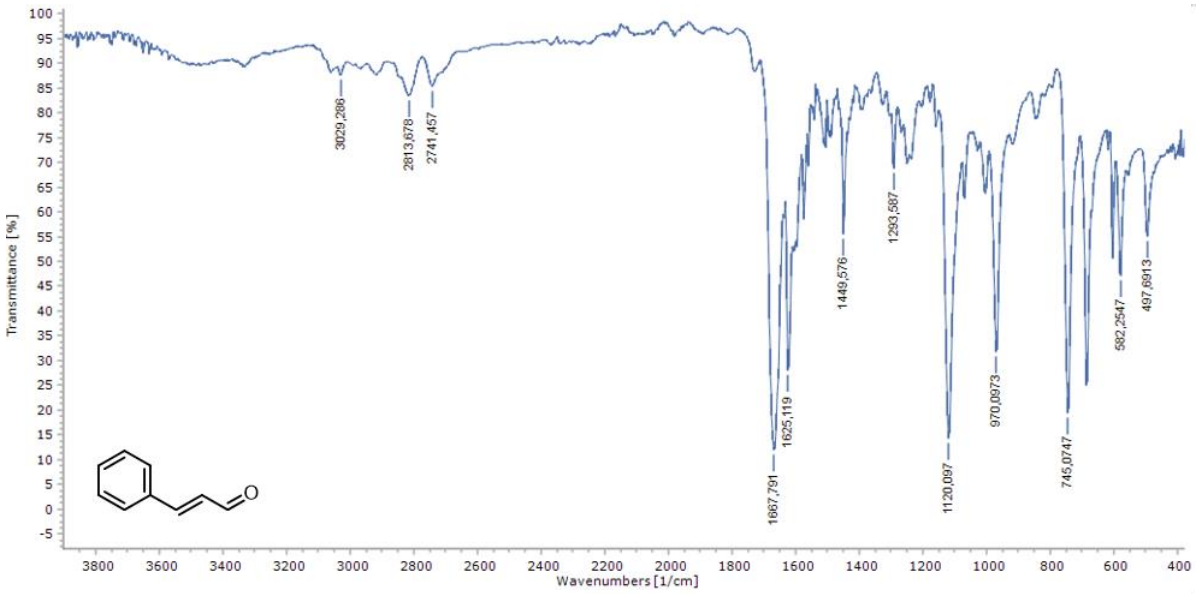


Fig. 4. IR spectrum (ATR sampling technique) of starting material (Cinnamaldehyde) for crossed aldol reaction.

Cinnamylidene acetone

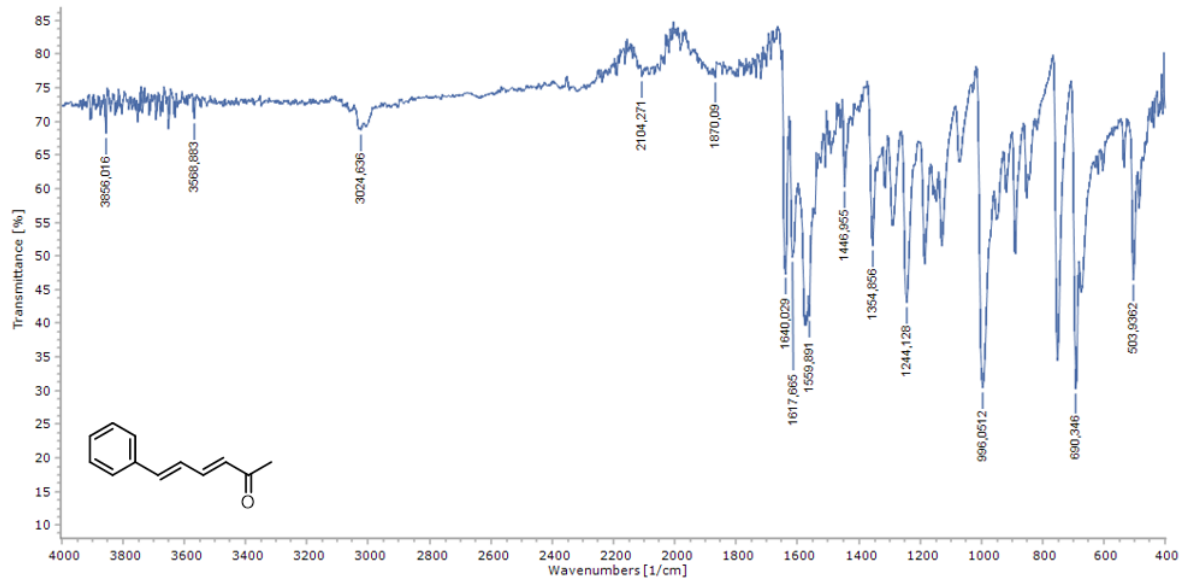


Fig. 5. IR spectrum (ATR sampling technique) of reaction product (Cinnamylidene acetone) from crossed aldol reaction.

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.

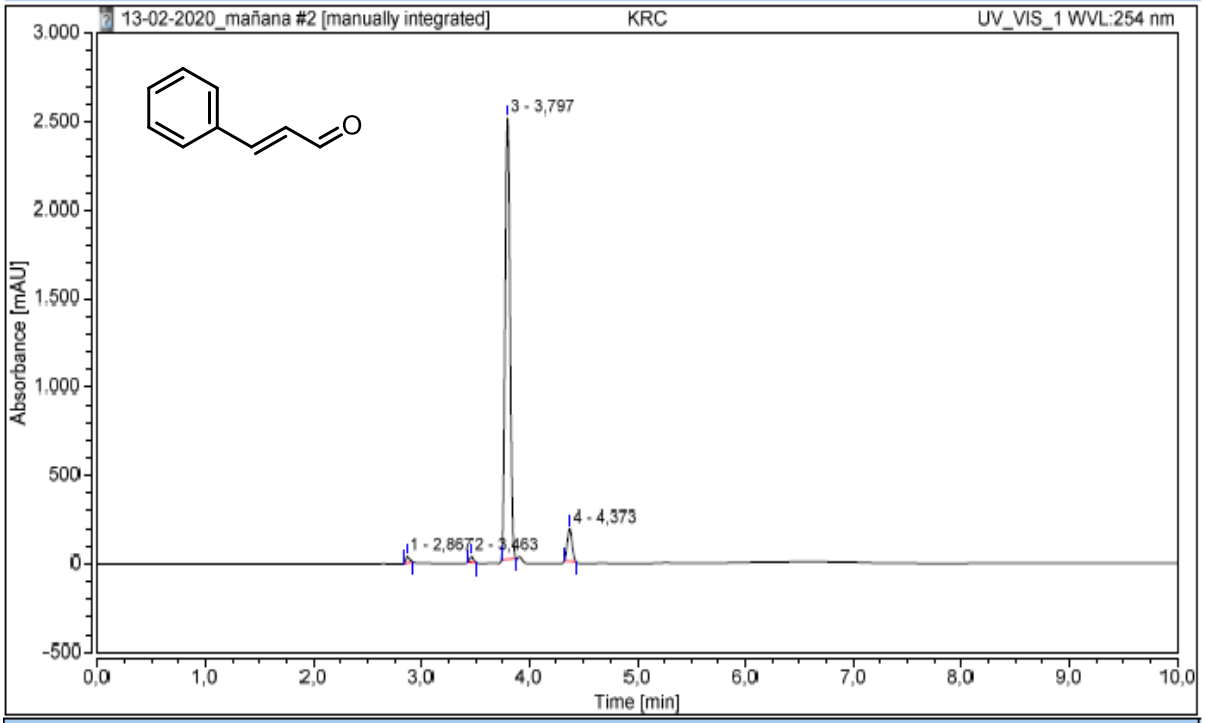
Cinnamaldehyde

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.

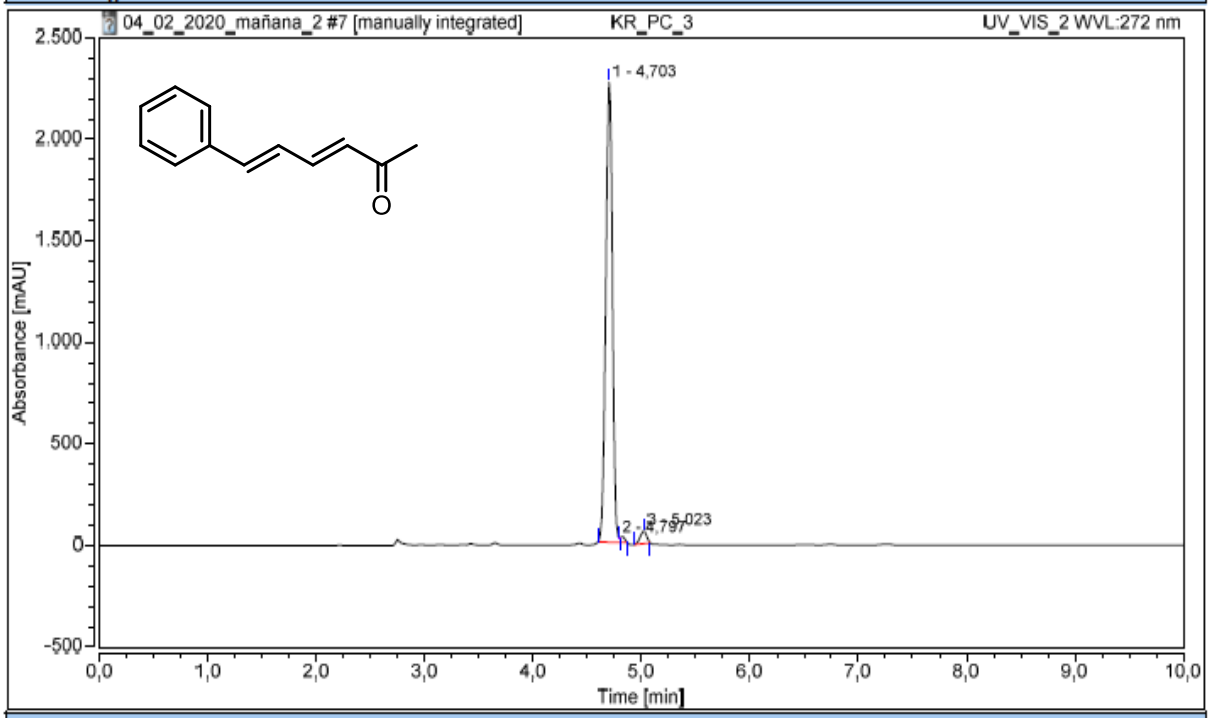
Cinnamylidene acetone

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

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