

# **LABORATORY OF ORGANIC CHEMISTRY**

**NATURAL PRODUCTS AND PHARMACEUTICALS**

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**Edited by Jan Milecki**

**UAM, Poznań 2013**

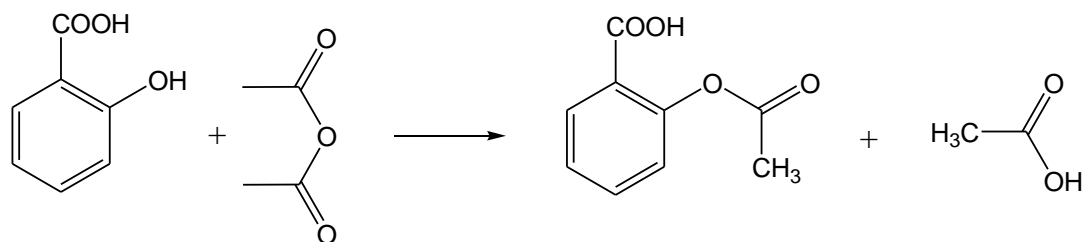
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**ASPIRIN (ACETYLSALICYLIC ACID)****Reagents:**

salicylic acid            5 g  
acetic anhydride        7.5 mL (0.73 mol)  
conc. sulfuric acid    1 mL  
model compound – a tablet of Aspirin<sup>®</sup>

**Instrumentation and glassware:**

Erlenmeyer flask 100 mL  
crystallizing dish  
heating mantle  
round bottom flask 50 mL  
condenser  
filtering flask with Büchner funnel  
beaker 100 mL  
glass rod  
Petri dish

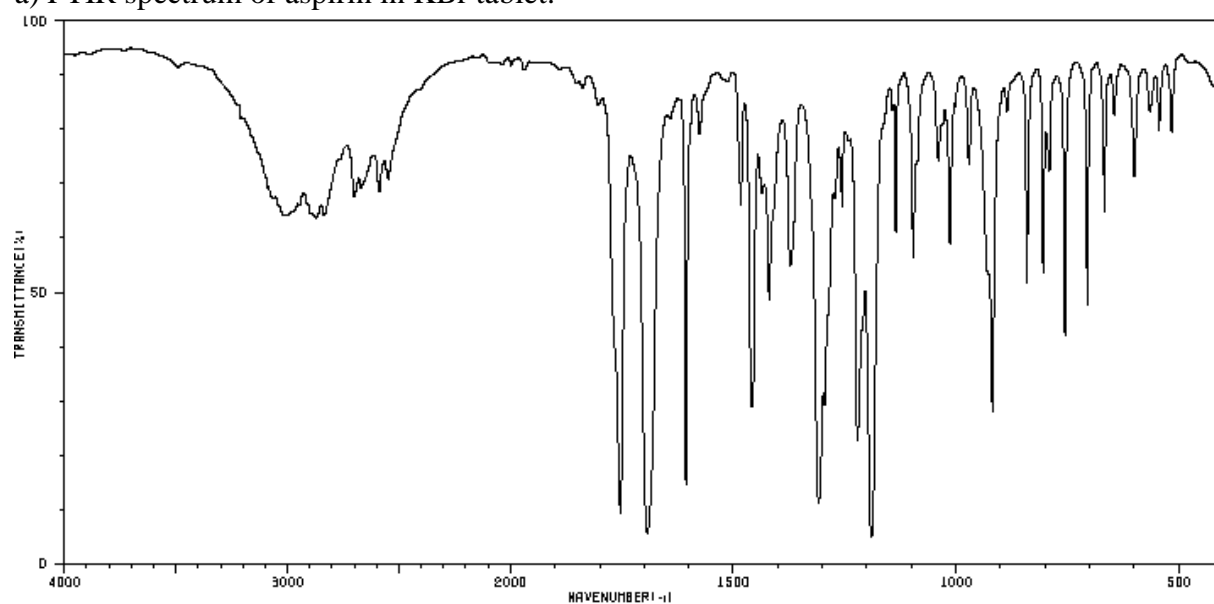
Place 5 g of salicylic acid and 7.5 mL of acetic anhydride in a conical flask (100 mL). Add carefully 3 drops of concentrated sulfuric acid. Stir the mixture for 20 min. and heat in a water bath at 60 °C until the substrate dissolves. After cooling down the solution, add 70 mL of water. Stir for additional 10 min and filter the crystals of product which separate. (acetylsalicylic acid) on the Büchner funnel and wash with cold water until the filtrate is neutral. The obtained crude product should be crystallized from 20 mL ethanol (heat under reflux). After dissolving pour the hot solution into a beaker (100 mL) with 35 mL of hot water and cool down slowly. Filter the product on Büchner funnel and dry on air. To recrystallize aspirin, dissolve the product in the mixture of diethyl ether and hexane (10 mL: 10 mL). Weigh the product, calculate the yield and measure the m.p. (lit. 135 °C).

**Thin layer chromatography (TLC):**

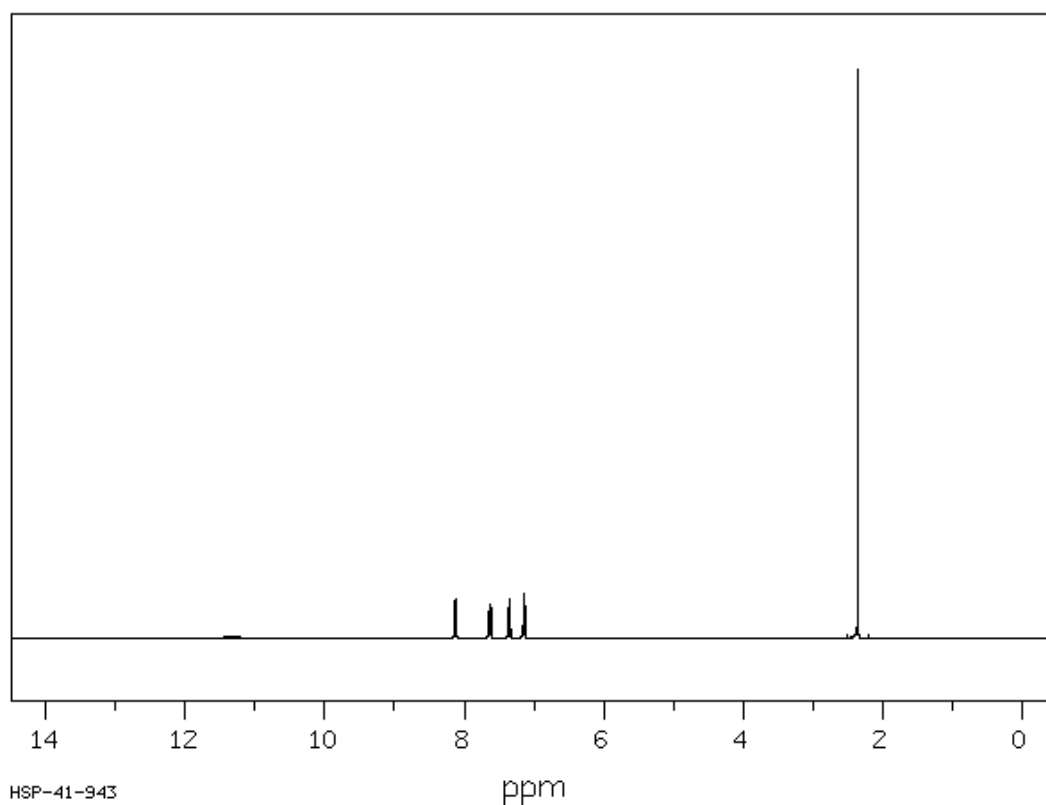
Apply the substrate and product (as a solution in MeOH) onto SiO<sub>2</sub> plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with chloroform/methanol (8:2). Remove the plate and allow the solvent to evaporate. The spot of aspirin is visible under the UV light. Mark the spot in pencil. Then, using forceps, dip the plate into a closed jar containing SiO<sub>2</sub> saturated with I<sub>2</sub>.

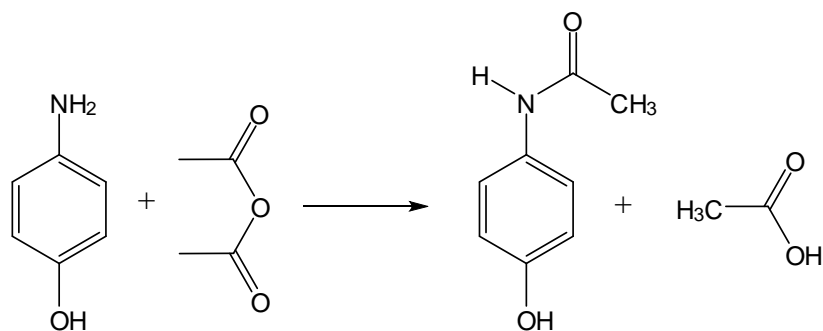
**SPECTRA**

a) FTIR spectrum of aspirin in KBr tablet.



b) <sup>1</sup>H NMR spectrum of aspirin in CDCl<sub>3</sub> (400 MHz).



**PARACETAMOL (ACETAMINOPHEN)****Reagents:**

*p*-aminophenol      5,5 g (0.05 mol)  
 acetic anhydride      6 mL (0.06 mol)  
 model compound – a tablet of APAP<sup>®</sup>  
    or Paracetamol<sup>®</sup>

**Instrumentation and glassware:**

heating mantle  
 round flask 50 mL  
 condenser  
 filtering flask with Büchner funnel  
 beaker 100 mL  
 glass rod  
 Petri dish

In a round-bottomed flask (100 mL) place 5.5 g of *p*-aminophenol, 15 mL of distilled water. To this mixture drop carefully 6 mL of acetic anhydride. Adjust the Liebig condenser and heat under reflux for 20 minutes. After the substrate has dissolved, cool down the solution, and the crystals of product should appear in the flask. Filter the product on the Büchner funnel and wash with cold water to pH=7 of the filtrate.

The obtained crude acetaminophen should be recrystallized from 20 mL water (heat under reflux). Next pour the hot solution into small beaker and cool down in ice-water bath. Filter the product on Büchner funnel, and dry on air on Petri dish.

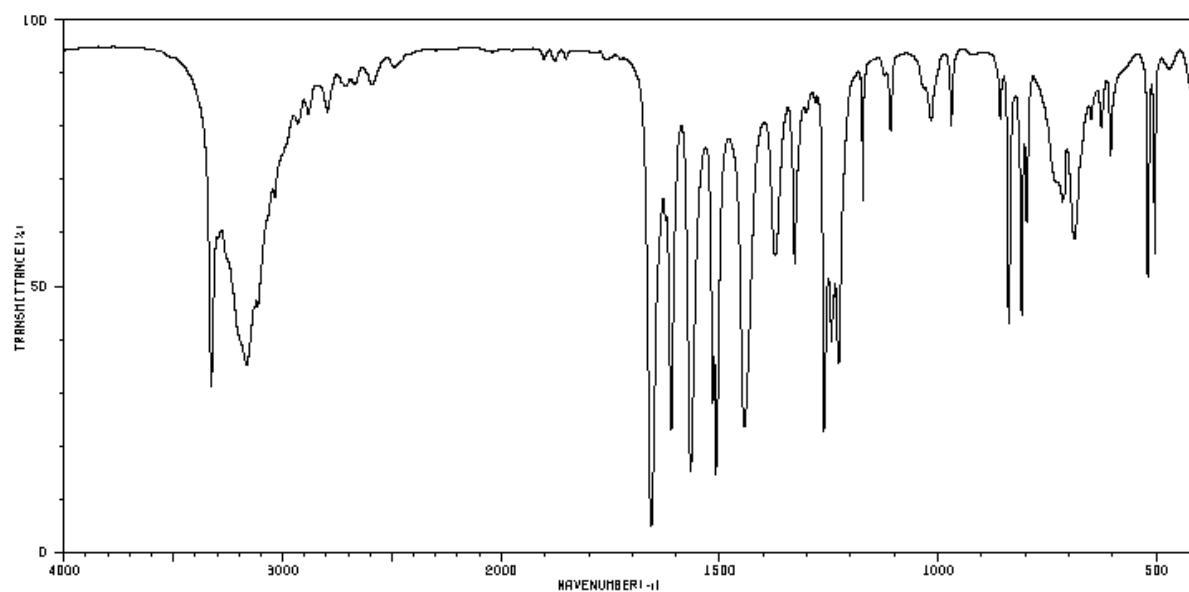
Weigh the product, calculate the yield and measure the m.p. (lit. 169 °C).

**Thin layer chromatography (TLC):**

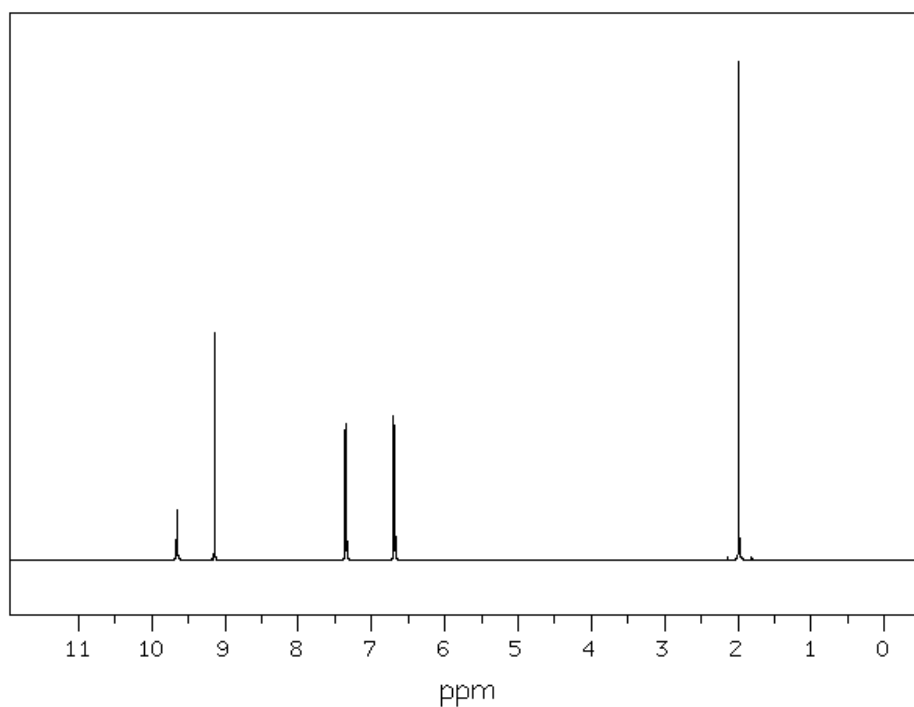
Apply the substrate and product (as a solution in MeOH) onto SiO<sub>2</sub> plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with chloroform/methanol (9:1). Remove the plate and allow the solvent to evaporate. The spot of paracetamol is visible under the UV light. Mark the spot in pencil. Then, using forceps, dip the plate into a closed jar containing SiO<sub>2</sub> saturated with I<sub>2</sub>.

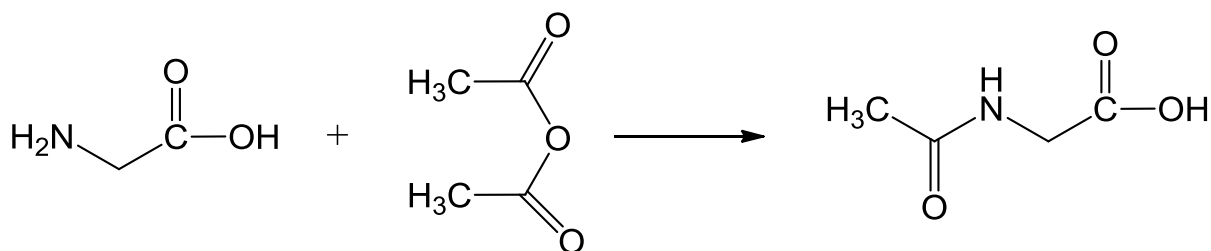
## SPECTRA

a) FT IR spectrum of paracetamol in KBr tablet



b)  $^1\text{H}$  NMR spectrum of paracetamol in  $\text{CDCl}_3$ .



**ACETYLGLYCINE****Reagents:**

glycine	0.0125 mol (0,94 g)
acetic anhydride	0.025 mol (2,4 mL)
distill water	15 mL

**Instrumentation and glassware:**

heating mantle  
round flask 50 mL  
condenser  
filtrating flask with Büchner funnel  
beaker 50 mL  
glass rod  
magnetic stirrer

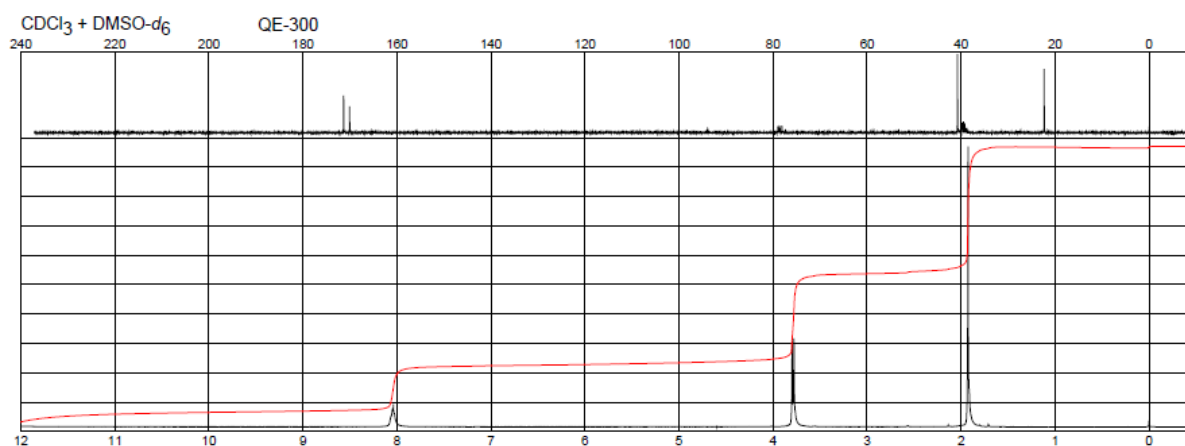
In a round-bottomed flask (50 mL) dissolve 0.0125 mol of glycine in 5 mL of distilled water. To this mixture add 0.025 mol of acetic anhydride in one portion and stir vigorously for 15-20 minutes. The solution becomes hot and some acetylglycine may crystallize. Cool down the mixture (preferably in refrigerator). The crystals of product should appear in a flask. Filter the product on the Büchner funnel and wash with cold water to pH=7 of the filtrate.

The obtained crude product should be recrystallized from 10 mL of boiling water (heat under reflux). Next pour the hot solution to small beaker and cool down in an ice bath (crystallizer with ice and water). Filter the product on Büchner funnel, and dry on air on Petri dish.

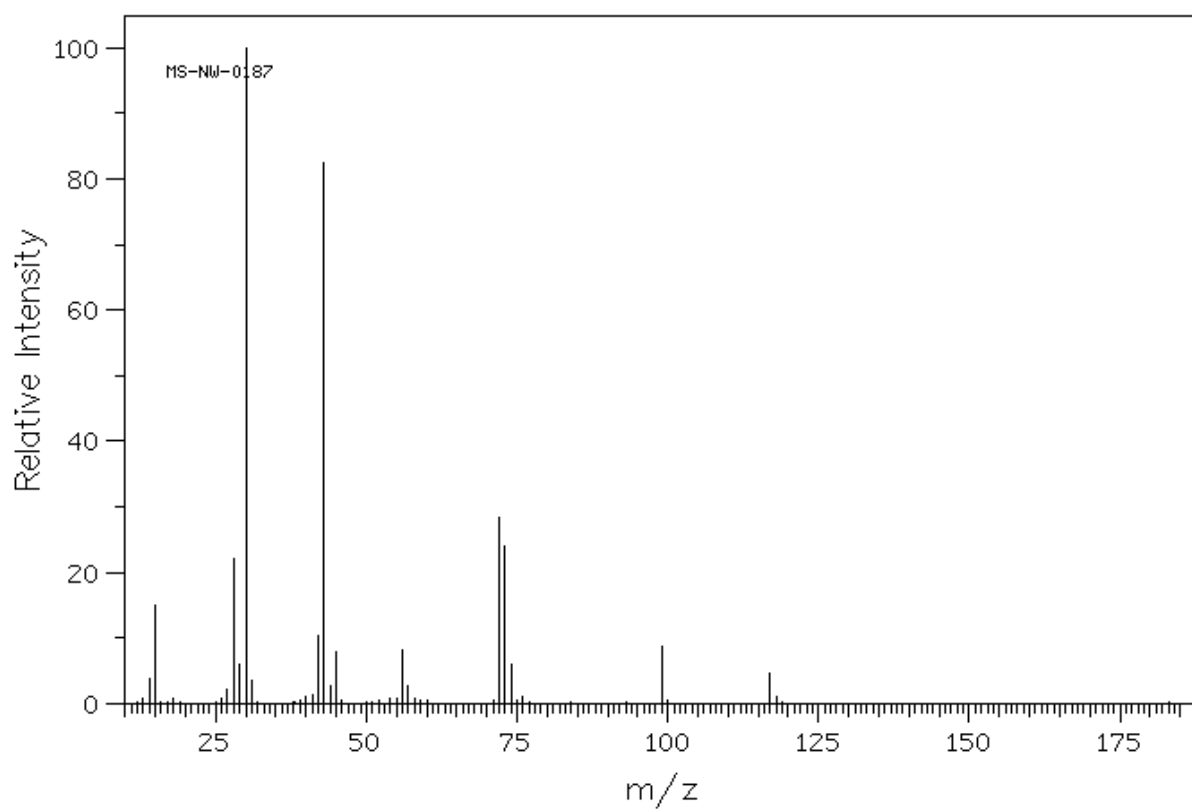
Weight the product, calculate the percentage yield and measure the m.p. (lit. 207-208 °C).

**Thin layer chromatography (TLC):**

Put the substrate and product onto  $\text{SiO}_2$  plate (as a solution in MeOH), then place the plate into developing chamber with chloroform/methanol (8:2). Remove the plate and allow the solvent to evaporate. Then, using forceps, dip the plate into the mixture of  $\text{SiO}_2/\text{I}_2$

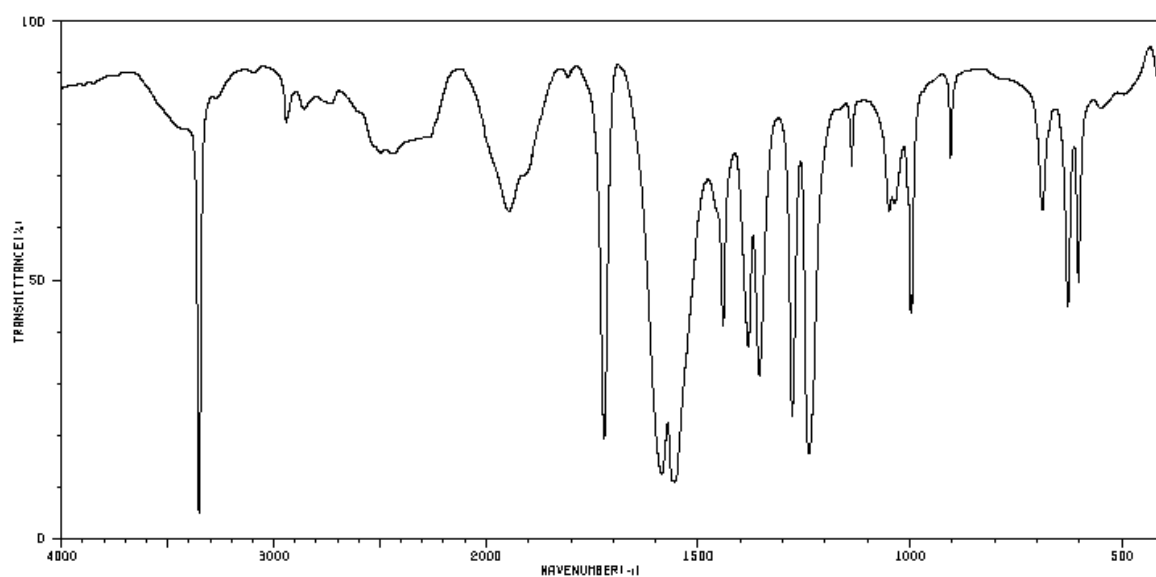
**SPECTRA**a)  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of acetylglycine

b) MS mass spectrum of acetylglycine

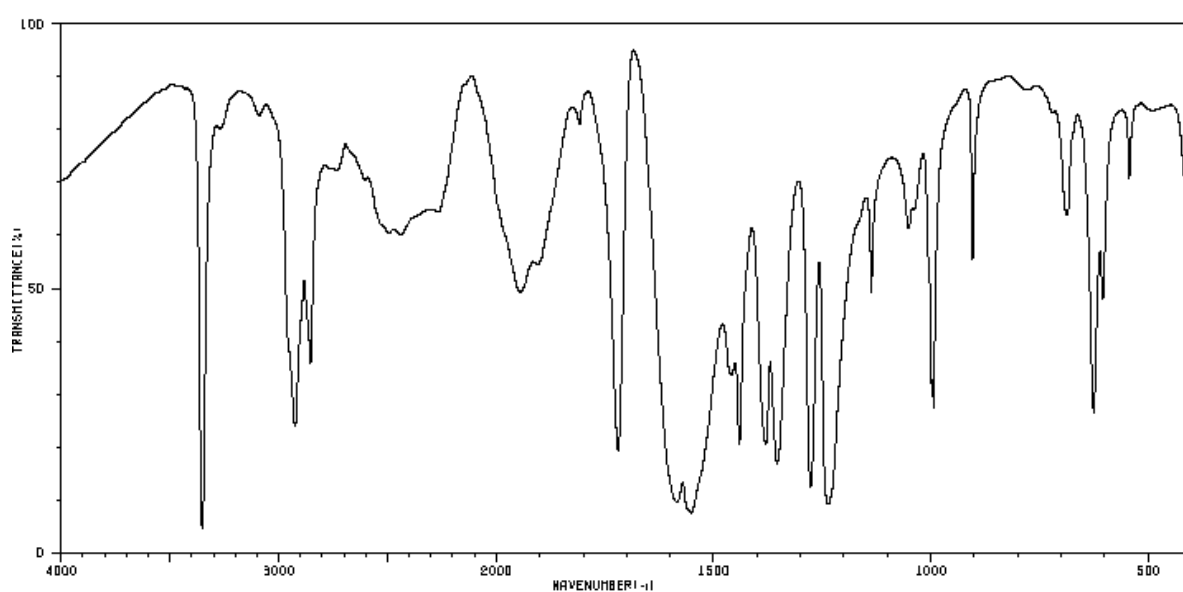


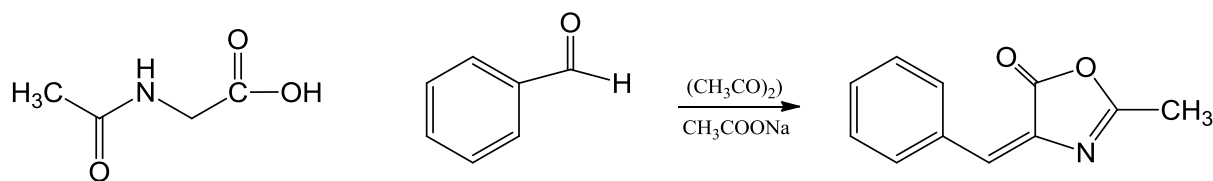


c) FT IR spectrum of acetylglycine in KBr tablet.



d) FT IR spectrum of acetylglycine in nujol.



**4-BENZYLIDENE-2-METHYLOXAZOL-5-ONE****Reagents:**

acetylglycine	0.0085 mol (0,98 g)
benzaldehyde	0.012 mol (1,2 mL)
anh. sodium acetate	0,5 g
acetic anhydride	0.02 mol (1,9 mL)

**Instrumentation and glassware:**

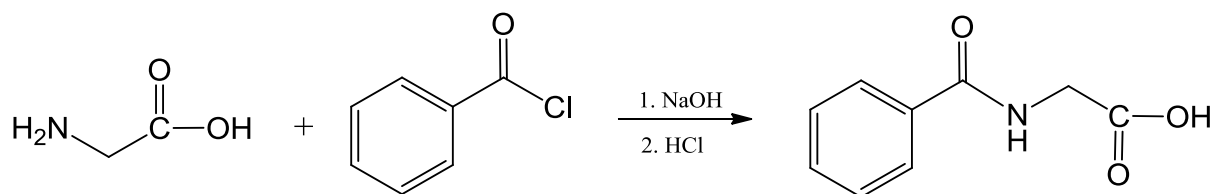
heating mantle  
 round flask 50 mL  
 condenser  
 filtration flask with Büchner funnel  
 beaker 50 mL  
 glass rod  
 magnetic stirrer

In a round-bottomed flask (100 mL) place acetylglycine, benzaldehyde and anh. sodium acetate. To this mixture drop carefully 6 mL of acetic anhydride. Adjust the Liebig condenser and heat under reflux for 1 hour. Cool down the solution (preferably for overnight) and the crystals of product should appear in a flask.

Stir the solid mass of yellow crystals with 6 mL of cold water, transfer to a Büchner funnel and wash well with cold water to pH=7 of the eluate. If the odor of benzaldehyde is still apparent, wash with a little ether. Recrystallize from chloroform. Filter the product on Büchner funnel, and dry on air on Petri dish. Weight the product, calculate the percentage yield and measure the m.p. (lit. 150-151 °C).

**Thin layer chromatography (TLC):**

Put substrate and product onto SiO<sub>2</sub> plate (as a solution in MeOH), then place the plate into chloroform/methanol (8:2). Remove the plate and allow the solvent to evaporate. The spot of the product is visible in the UV light. Mark the spot in pencil. Then, using forceps, dip the plate into the mixture of SiO<sub>2</sub>/I<sub>2</sub>

**BENZOYLGLYCINE (HIPPURIC ACID)****Reagents:**

glycine	0.03 mole (2.3 g)
10% NaOH	19 mL
benzoyl chloride	0.03 mole (3.5 mL)
conc. HCl	5 mL
Congo red paper	
chloroform	10 mL

**Instrumentation and glassware:**

heating mantle  
 round flask 50 mL  
 condenser  
 filtrating flask with Büchner funnel  
 beaker 50 mL  
 glass rod  
 magnetic stirrer

In conical flask prepare 10% of sodium hydroxide solution and dissolve in it 0.03 mole of glycine. Add 0.03 mole of benzoyl chloride in 5 portions to the solution. Stopper the vessel and shake vigorously after each addition until all the chloride has reacted. Transfer the solution to a beaker and rinse the conical flask with a little water. Place a few pieces of crushed ice to the solution and add slowly 5 mL of HCl with stirring until the mixture is acid to Congo red paper.

Collect the resulting crystalline precipitate of benzoylglycine, which is contaminated with a little benzoic acid, upon a Büchner funnel, wash with cold water and drain well. Place the solid in a round bottom flask with 10 mL of chloroform and heat under reflux. This process extracts any benzoic acid which may be present. Allow the mixture to cool down. Filter the product on Büchner funnel, and dry on air on Petri dish.

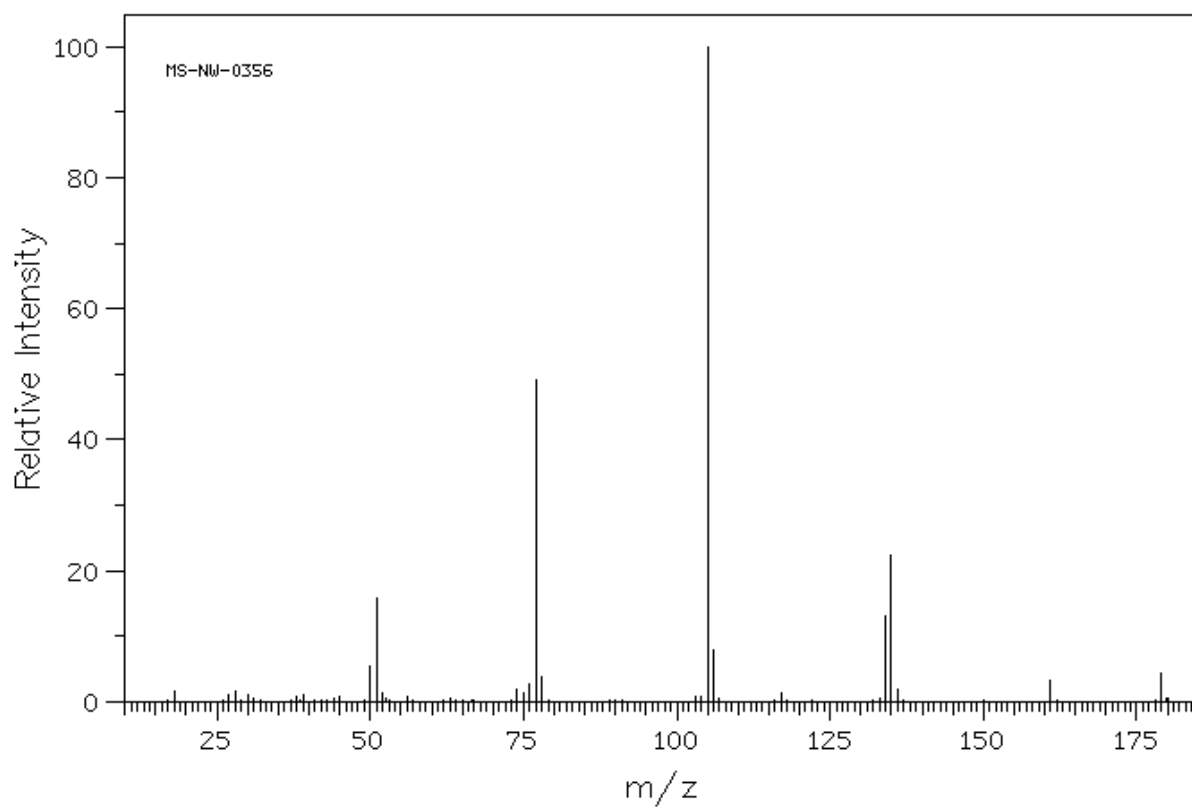
Weight the product, calculate the percentage yield and measure the m.p. (lit. 186-187 °C).

Thin layer chromatography (TLC):

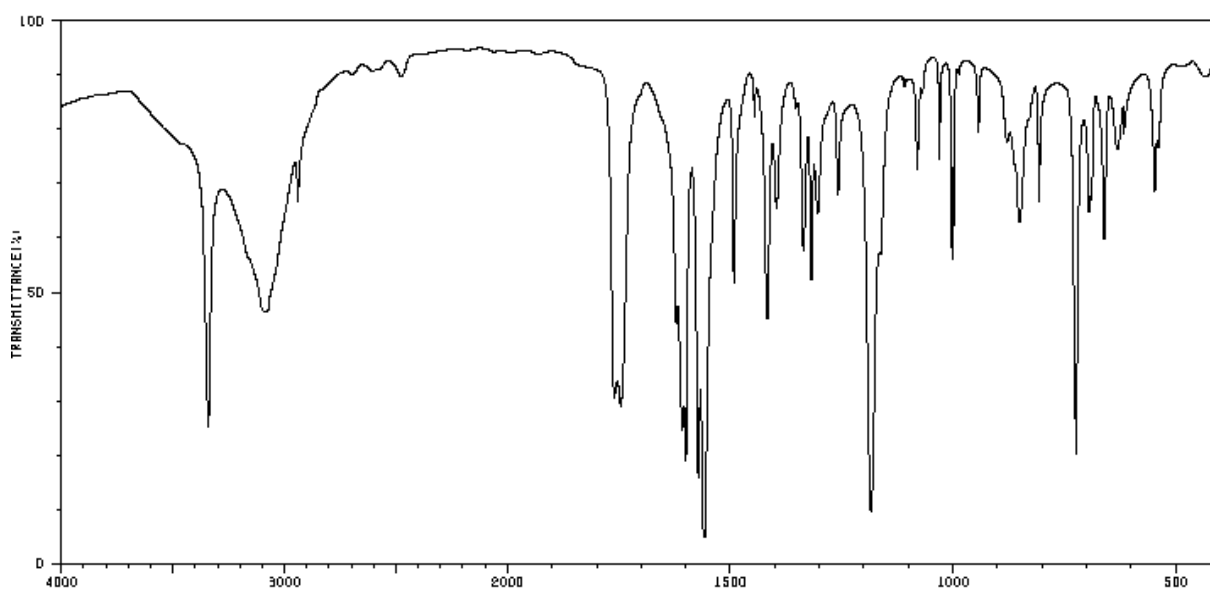
Put substrate and product onto SiO<sub>2</sub> plate (as a solution in MeOH), then place the plate into developing chamber with chloroform/methanol (8:2). Remove the plate and allow the solvent to evaporate. The spot of hippuric acid is visible in the UV light. Mark the spot in pencil. Then, using forceps, dip the plate into the mixture of SiO<sub>2</sub>/I<sub>2</sub>

**SPECTRA**

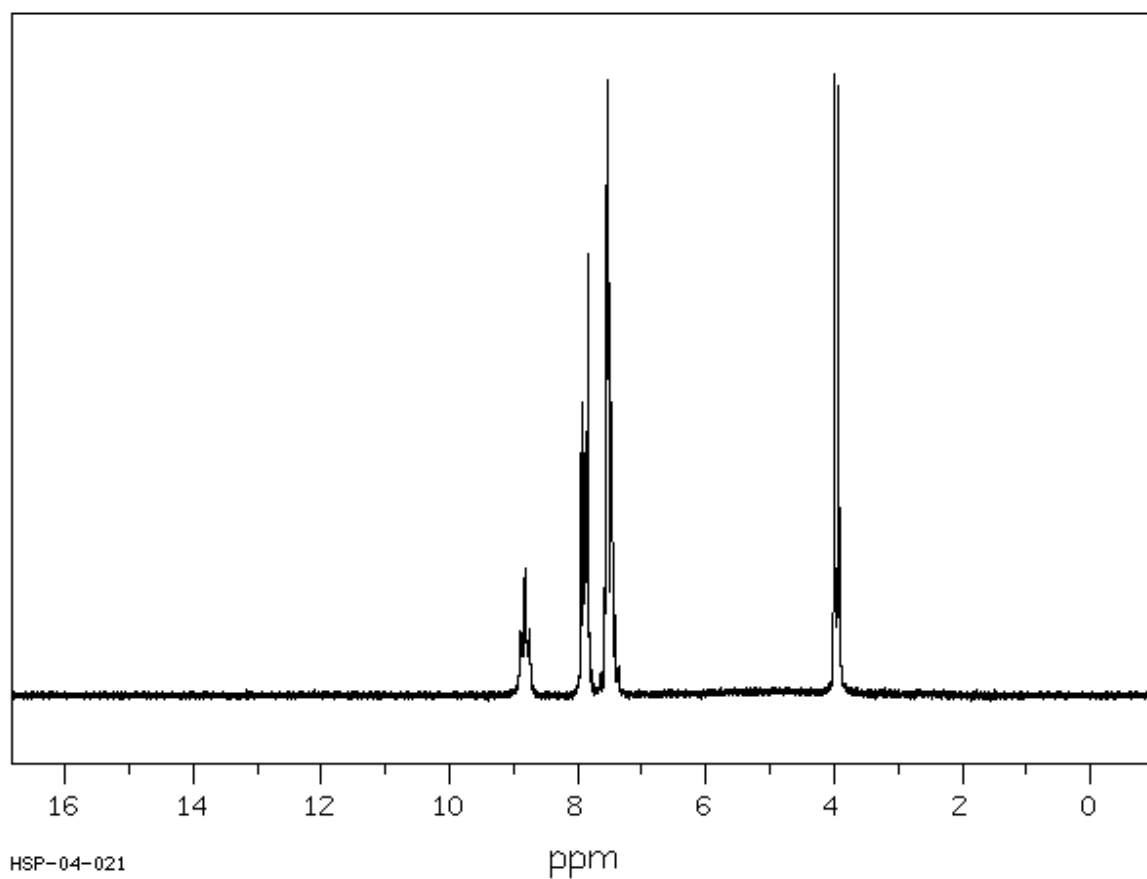
## a) EIMS spectrum of hippuric acid



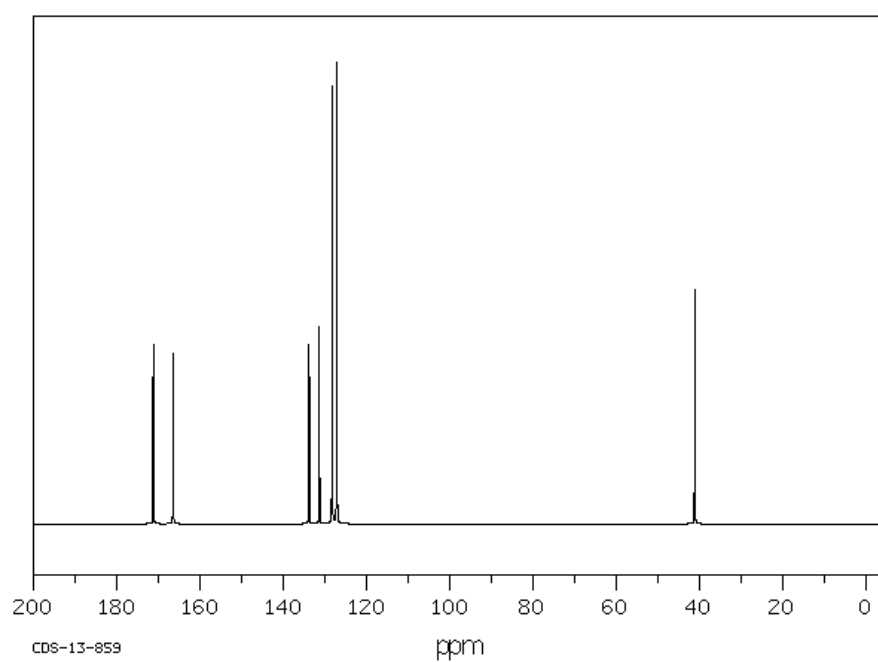
## b) IR spectra of hippuric acid in KBr.

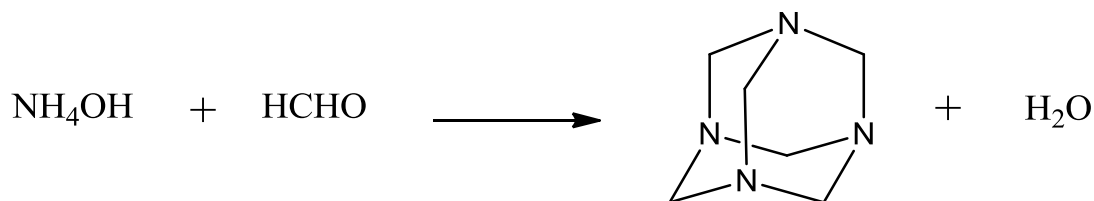


c)  $^1\text{H}$ -NMR spectrum of hippuric acid in  $\text{DMSO-d}_6$  (90Hz)



d)  $^{13}\text{C}$  NMR spectrum of hippuric acid in  $\text{DMSO-d}_6$



**HEXAMINE (UROTROPINE)****Reagents:**

conc. ammonia            37 mL  
37% formaldehyde       9 mL  
anh. ethanol

**Instrumentation and glassware:**

round-bottom flask 100 mL  
filtering flask with Büchner funnel  
measuring cylinder  
Petri dish

Place 10 mL of 37% formaldehyde and 9 mL of ammonia into a round bottomed flask (100 mL). Evaporate the solution on a rotavapor. During evaporation a white residue (urotropine) is formed. Add another portion of ammonia (14 mL) to dissolve the solid.

Once more place the flask onto a rotavapor and evaporate the solvent. Repeat that step once more with an additional portion of ammonia (14 mL). After evaporating, add 15 mL of anh. ethanol and dissolve the solid by heating under reflux. Filter the hot mixture on the Büchner funnel. Add 10 mL diethyl ether to the filtrate and cool down. Filter the precipitated urotropine, wash it with anhydrous ethanol, drain well and then dry on air.

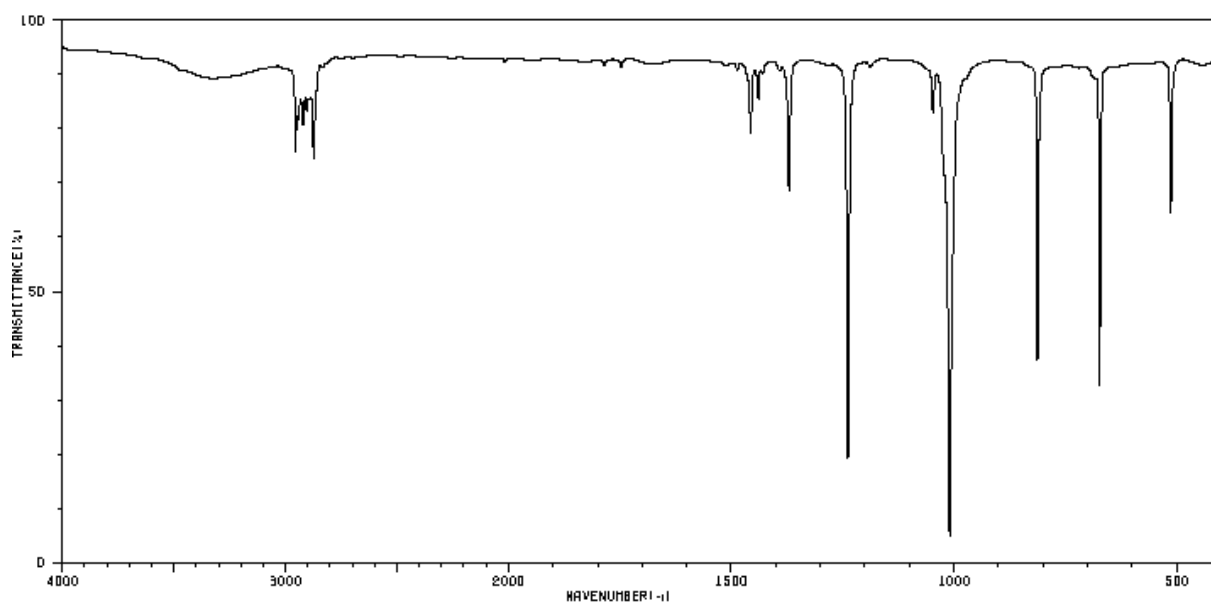
Weigh the product, calculate the yield and measure the m.p. (lit. 214-215 °C).

**Thin layer chromatography (TLC):**

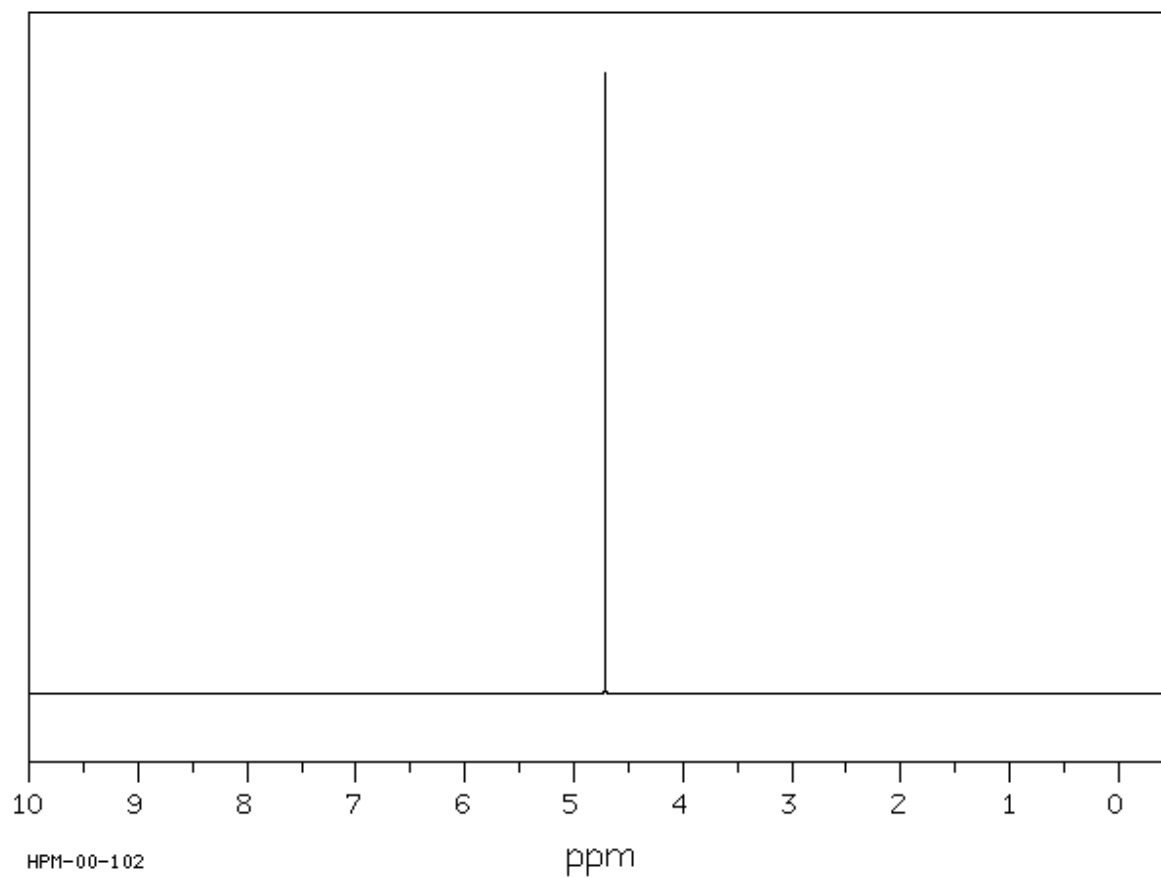
Apply the substrate and product onto SiO<sub>2</sub> plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with ethanol/CH<sub>2</sub>Cl<sub>2</sub> (8:1). Remove the plate and allow the solvent to evaporate. Then, using forceps, dip the plate into a closed jar containing SiO<sub>2</sub> saturated with I<sub>2</sub>.

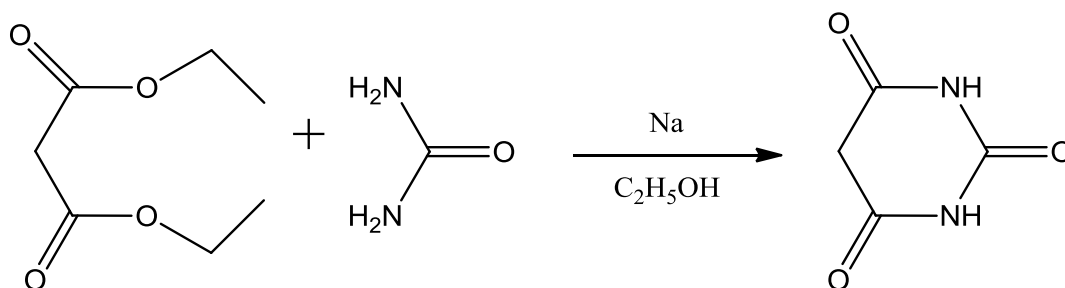
**SPECTRA**

a) IR spectrum of hexamine in KBr tablet.



b) <sup>1</sup>H-NMR spectrum of hexamine in CDCl<sub>3</sub>. (300 Hz)



**BARBITURIC ACID****Reagents:**

sodium	1.2 g
abs. ethanol	50 mL
diethyl malonate	8 g
urea	3 g
HCl	5 mL

**Instrumentation and glassware:**

round-bottom flask 250 mL  
 condenser  
 filtering flask with Büchner funnel  
 measuring cylinder

Place 1.2 g of clean sodium metal into a 250 mL round-bottomed flask, fitted with a reflux condenser,. Add 25 mL of absolute ethanol in one portion. If the reaction is unduly vigorous, immerse the flask momentarily in ice.

When all the sodium has reacted, add 8 g (7,6 mL) of diethyl malonate, followed by a solution of 3 g of dry urea in 25 ml of hot absolute ethanol (70 °C). Shake the mixture well, and reflux it for 1 hour in an oil bath heated to 110 °C. White solid separates.

Add 45 mL of hot (50 °C) water to the reaction mixture followed with concentrated hydrochloric acid (ca. 5 mL), with stirring, until the solution is acidic. Filter the resulting mixture and leave clear solution in the refrigerator overnight. Filter the solid on the Büchner funnel, wash it with cold water, drain well and then dry at 100 °C for 2 hours. Weigh the product, calculate the yield of barbituric acid and measure the m.p. (lit. melts with decomposition at 245 °C).

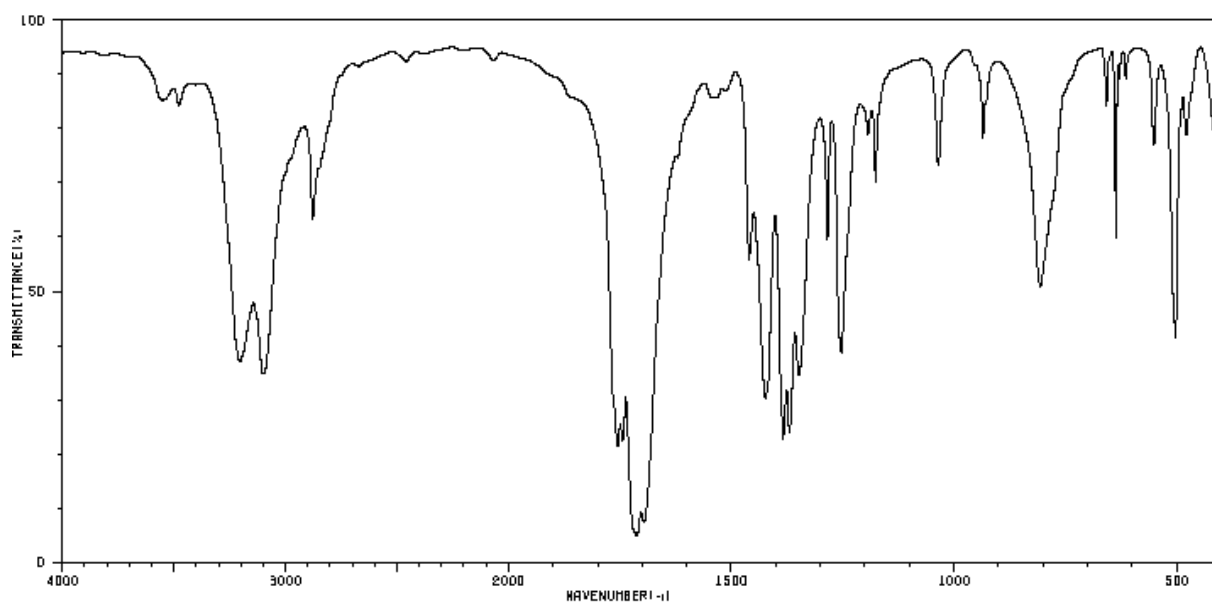
**Thin layer chromatography (TLC):**

Apply the substrate and product onto SiO<sub>2</sub> plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with ethanol/CH<sub>2</sub>Cl<sub>2</sub> (8:1). Remove the plate and allow the solvent to evaporate. The spot of the product is visible under the UV light. Mark the spot in pencil. Then, using forceps, dip the plate into a closed jar containing SiO<sub>2</sub> saturated with I<sub>2</sub>.

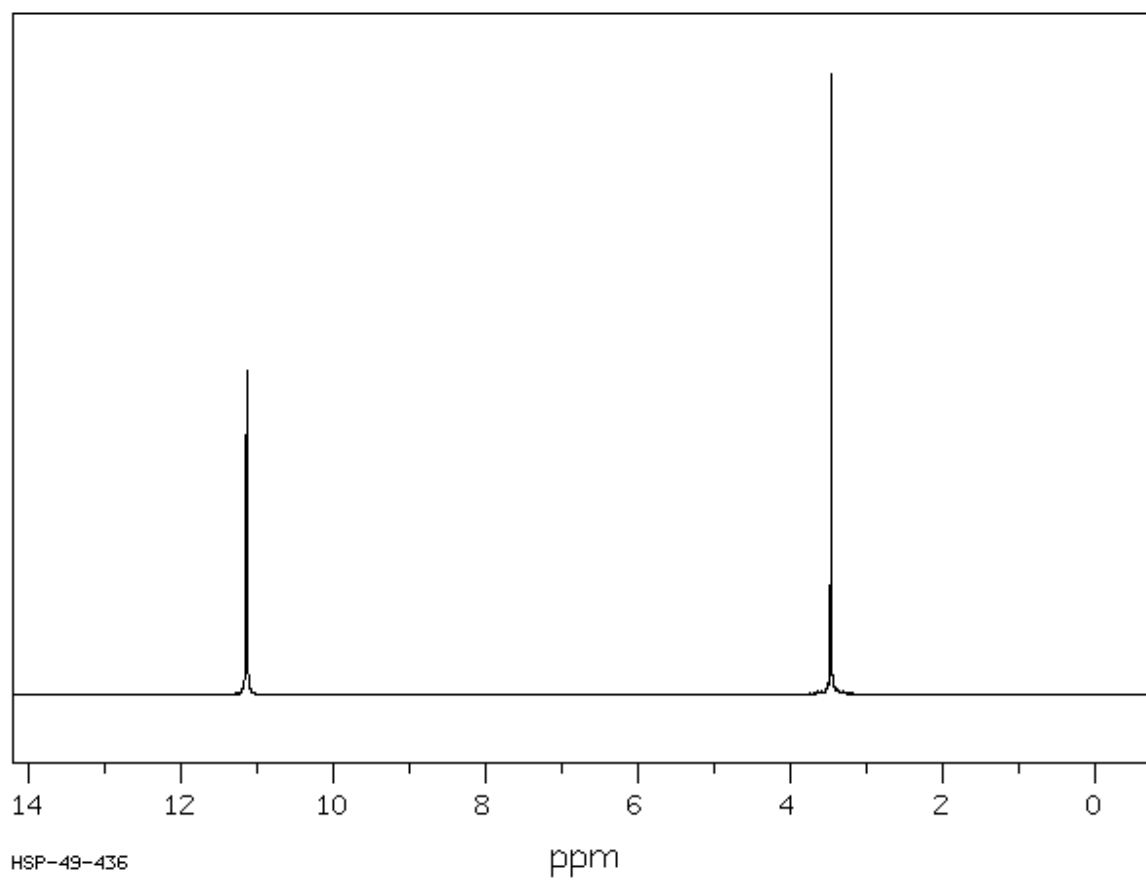


**SPECTRA**

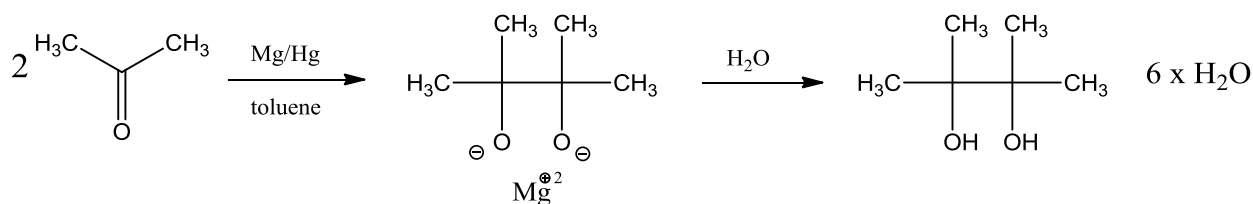
a) IR spectrum of barbituric acid in KBr.



b)  $^1\text{H}$ -NMR spectrum of barbituric acid in  $\text{DMSO-d}_6$  (400 MHz).



## PINACOL HYDRATE

**Reagents:**

magnesium turnings	2.5 g
mercury (II) chloride	3g
toluene	50 mL
anh. acetone	15 mL

**Instrumentation and glassware:**

two-necked round-bottom flask 250 mL  
 reflux condenser  
 stirrer  
 water bath  
 filtering flask with Büchner funnel  
 dropping funnel 50 mL  
 Erlenmeyer flask 125 mL  
 graduated cylinder 25 mL  
 Petri dish

**Caution:** *Pinacol is irritating.*

Place 2.5 g of dry magnesium turnings and 25 mL of toluene in a 250-mL in two-necked round-bottomed flask fitted with a dropping funnel and a condenser carrying calcium chloride guard-tubes. Place a solution of 3 g. of mercury (II) chloride (**POISONUS!**) in 15 mL of dry acetone in the dropping funnel and run in about ¼ of this solution. Shake the flask with a rotary motion to give the contents a swirling movement until the reaction started.

If the reaction does not commence in a few minutes warm carefully the flask on a water bath and be ready to cool the flask in cold water bath to moderate the reaction.

After the reaction had run for 15 to 20 minutes, add additional portions of acetone and shake periodically the flask to avoid the formation of a cake inside the flask.

When reaction has slowed down, warm the flask on water bath for 1-2 hours to maintain a rapid reflux rate. During this period the magnesium pinacolate swells up and nearly fills the flask. Cool slightly the flask and disconnect from the condenser and shake until the solid mass is well broken up. Attach the condenser and reflux for about 1 hour, or until all the magnesium has disappeared.

Then add 10 mL of water through the dropping funnel and heat again on the water bath for 1 hour with occasional shaking. This converts the magnesium pinacolate into pinacol and precipitate of  $\text{Mg}(\text{OH})_2$ . Cool down the mixture and filter on the Büchner funnel. Return the solid to the flask and reflux with 15 mL of toluene for 10 min. in order to extract remaining

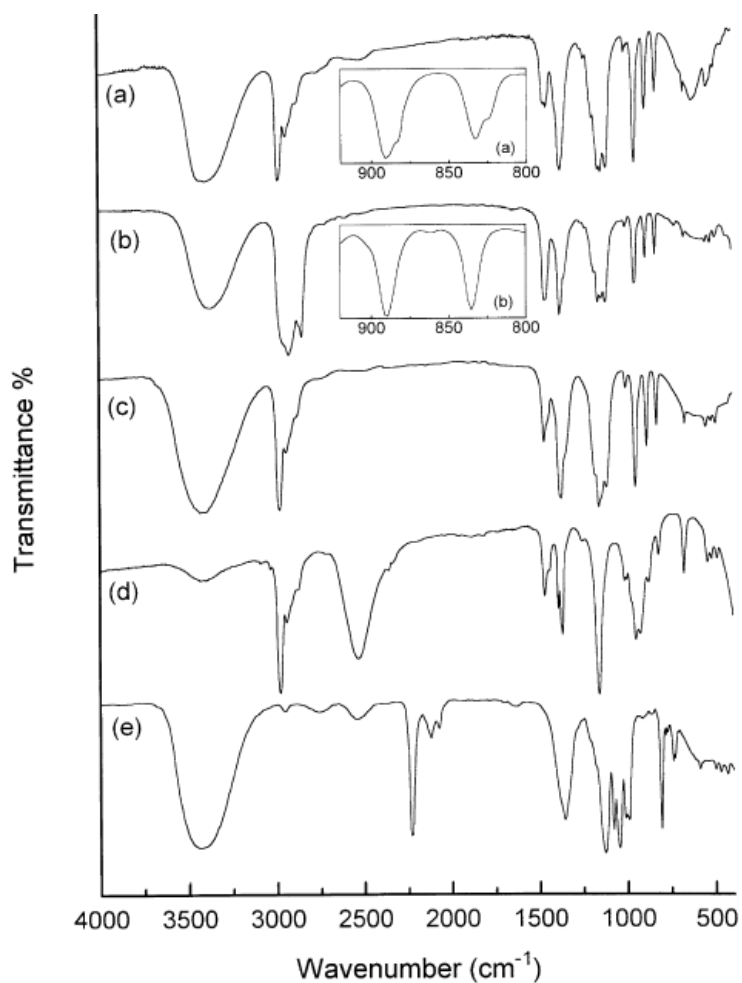
pinacol. Filter it again on Büchner. Combine the extracts and concentrate them on the rotary evaporator to remove the toluene. To the solution add 10 mL of water, cool in ice-bath and stir vigorously the mixture for 30 min. Filter pinacol hydrate on Büchner funnel and wash the product with toluene to remove impurities.

Dry the pinacol hydrate by exposure to air at room temperature.

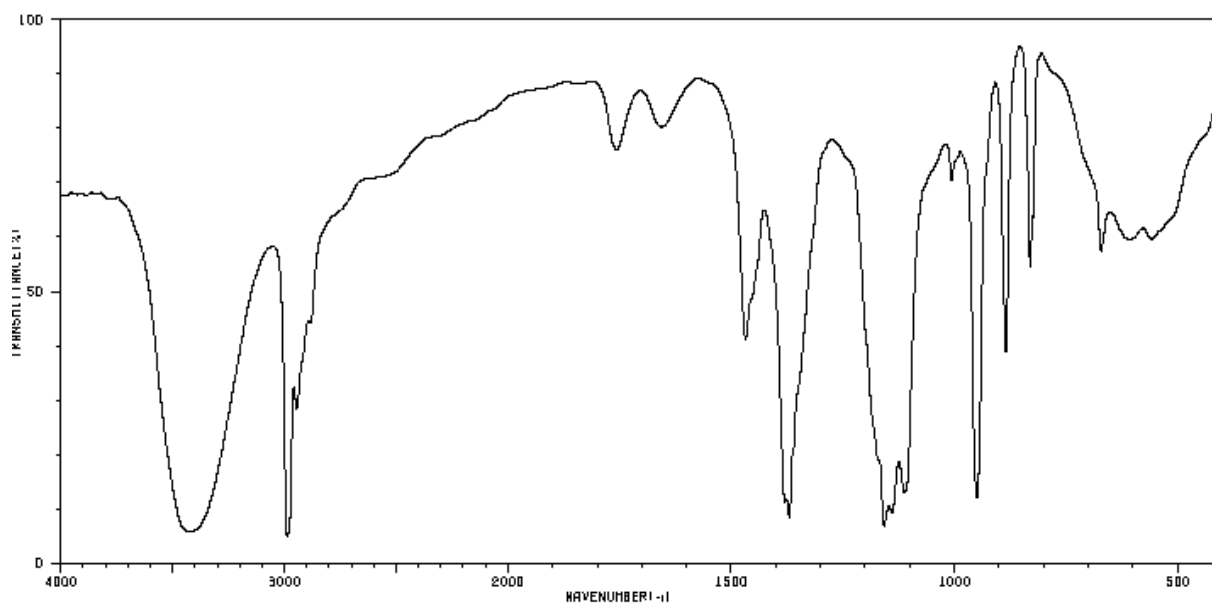
The yield 90%, m.p. 46-57 °C.

## SPECTRA

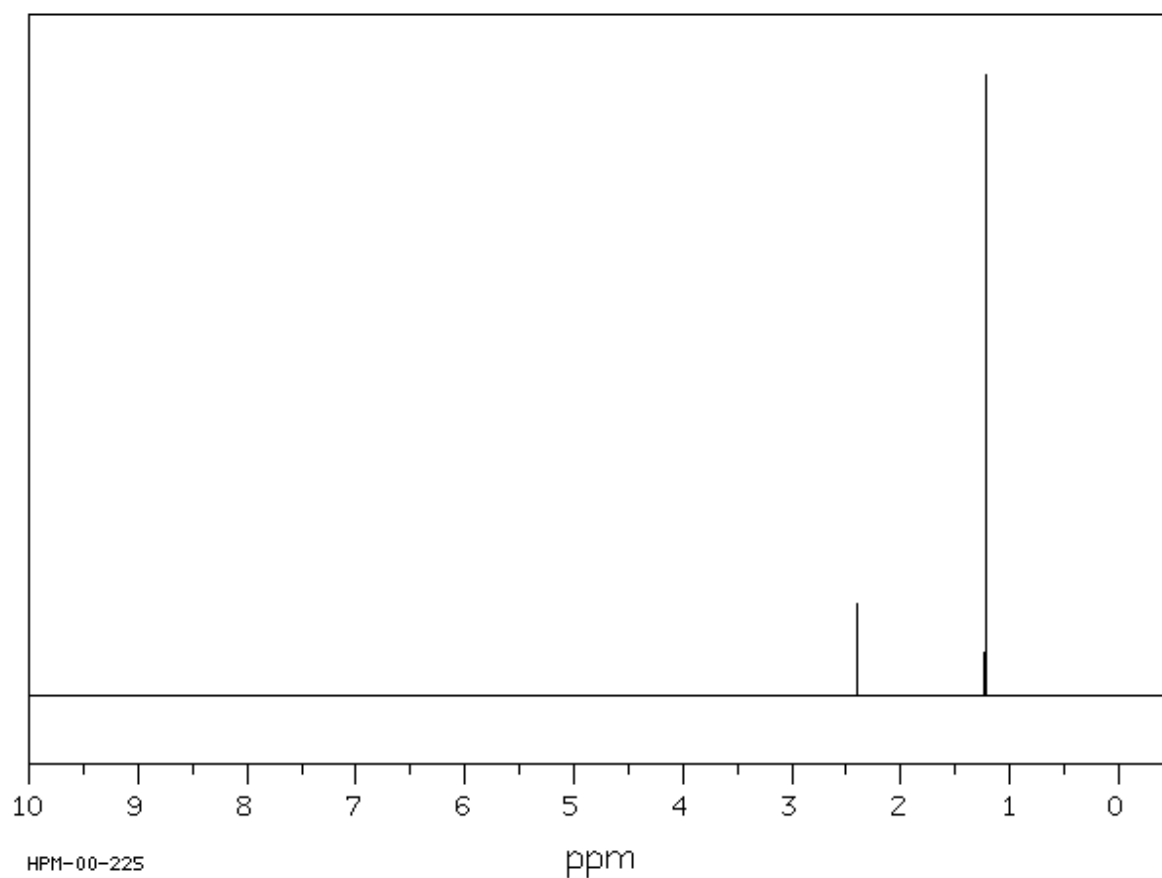
IR spectra of pinacol and its deuterium derivatives: a) between KBr plates, b) in nujol, c) neat liquid, d)  $(\text{CH}_3)_2\text{C}(\text{OD})\text{-C}(\text{OD})(\text{CH}_3)_2$  as neat liquid, e)  $(\text{CD}_3)_2\text{C}(\text{OH})\text{-C}(\text{OH})(\text{CD}_3)_2$  neat liquid. The conformationally sensitive bands of the A and B forms are shown in the expanded spectra a) and b), respectively.



a) IR spectrum of pinacol in KBr

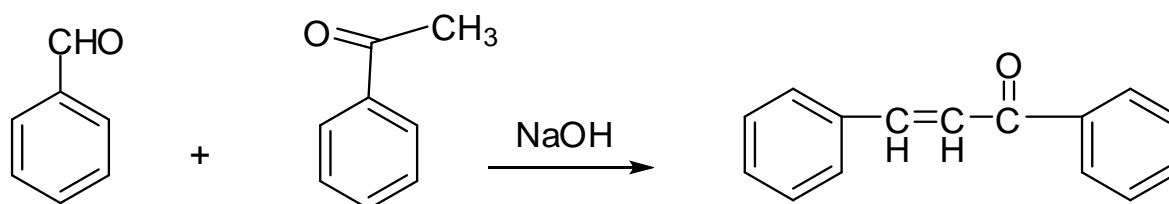


b)  $^1\text{H}$ -NMR spectrum of pinacol in  $\text{CDCl}_3$  (300 MHz).



## CHALCONE

(BENZYLIDENEACETOPHENONE)

**Reagents:**

sodium hydroxide 0.055 mol  
ethanol 12 mL  
acetophenone 0.043 mol  
benzaldehyde 0.043 mol (d=1,0415 g/mL)

**Instrumentation and glassware:**

two-necked round-bottom flask 100 mL  
condenser  
dropping funnel (50 mL)  
magnetic stirrer  
thermometer  
filtering flask with Büchner funnel  
water bath

**WARNING! Work under hood! Wear the gloves!**

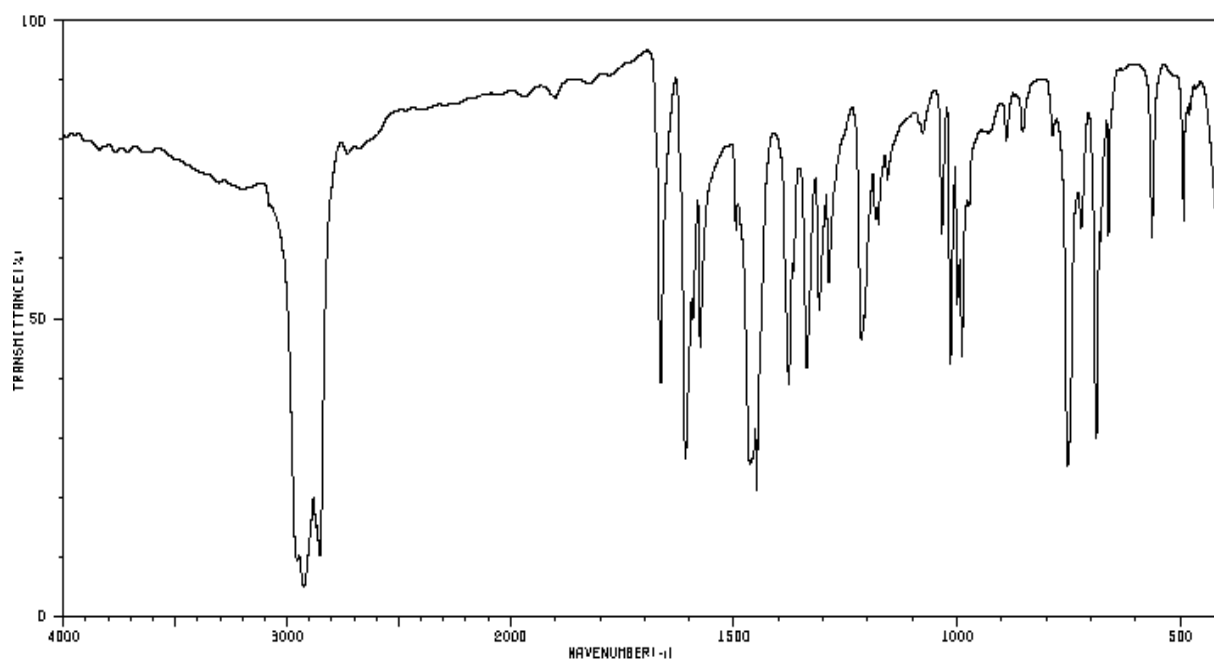
To 100 mL two necked round-bottom flask with magnetic stirrer and condenser, place solution of 0.055 mol sodium hydroxide in 20 mL of distilled water and 12,25 mL of ethanol. Place flask in the bath with crushed ice and add dropwise by dropping funnel 0.043 mol of acetophenone and then 0.043 mol of benzaldehyde. Keep the temperature of reaction mixture at about 25°C (the proper range of temperature of reaction mixture is 15-30°C) stir vigorously till the forming product prevents stirring (usually after 2-3 hours). Remove magnetic stirrer and left the reaction overnight in the fridge in bath with ice. Filter on the Büchner funnel and wash with cool water to neutral pH. Then wash with small portion of ethanol (8 mL). After drying in the air 8,8 g of crude product - chalcone of m.p. 50-54°C is obtained. Recrystallize the yellow product from ethanol (usually about 5 mL for each 1 g of chalcone). Weigh the product, calculate the yield of pure chalcone and measure the m.p. (lit. m.p. 56-57°C).

**Thin layer chromatography (TLC):**

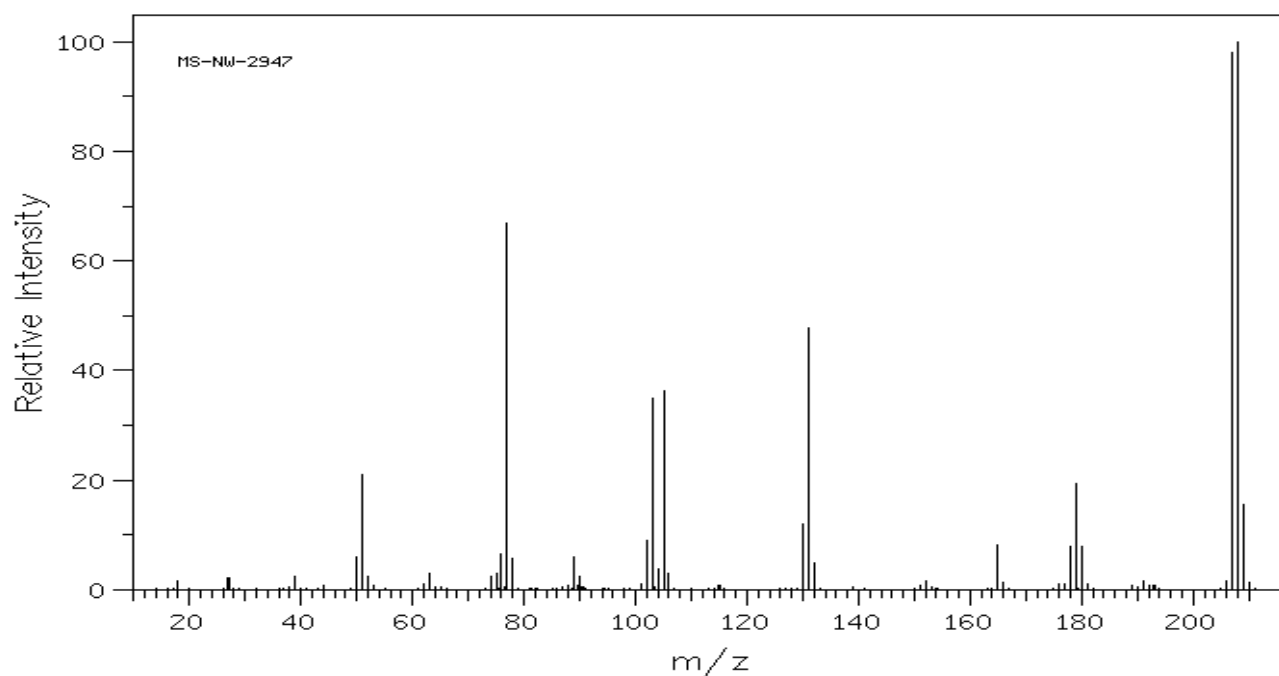
Apply the substrate and product onto SiO<sub>2</sub> plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with ethanol/CH<sub>2</sub>Cl<sub>2</sub> (8:1). Remove the plate and allow the solvent to evaporate. The spot of the product is visible under the UV light. Mark the spot in pencil. Then, using forceps, dip the plate into a closed jar containing SiO<sub>2</sub> saturated with I<sub>2</sub>.

## SPECRTA

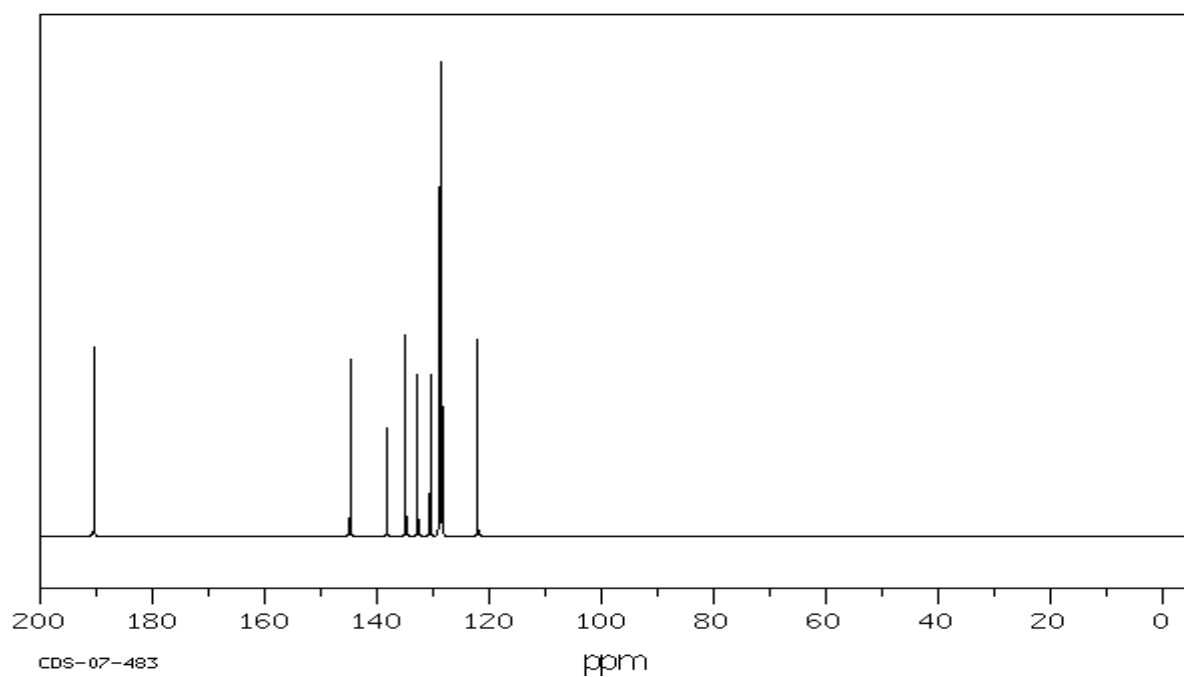
a) FT IR spectrum of chalcone in nujol



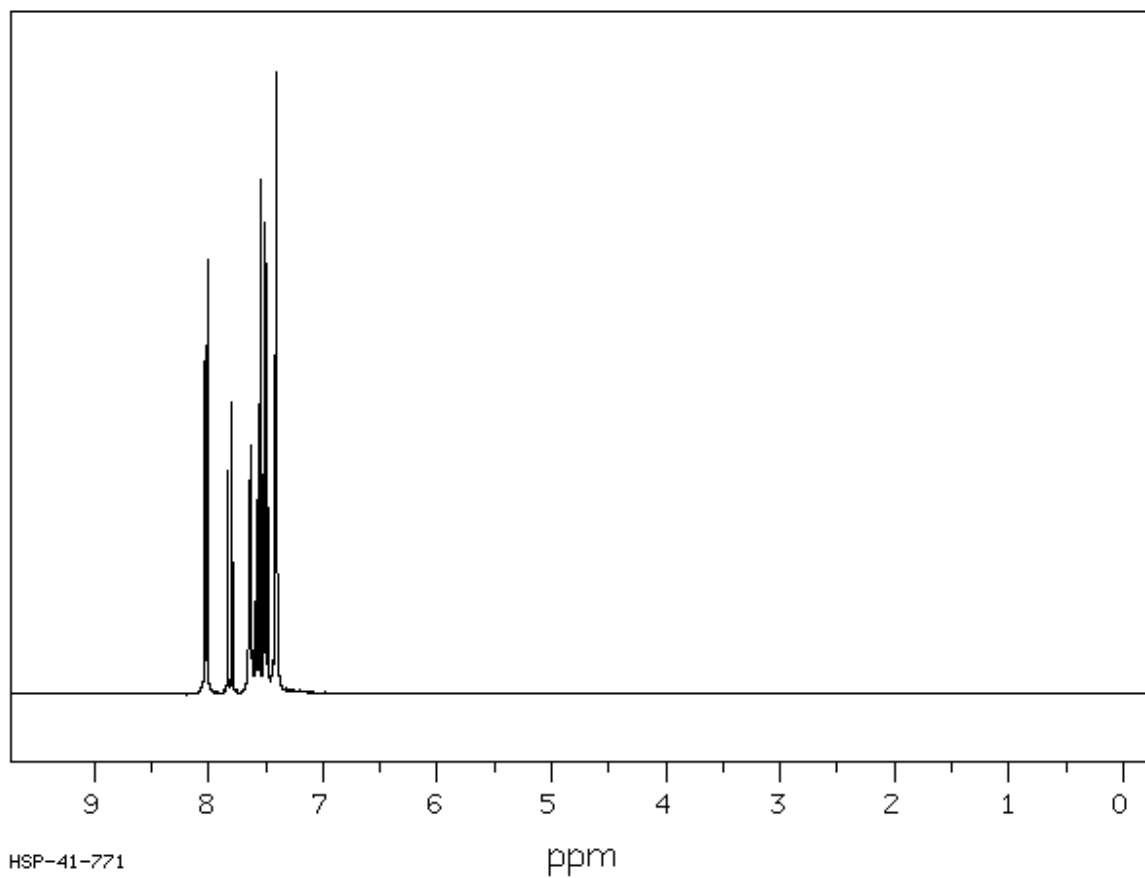
b) MS spectrum of chalcone

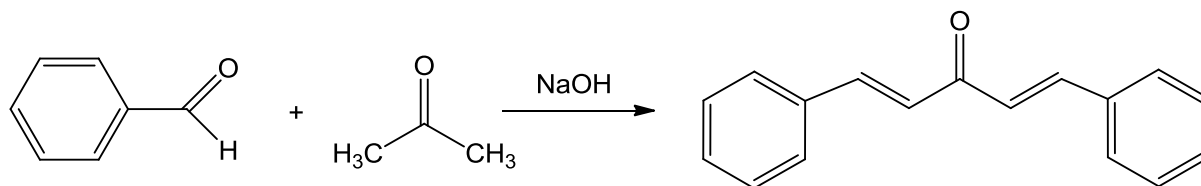


c)  $^{13}\text{C}$  NMR spectrum of chalcone in  $\text{CDCl}_3$



d)  $^1\text{H}$  NMR spectrum of chalcone in  $\text{CDCl}_3$  (400Hz)



**DIBENZYLIDENEACETONE****Reagents:**

benzaldehyde	0,02 mol (d=1,0415 g/mL)
acetone	0,01 mol (d=0.791g/mL)
NaOH	2 g
ethanol	27 mL
ethyl acetate	5 mL

**Instrumentation and glassware:**

three-necked round-bottomed flask 250 mL  
dropping funnel (10 mL)  
thermometer  
filtering flask with Büchner funnel  
water bath  
stirrer  
beaker 100 mL  
Petri dish

In a three-necked round-bottomed flask (250 mL) with magnetic stirrer, thermometer and dropping funnel place the cold solution of NaOH in 34 mL of water and 27 mL of ethanol. Prepare the mixture (**A**) of benzaldehyde and acetone (calculate the amount of the reagents!) and place it into the dropping funnel. After cooling down the solution (in water bath keep temp. 20-25 °C), drop half of the mixture **A**. Stir vigorously and after 15 minutes add additional portion of this mixture. Stir additionally 30 minutes. Then shake the flask with reaction mixture.

Filter the yellow solid on Büchner funnel, wash with water up to pH=7 (check pH for base with pH paper) and dry on air on Petri dish. Recrystallize the crude product from ethyl acetate or ethanol (5 mL). Filter the crystals on Büchner funnel and dry on air. Weight the product, calculate the percentage yield and measure the m.p. (lit. 112-113 °C).

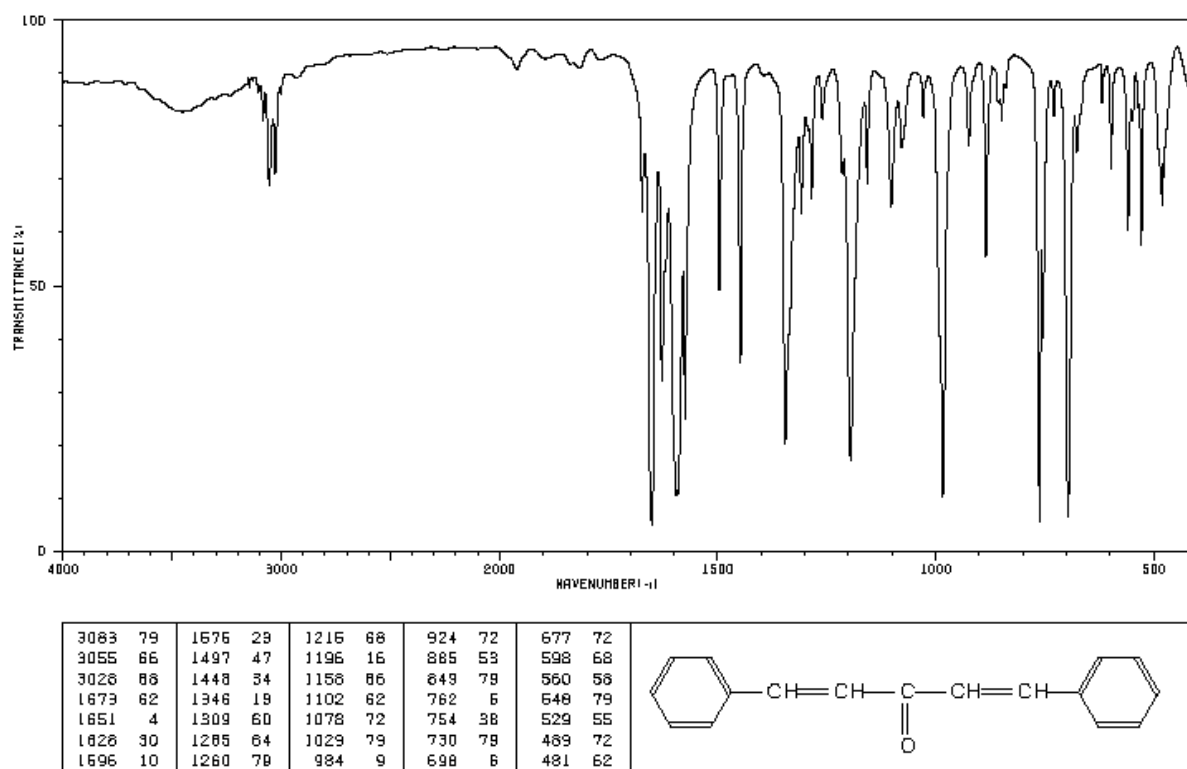
**Thin layer chromatography (TLC):**

Apply the substrate and product onto SiO<sub>2</sub> plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with CHCl<sub>3</sub>/MeOH (10:0.5). Remove the plate and allow the solvent to evaporate and inspect under UV light. Mark the spots with pencil. Then, using forceps, dip the plate into a closed jar containing SiO<sub>2</sub> saturated with I<sub>2</sub>.

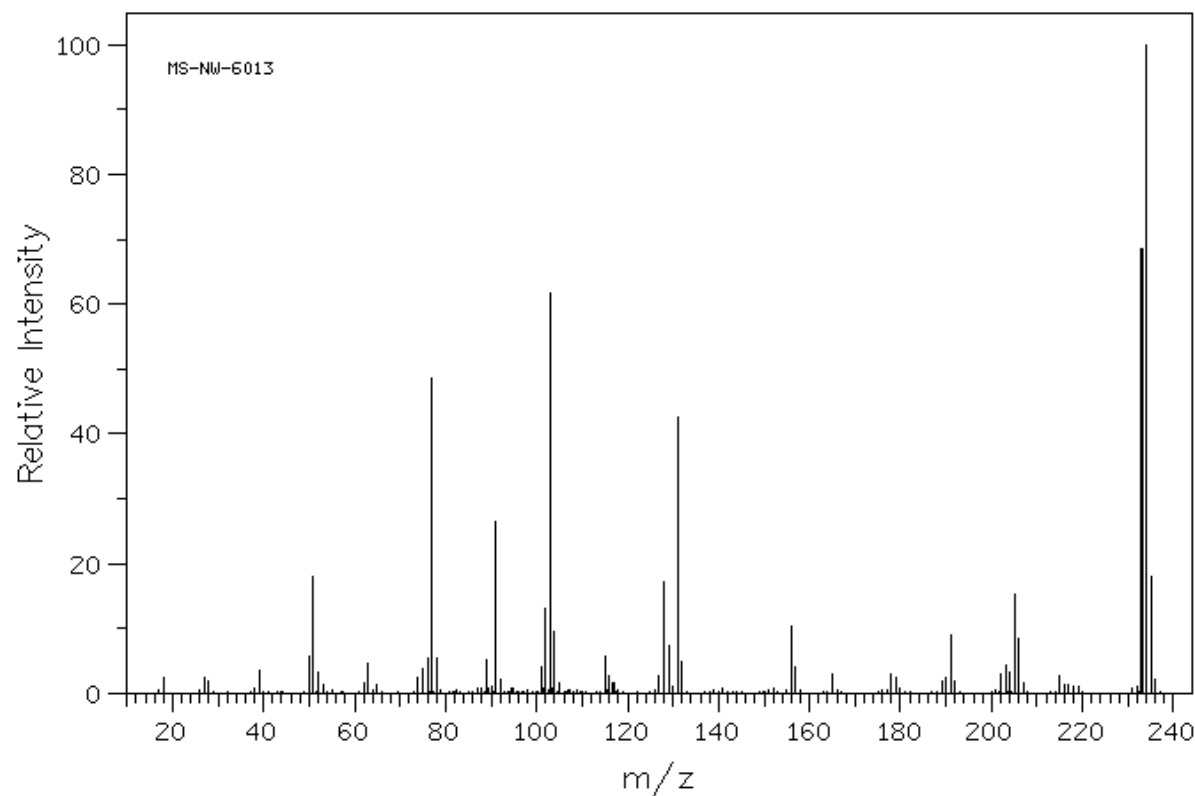


## SPECRTA

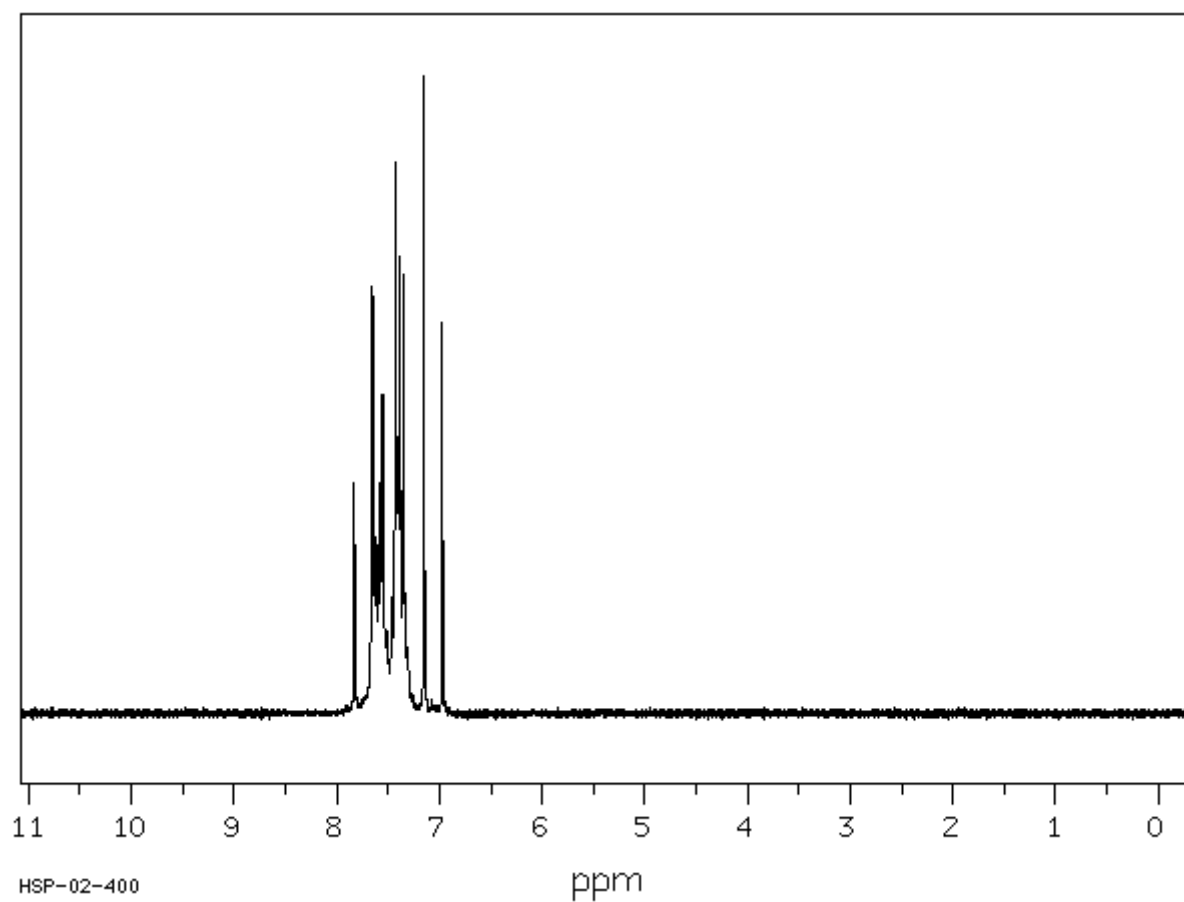
a) FT IR spectrum in KBr tablet.



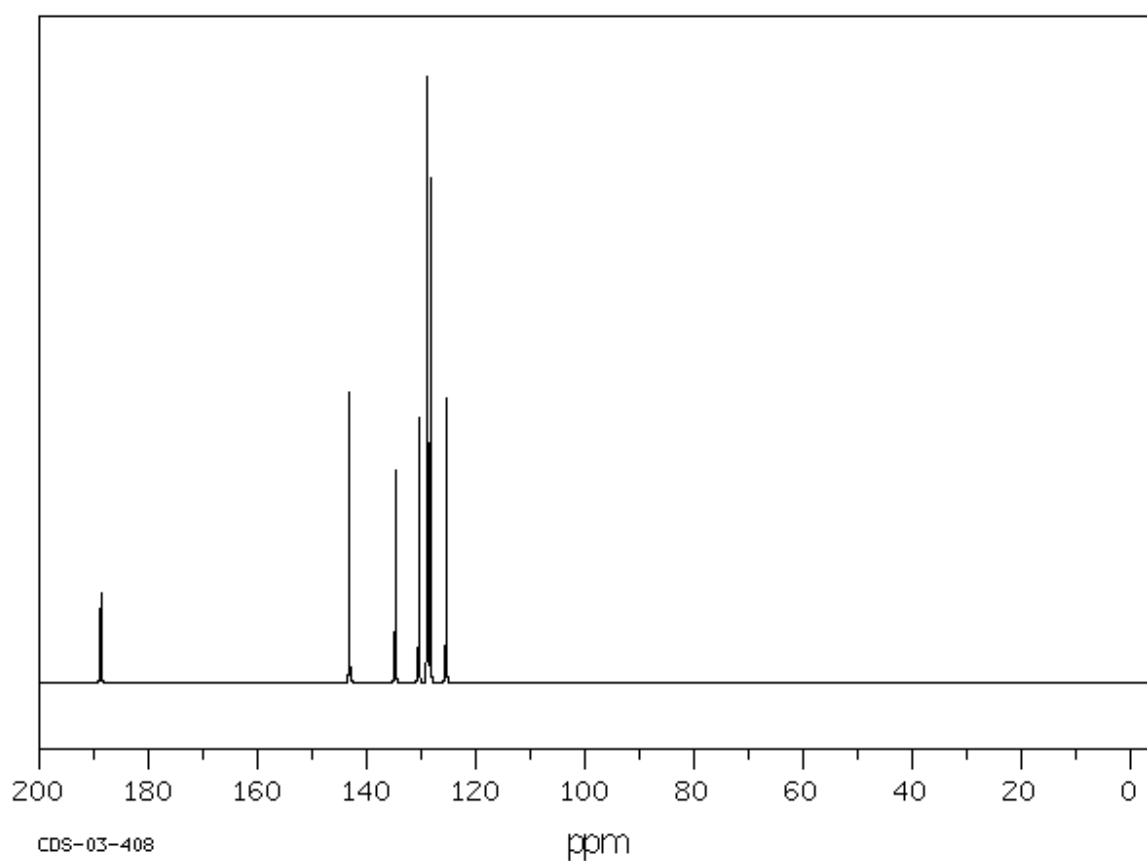
b) MS spectrum

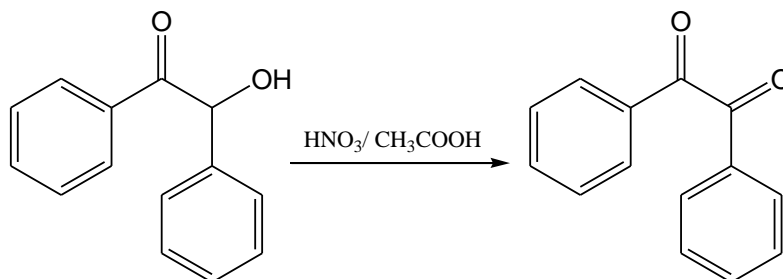


c)  $^1\text{H}$  NMR spectrum in  $\text{CDCl}_3$



d)  $^{13}\text{C}$  NMR spectrum in  $\text{CDCl}_3$



**PHENYTOINE (2 steps synthesis)****Step 1 - DIBENZOYL****Reagents:**

benzoine	4 g
anh. acetic acid	20 mL
conc. nitric acid	10 mL
ethanol	10 mL
pH paper	
$\text{CH}_2\text{Cl}_2$	

**Instrumentation and glassware:**

round-bottomed flask 100 mL  
crystallizing dish  
heating mantle  
stirrer  
gas supply pipe  
beaker 200 mL  
filtering flask with Büchner funnel  
Petri dish

Place 4g of benzoine and 20 mL of AcOH in 100 mL flask. Operating under the hood add 10mL nitric acid and reflux the mixture for 1 hour. Check the reaction progress with TLC (use  $\text{CH}_2\text{Cl}_2$  as mobile phase).

After the substrate reacted completely cool the mixture and pour it into the 200 mL beaker with 40 g of ice and 20 mL of water. Stir until the separated oil solidifies completely. Filter the crude dibenzoyl on Büchner funnel and wash thoroughly with water (check the filtrate for acid with pH paper). Product can be recrystallized from ethanol (10 mL).

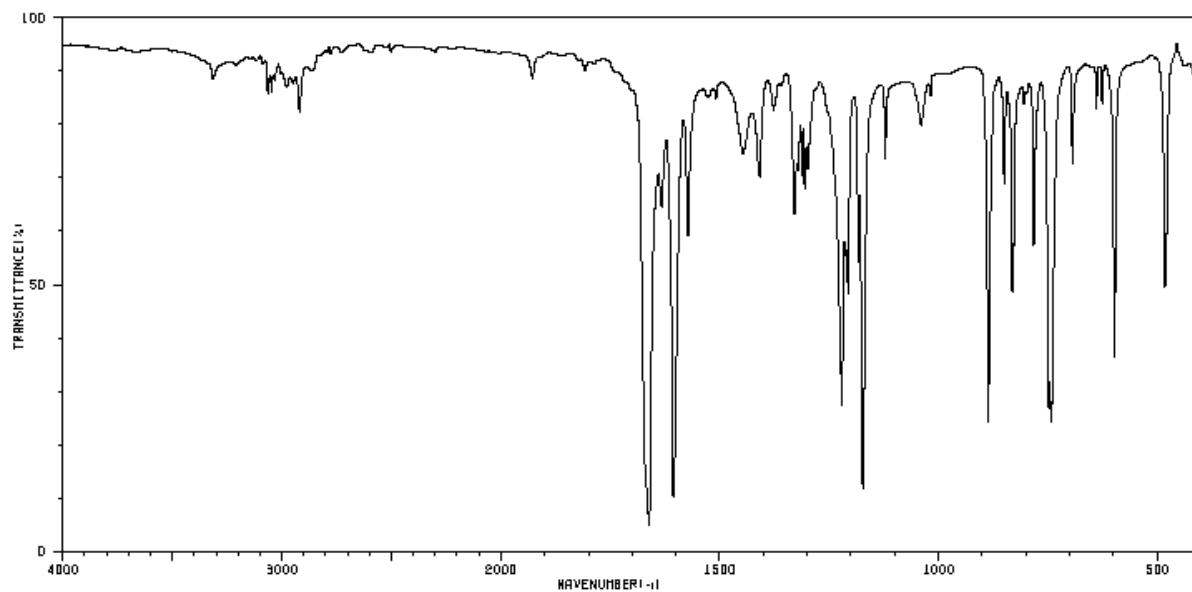
Weight the product, calculate the yield and measure the m.p. (lit. 94-96 °C).

**Thin layer chromatography (TLC):**

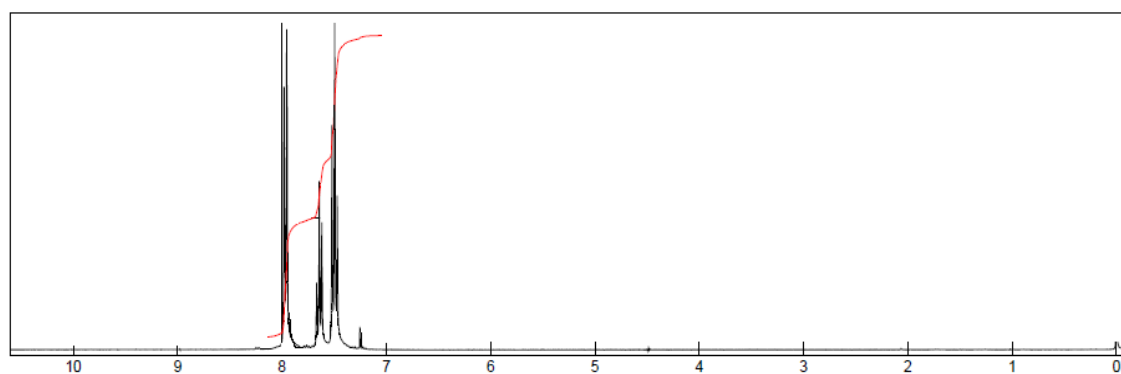
Apply the substrate and product onto  $\text{SiO}_2$  plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with  $\text{CH}_2\text{Cl}_2$  or hexane/ethyl acetate (8:2). Remove the plate and allow the solvent to evaporate and inspect under UV light. Mark the spots with pencil. Then, using forceps, dip the plate into a closed jar containing  $\text{SiO}_2$  saturated with  $\text{I}_2$ .

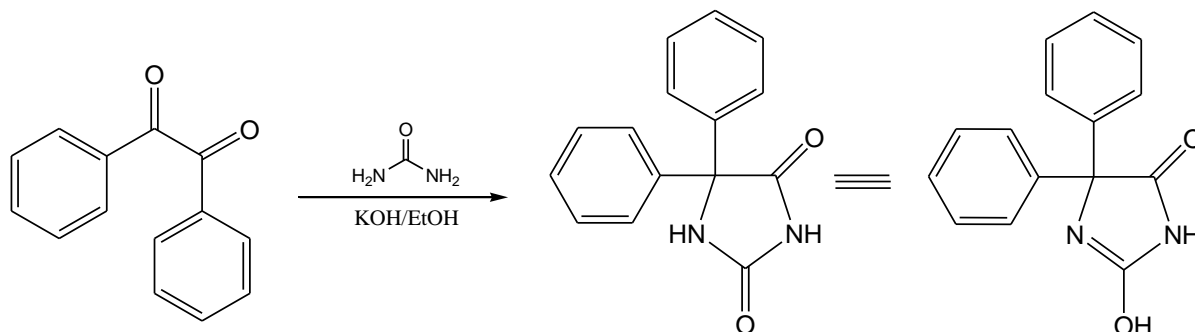
## SPECTRA

a) FT IR spectrum of dibenzoyl in KBr tablet.



b)  $^1\text{H}$ -NMR spectrum of dibenzoyl (400 MHz).



**5,5-DIPHENYLOIMADAZOLIDINO-2,4-DION****Step 2 - PHENYTOINE****Reagents:**

dibenzoyl	2 g
urea	0.96 g
nitric acid (conc.)	10 mL
95 % ethanol	50 mL + 16 mL
KOH	9.4 mL
$\text{CH}_2\text{Cl}_2$	10 mL

**Instrumentation and glassware:**

round-bottom flask 100 mL  
 reflux condenser  
 crystallizing dish 500 mL  
 heating mantle  
 stirrer  
 beaker 400 mL  
 filtering flask with Büchner funnel  
 graduated cylinders 100 mL and 50 mL  
 Petri dish

**Use rubber gloves when working with chemicals!**

Place 2.0 g of dibenzoyl in 100 mL flask, add 0.96 g urea and 50 mL of ethanol. Stir until reagents dissolve. Add 6 mL of 9.4 M KOH and reflux for 2 hrs. Check the reaction progress with TLC ( $\text{CH}_2\text{Cl}_2$  as mobile phase).

After the reaction is completed, filter off the precipitate and pour the filtrate into 400 mL beaker and add ice-water mixture (50 g +50 mL). Put the beaker into ice bath made from the crystallizing dish and add dropwise 10% HCl until the pH of the mixture reaches 4-5.

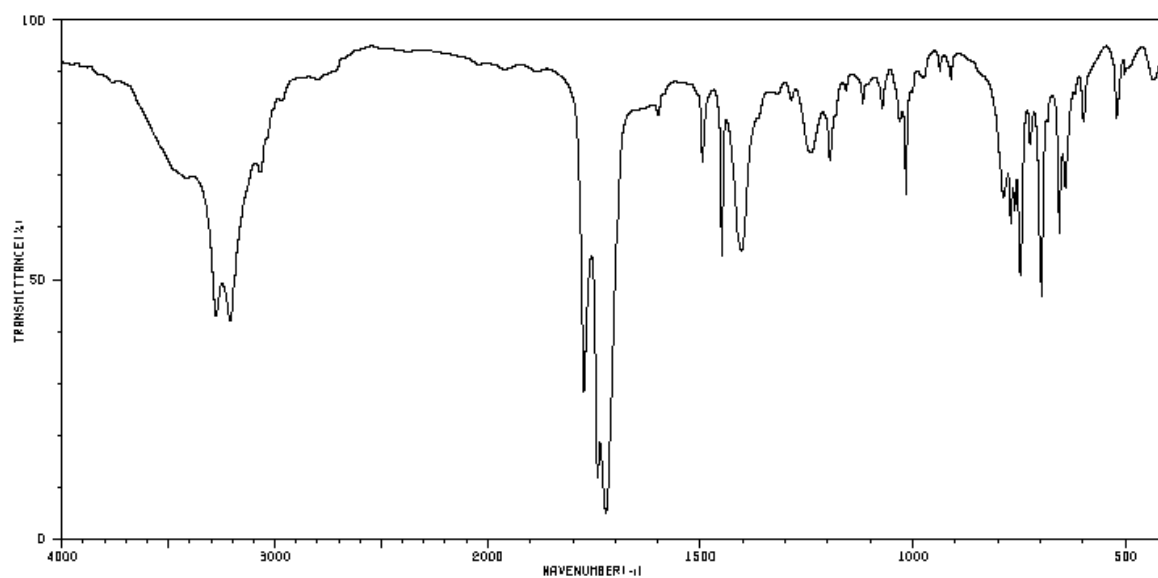
Filter the ice-cold mixture. Recrystallize crude product from the ethanol-water mixture (8:2). Weigh the product, calculate the yield and check the melting point (lit. 293-294 °C).

Thin layer chromatography (TLC):

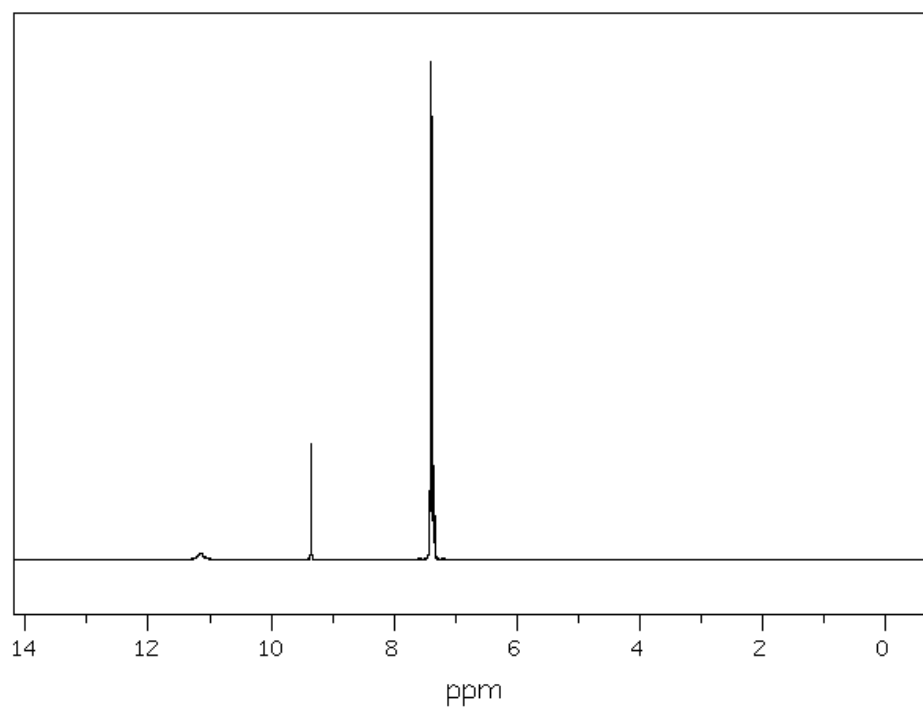
Apply the substrate and product onto  $\text{SiO}_2$  plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with  $\text{CH}_2\text{Cl}_2$  or hexane/ethyl acetate (8:2). Remove the plate and allow the solvent to evaporate and inspect under UV light. Mark the spots with pencil. Then, using forceps, dip the plate into a closed jar containing  $\text{SiO}_2$  saturated with  $\text{I}_2$ .

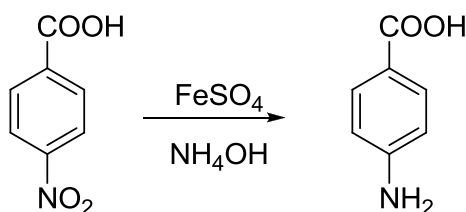
## SPECTRA

a) FTIR spectrum of phenytoine in KBr.



b)  $^1\text{H}$  NMR spectrum of phenytoine in DMSO- $\text{d}_6$  (400 MHz).



**BENZOCAINE (2 steps synthesis)****Step 1. 4-AMINOBENZOIC ACID****Reagents:**

4-nitrobenzoic acid	2,1 g
12% $\text{NH}_4\text{OH}$	15 mL
25% $\text{NH}_4\text{OH}$	20 mL
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	44,6 g
Citric acid	
Ethyl acetate	
$\text{CH}_2\text{Cl}_2$	

**Instrumentation and glassware:**

Erlenmeyer flask 250 mL  
 round-bottom flask 250 mL  
 filtering flask with Büchner funnel  
 round-bottom flask 50 mL  
 graduated cylinders 100 mL  
 beaker 250 mL  
 stirrer  
 crystallizing dish 500 mL

In a round-bottomed flask (250 mL) with magnetic stirrer place 44,6 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 50 mL  $\text{H}_2\text{O}$  and boil it.

In the meantime, dissolve in Erlenmeyer flask (250 mL) 21 g of 4-nitrobenzoic acid in 15 mL 12%  $\text{NH}_4\text{OH}$  (place the flask in the water bath and heat slightly to dissolve the solid compound). Add dropwise this solution to the flask with salt  $\text{FeSO}_4$  and stir vigorously till the forming product prevents stirring. Then shake the flask with reaction mixture.

After 20 minutes, add slowly 15 mL of 25%  $\text{NH}_4\text{OH}$  up to pH=9 (check pH for base with pH paper). Stir additionally 20 minutes. Then the hot mixture filter on Büchner funnel.

**Important is the solution!!!!** Acidify it with citric acid to pH=4 and move the mixture to separatory funnel and extract (5x30 mL) with the mixture ethyl acetate/ $\text{CH}_2\text{Cl}_2$  1:2 (v/v). Combine the organic layers and dry the extract over  $\text{MgSO}_4$ , then filtrate through the funnel with cotton plug to remove  $\text{MgSO}_4$ . Transfer the combined fractions into a clean, dry and **weighted** round-bottom flask (100 mL) and concentrate the solution on rotary evaporator.

The crude yellow crystals of 4-aminobenzoic acid will be used to the second step of reaction. Measure the m.p. (lit. 192 °C).

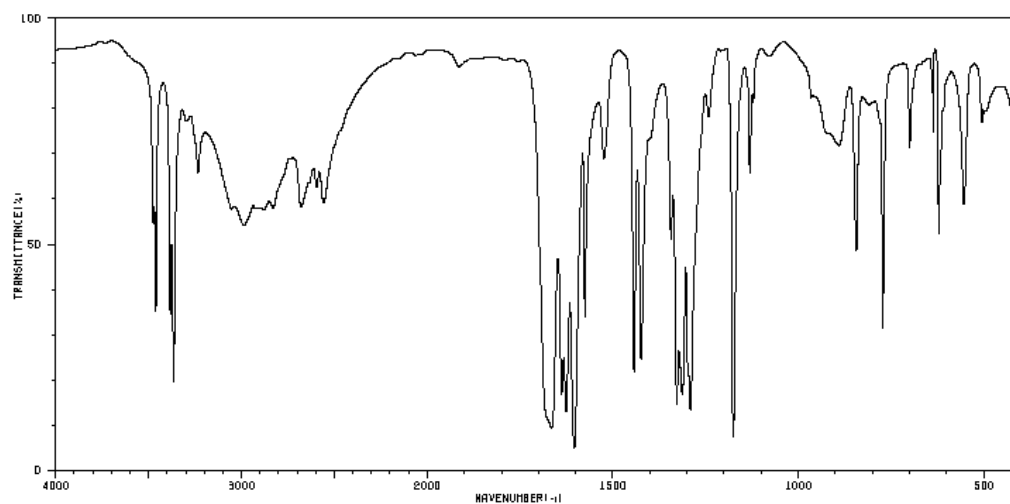
**Warn! The product dissolve easily in water, ethanol and ether!**

Thin layer chromatography (TLC):

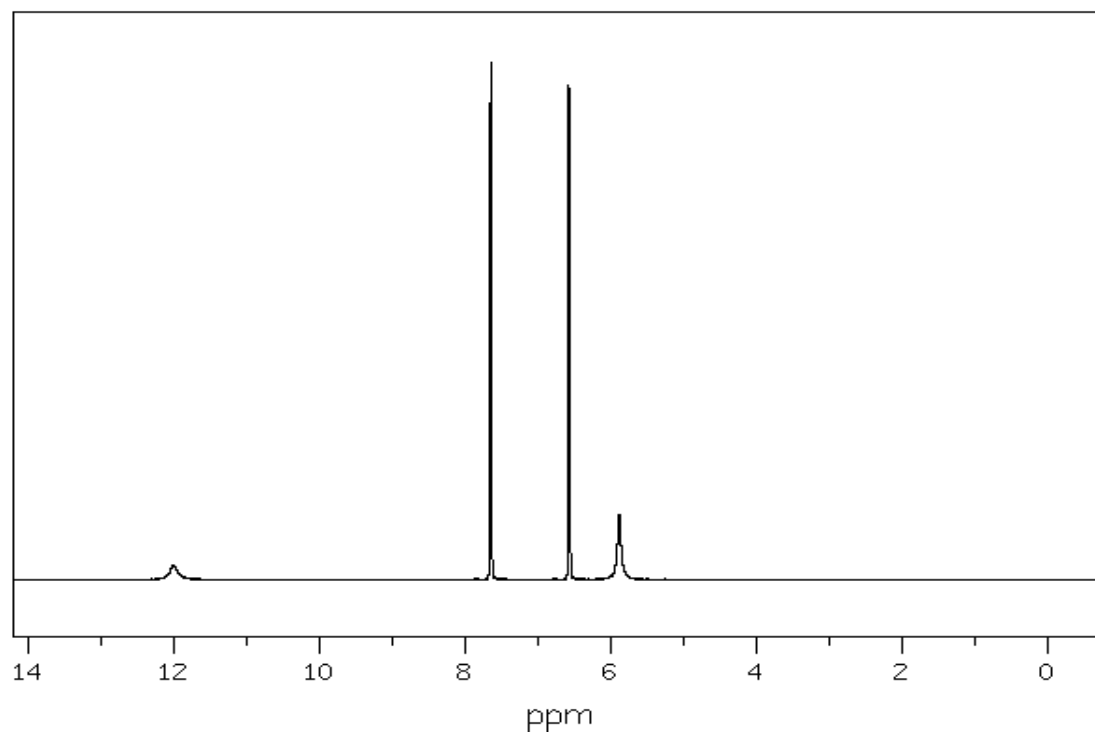
Apply the substrate and product onto  $\text{SiO}_2$  plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with  $\text{CH}_2\text{Cl}_2$  /MeOH (8:2). Mark the spots with pencil under UV light.

## SPECTRA

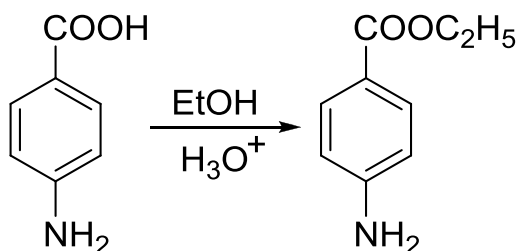
a) FTIR spectrum in KBr tablet .



b)  $^1\text{H}$ -NMR spectrum in  $\text{DMSO-d}_6$  (400 MHz).





**Step 2. ETHYL 4-AMINO BENZOATE (BENZOCAINE)****Reagents:**

4-aminobenzoic acid    1.0 g  
abs. EtOH                8 mL  
 $\text{H}_2\text{SO}_4$  (10% oleum)    0.6 mL  
5%  $\text{NH}_4\text{OH}$             ~5 mL  
pH papers

**Instrumentation and glassware:**

round-bottom flask 100 mL or 50 mL  
Liebig condenser  
separatory funnel  
Erlenmeyer flask 100 mL  
filtering flask with Büchner funnel  
graduated cylinder 100 mL  
beaker 100 mL  
heating mantel  
Petri dish

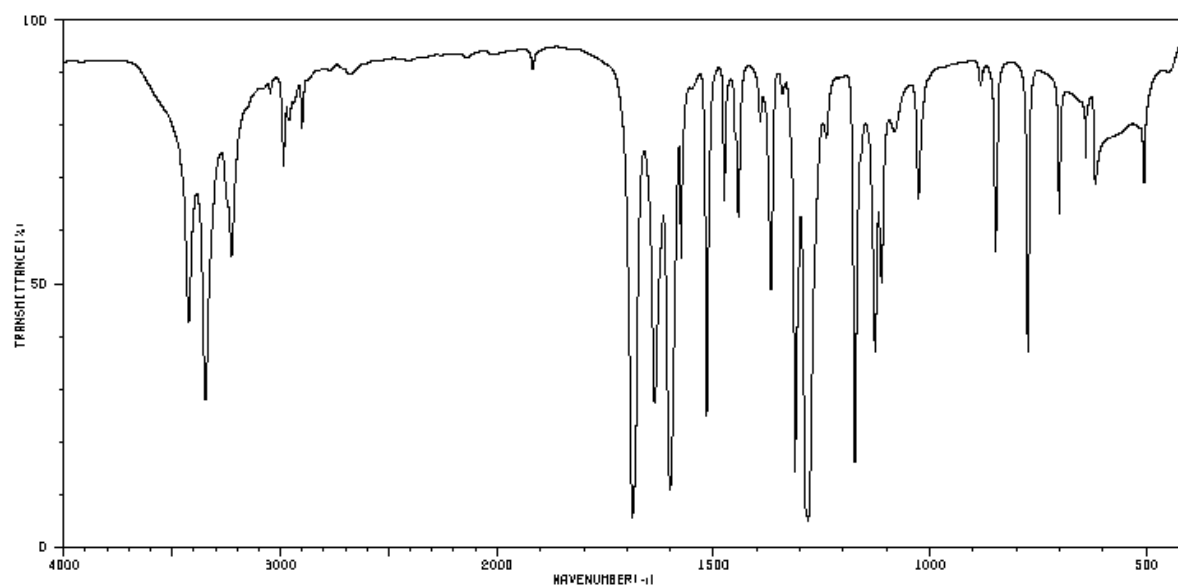
In a round-bottomed flask (100 mL) place 1 g of 4-aminobenzoic acid, 8 mL anh. ethanol and 0,6 mL of 10% oleum. Adjust the Liebig condenser and heat under reflux for 4 hours. Cool down the mixture and alkalize with aq.  $\text{NH}_4\text{OH}$  to pH=10. Move the mixture to separatory funnel and extract with ethyl acetate (3x30 mL). Combine the organic layers and wash this fraction with NaCl solution. Dry the extract over  $\text{MgSO}_4$ , then filtrate through the funnel with cotton plug to remove  $\text{MgSO}_4$ . Transfer the combined fractions into a clean and dry round-bottom flask (100 mL) and concentrate the solution on rotary evaporator. The crude product recrystallize from chloroform. Filter the crystals on Büchner funnel, and dry on air on Petri dish. Weight the product, calculate the percentage yield and measure the m.p. (lit. 92 °C).

**Thin layer chromatography (TLC):**

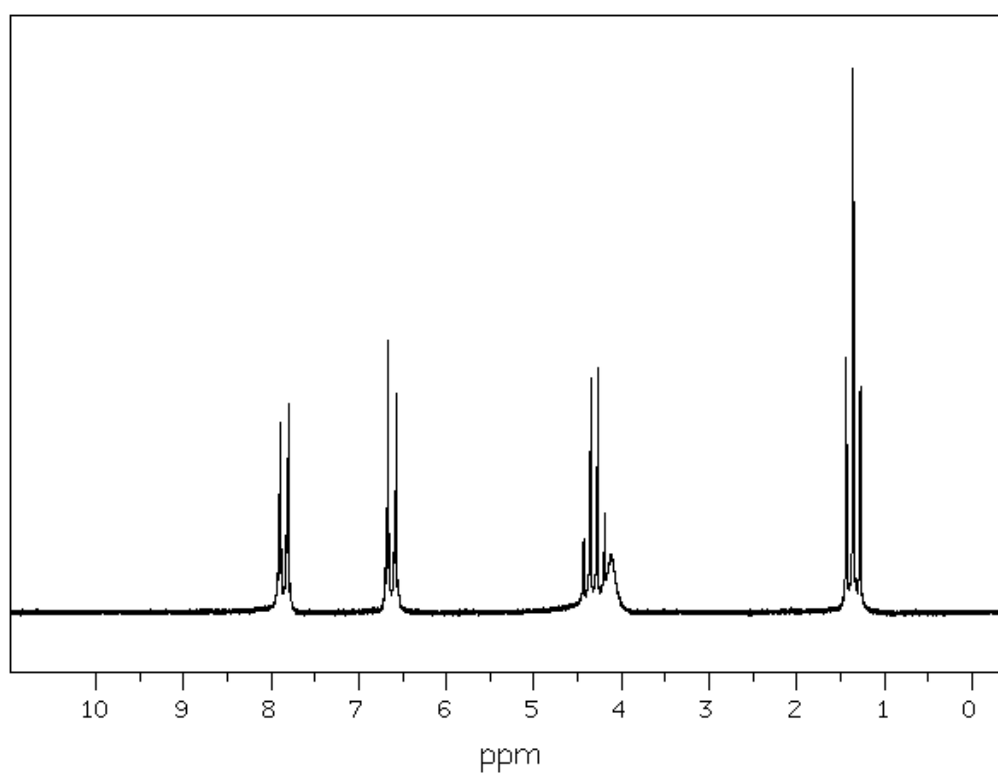
Apply the substrate and product onto  $\text{SiO}_2$  plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with  $\text{CH}_2\text{Cl}_2$  / MeOH (8:2). Remove the plate and allow the solvent to evaporate and inspect under UV light. Mark the spots with pencil. Then, using forceps, dip the plate into a closed jar containing  $\text{SiO}_2$  saturated with  $\text{I}_2$ .

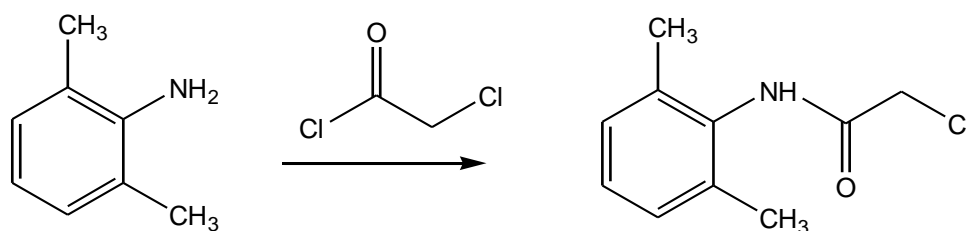
## SPECTRA

a) FTIR spectrum of benzocaine in KBr tablet.



b)  $^1\text{H}$ -NMR spectrum of benzocaine in  $\text{CDCl}_3$



**LIDOCAINE (2 steps synthesis)****Step 1 – 2-CHLORO-2,6-DIMETHYLACETANILIDE****Reagents:**

2,6-dimethylaniline	0.5 g
anh. CH <sub>3</sub> COOH	3.6 mL
chloroacetyl chloride	0.37 mL
sodium acetate	0.75 g
toluene	4.5 mL
diethylamine	0.42 mL
pH papers	

**Instrumentation and glassware:**

crystallizing dish 500 mL  
 stirrer with heating  
 Erlenmeyer flask 50 mL  
 Erlenmeyer flask 25 mL  
 filtering flask with Büchner funnel  
 graduated cylinder 25 mL  
 Petri dish

**Carry out the following reaction in the fume hood!**

In a clean, dry 50-mL Erlenmeyer flask, mix 0.5 g of 2,6-dimethylaniline, 3.6 mL of glacial acetic acid, and 0.37 mL of chloroacetyl chloride ( in that order), under the fume hood.

Carefully warm this mixture in a hot water bath with swirling for 4 minutes (use hot tap water), remove from the bath, and add a solution of 0.75 g of sodium acetate in 15 mL of distilled water (previously prepared in a 25 mL Erlenmeyer flask).

Cool down the mixture in an ice bath for a few minutes, and collect the product on a Büchner funnel. Rinse the solid with small portions of water until acetic acid odor is gone and dry it by pressing and drawing air through the filter cake on the funnel for about 15 min. Transfer the product to a filter paper and let it air-dry.

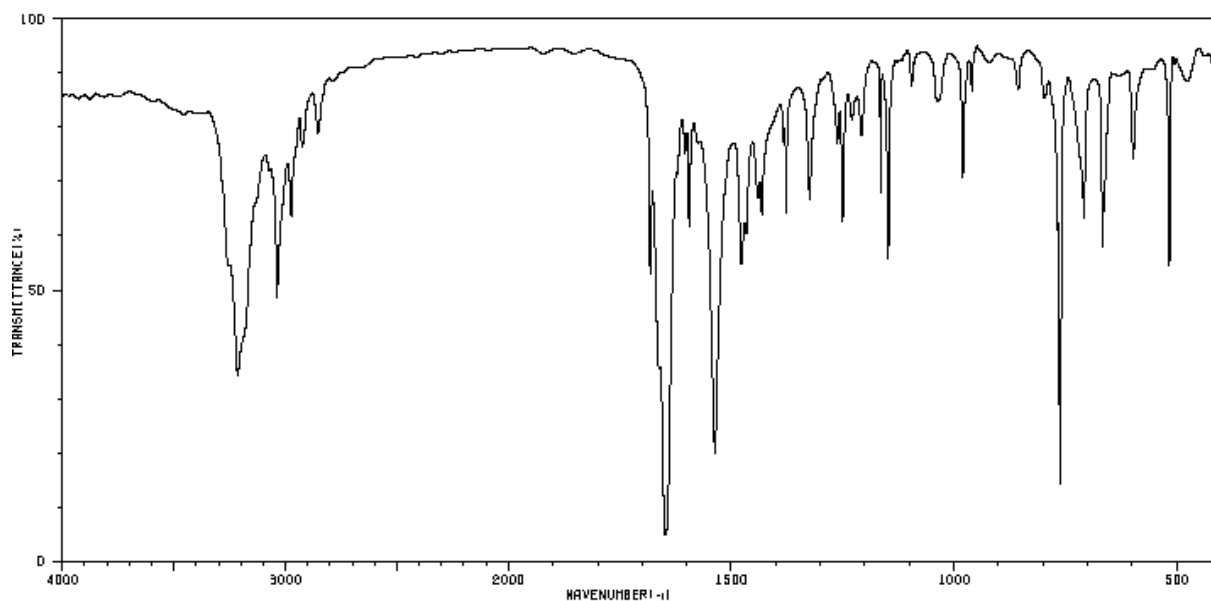
Weight the product, calculate the percentage yield and measure the m.p. (lit. 145-146 °C).

**Thin layer chromatography (TLC):**

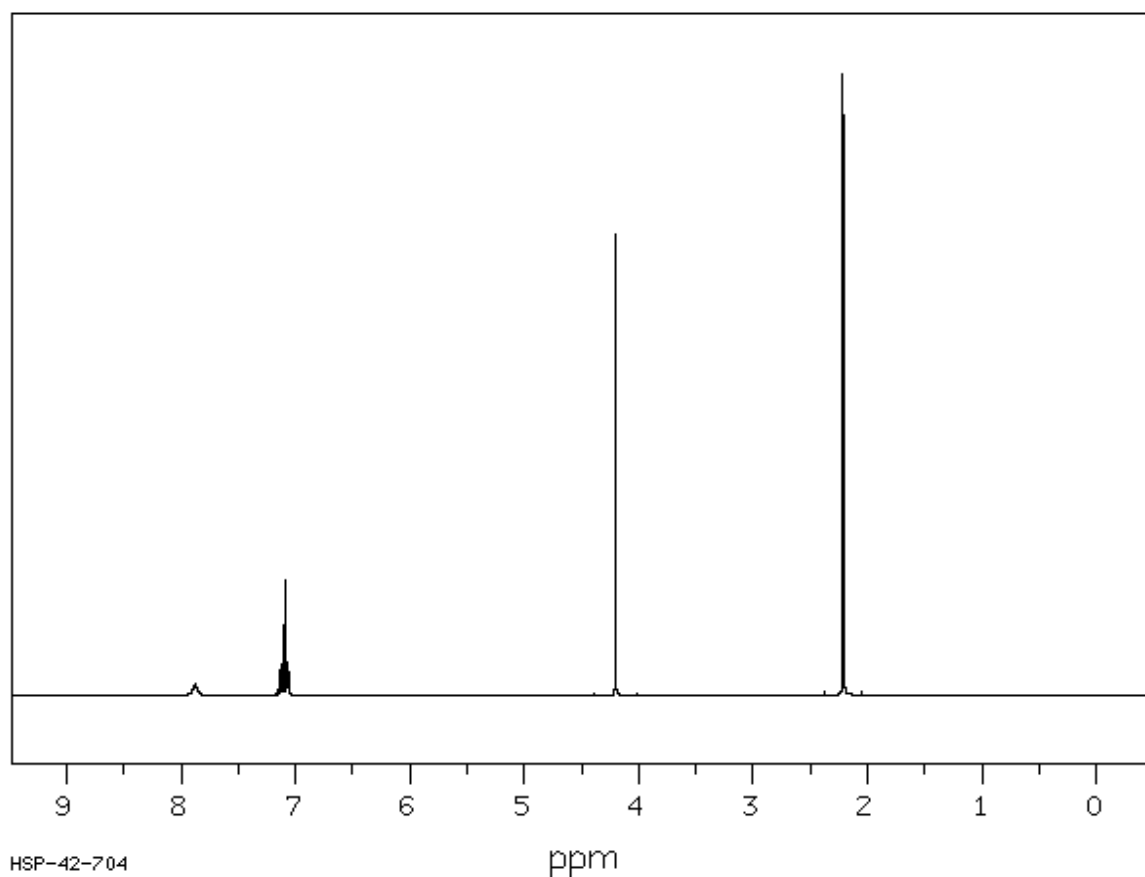
Apply the substrate and product onto SiO<sub>2</sub> plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with hexane/ethyl acetate (9.5:0.5). Remove the plate and allow the solvent to evaporate and inspect under UV light. Mark the spots with pencil. Then, using forceps, dip the plate into closed jar containing SiO<sub>2</sub> saturated with I<sub>2</sub>.

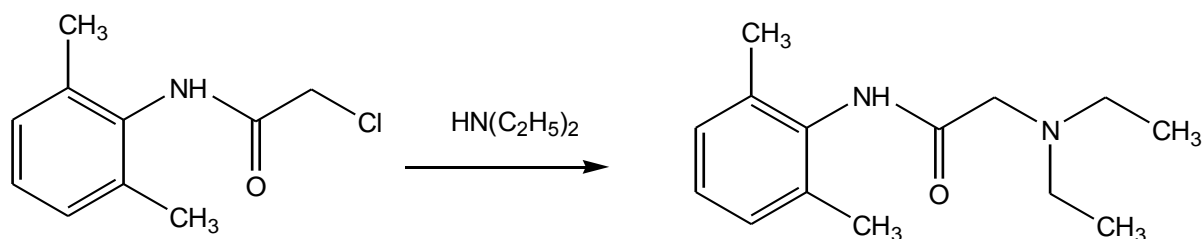
**SPECTRA**

a) FTIR spectrum of 2-chloro-2,6-dimethylacetanilide in KBr tablet.



b) <sup>1</sup>H-NMR spectrum of 2-chloro-2,6-dimethylacetanilide in CDCl<sub>3</sub> (400 MHz).



**STEP 2 – 2 DIETHYLAMINO-2,6-DIMETHYL-ACETANILIDE (LIDOCAINE)****Reagents:**

2-chloro-2,6-dimethylacetanilide	0.3 g
toluene	4.5 mL
diethyloamine	0.42 mL
diethyl ether	20 mL
pH papers	

**Glassware:**

round-bottom flask 10 mL  
 reflux condenser  
 stirrer and heating mantel  
 graduated pipette  
 filtering flask with Büchner funnel  
 separatory funnel 100 mL  
 Erlenmeyer flask 125 mL  
 graduated cylinder 25 mL  
 funnel  
 Petri dish

In a 10 mL round-bottom flask, place 0.3 g of obtained 2-chloro-2,6-dimethylacetanilide, 4.5 mL of toluene and a stirring bar. Add 0.42 mL of diethylamine to the reaction mixture. Attach a reflux condenser and reflux vigorously for 90 minutes. Then cool down the mixture and store until the next lab period.

Dissolve the formed solid under reflux. Cool the mixture and filter out the crystals formed on a funnel and **collect the filtrate**. If you do not observe any precipitate just transfer the filtrate to separatory funnel and wash with water (2x 5 ml) and extract with dil. HCl (1:5, 3x5 ml). Combine acid extracts, made them strong alkaline with 30% KOH (ca. 5 ml). Cool in ice, dark-yellow lidocaine separates. Filter the crystals and extract aqueous phase with hexane (2x10 ml). Combine extracts and crude crystals which dissolve and dry above anh.  $K_2CO_3$ , filter the drying agent using cotton tipped glass funnel. Add decolorizing carbon and heat under reflux. Cool down, filter out the carbon and concentrate to approx. 3 mL. Lidocaine crystallizes as colorless crystals. Filter the crystals and dry them on air.

Weigh the product, calculate the percentage yield and measure the m.p. (lit. 68-69 °C).

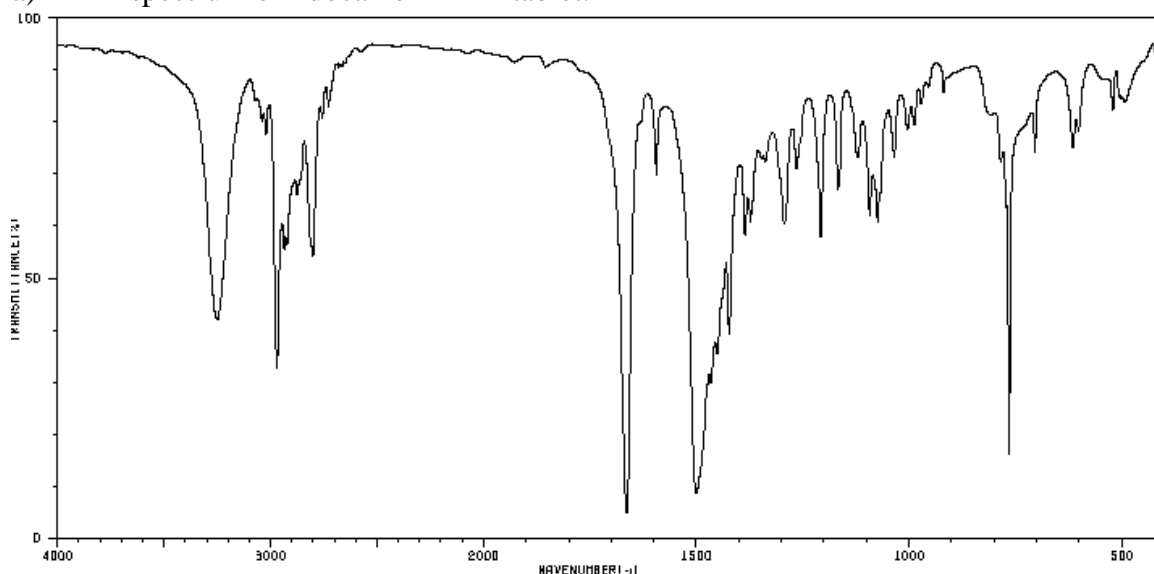
**Thin layer chromatography (TLC):**

Apply the substrate and product onto  $SiO_2$  plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with hexane/ethyl acetate (9.5:0.5). Remove the plate and allow the solvent to evaporate and inspect under UV light. Mark the spots with pencil. Then, using forceps, dip the plate into closed jar containing  $SiO_2$  saturated with  $I_2$ .

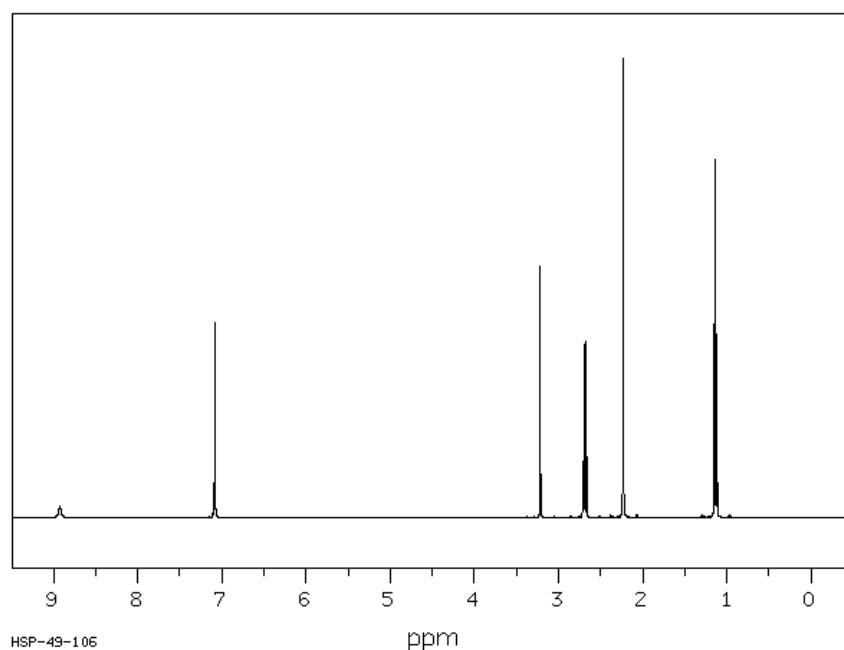
The crude lidocaine is reconverted to the crystalline salt, lidocaine hydrogen sulfate, by dissolving it in diethyl ether (10 mL of solvent per g of solute) and then adding a solution of 2 mL of 2.2 M sulfuric acid in ethanol per g of solute. Mix the solutions thoroughly and scratch at the air-liquid interface to induce crystallization.. Dilute the mixture with an equal volume of acetone to facilitate filtration. Isolate the precipitated salt by vacuum filtration. Rinse the product with acetone and then air-dry. Weight the product, calculate the percentage yield and measure the m.p. (lit. 210-212 °C).

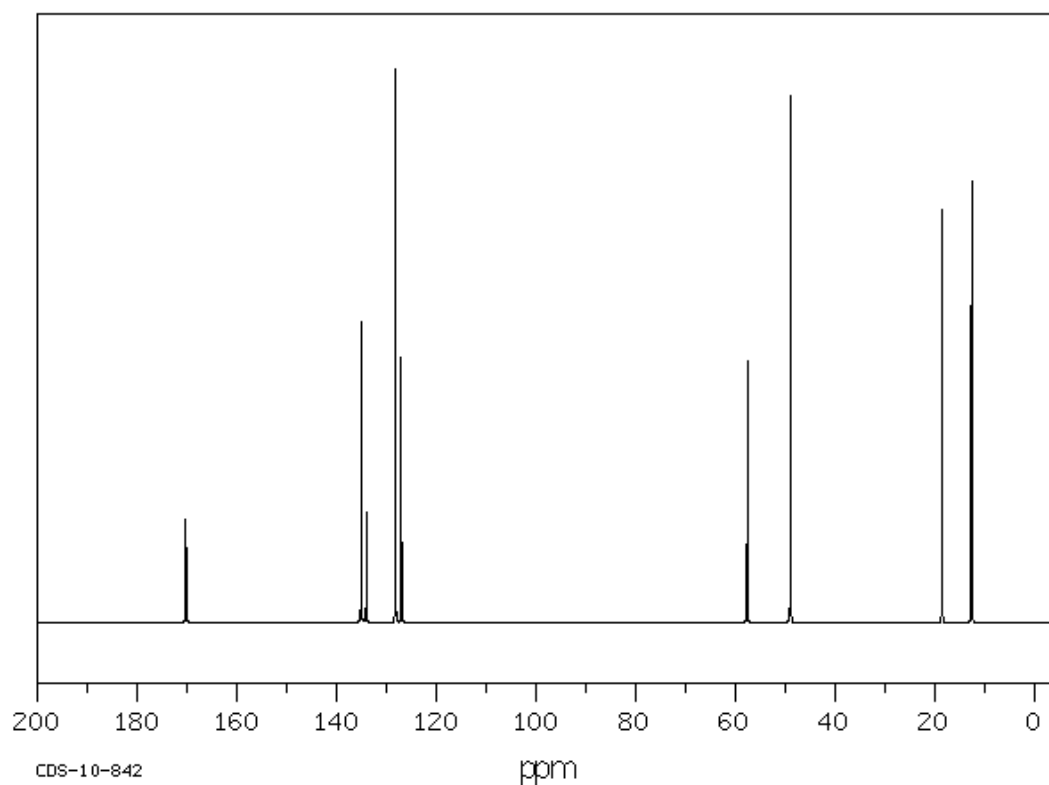
## SPECTRA

a) FT IR spectrum of lidocaine in KBr tablet.

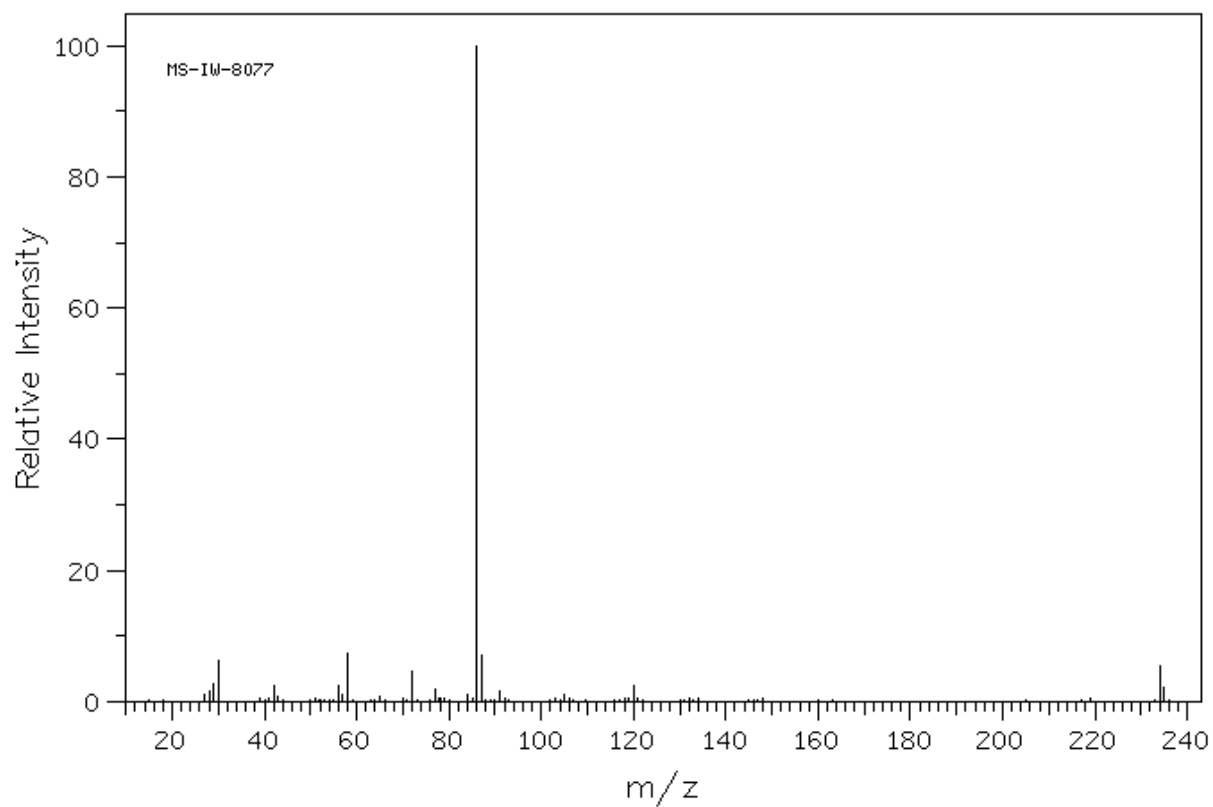


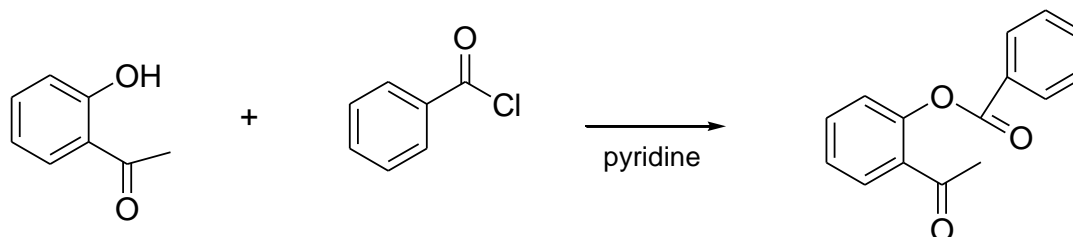
b) <sup>1</sup>H-NMR spectrum of lidocaine in CDCl<sub>3</sub> (400 MHz)



c)  $^{13}\text{C}$  NMR spectrum of lidocaine in  $\text{CDCl}_3$ 

## d) MS spectrum of lidocaine



**FLAVONE (3 steps synthesis)****STEP 1      2-BENZOILOXOACETOPHENONE****Reagents:**

<i>o</i> -hydroxyacetophenone	3.4 g
benzoyl chloride	4 mL
pyridine	5 mL
1M hydrochloric acid	120 mL
methanol	15 mL

**Instrumentation and glassware:**

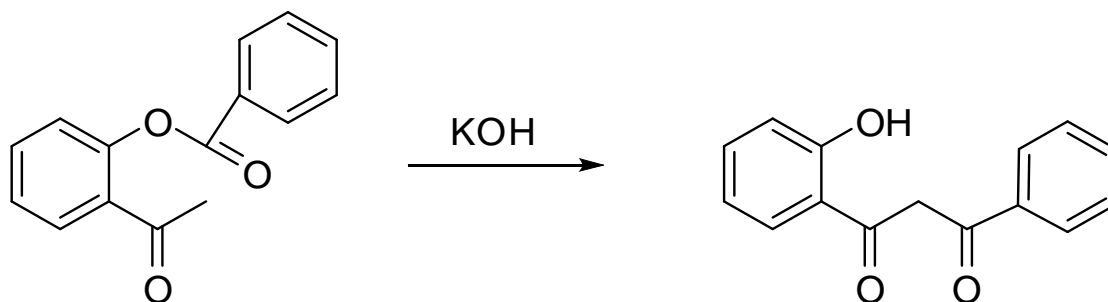
conical flask with stopper 50 mL  
 beaker 250 mL  
 filtering flask with Büchner funnel

Place 3.4 g *o*-hydroxyacetophenone into conical flask (50 mL) add 4 mL benzoyl chloride and 5 mL anhydrous, freshly distilled pyridine and close the flask with stopper. **All operations should be done under efficiently working hood and with protecting gloves!** Flask should be shaken till its content is mixed. The temperature of reaction mixture will raise. After 20 min. carry the reaction mixture into a beaker with 120 mL of 1M hydrochloric acid with 50 g of crushed ice. Filter the product on Büchner funnel and wash with 5 mL of methanol cooled in ice bath and then with 5 mL of distilled water. To recrystallize product dissolve it in methanol (6 - 8 mL), heat and then cool down the mixture in ice bath and filter the product under reduced pressure. Weigh and calculate the yield. Measure melting point of *o*-benzoyloxyacetophenone (lit. m.p. 87- 88°C).

**Thin layer chromatography (TLC):**

on SiO<sub>2</sub>, CHCl<sub>3</sub>/hexane (8:2), the intensity of the spots check under UV light before developing the plate in the mobile phase. Remove the plate and allow the solvent to evaporate. The spots of alkaloids mixture is visible in the UV light. Mark the spots in a pencil.



**STEP 2** 2-HYDROXYDIBENZOYLMETHANE**Reagents:**

<i>o</i> -benzoyloxyacetophenone	4 g
pyridine	15 mL
granulated potassium hydroxide	1,4 g
10% acetic acid	21 mL
methanol	

**Instrumentation and glassware:**

Round-bottomed flask 50 mL  
 condenser  
 water bath  
 magnetic stirrer  
 glass rod  
 filtering flask with Büchner funnel

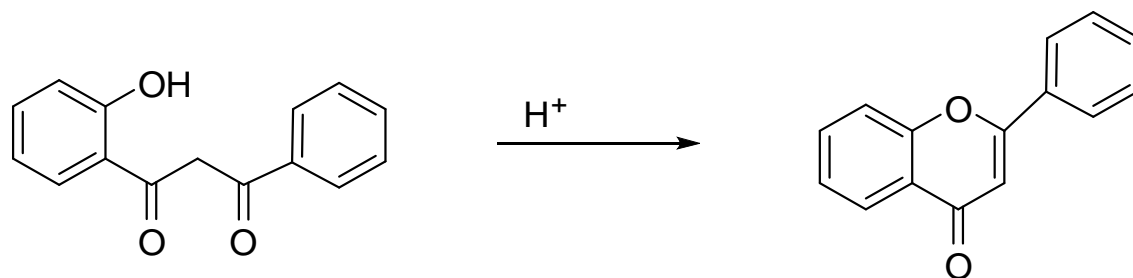
**All operations should be done under efficiently working hood!**

Place 4 g of *o*-benzoiloxoacetophenone in 15 mL of pyridine in round-bottomed flask (50 mL) equipped with condenser and placed in a water bath and magnetic stirrer and heat under reflux to 50°C. Then add 1,4 g of granulated potassium hydroxide. Mix the reaction mixture for 15 min., if the yellow precipitate of potassium salt prevents stirring, then mix with glass rod. Cool down the reaction mixture to the room temperature and add 21 mL of 10% acetic acid with stirring. Filter yellow precipitate on Büchner funnel and dry at the 50°C. Weight and calculate the yield. Measure the melting point of the *o*-hydroxydibenzoylmethane (lit. m.p. 117-120°C). Product obtained is pure enough to be used in the next synthesis step. After recrystallization with methanol melting point of *o*-hydroxydibenzoylmethane is 121-122°C.

Thin layer chromatography (TLC):

on SiO<sub>2</sub>, CHCl<sub>3</sub>/hexane (8:2), the intensity of the spots check under UV light before developing the plate in the mobile phase. Remove the plate and allow the solvent to evaporate. The spots of alkaloids mixture is visible in the UV light. Mark the spots in a pencil.

## STEP 3 FLAVONE

**Reagents:**

*o*-hydroxydibenzoylmethane 3 g  
glacial acetic acid 17 mL  
sulfuric acid conc. 0,7 mL  
ice and distilled water

**Instrumentation and glassware:**

round bottomed flask 50 mL  
beaker 200 mL  
condenser  
water bath  
filtering flask with Büchner funnel

To the solution of 3 g *o*-hydroxydibenzoylmethane in 17 mL glacial acetic acid placed in round bottomed flask (50 mL) equipped with condenser and water bath add, with stirring, 0,7 mL concentrated sulfuric acid. Heat the solution for 1 h shaking the flask gently from time to time. In the next step carry the reaction mixture into a beaker (200 mL) with 80 g crushed ice and keep the mixture aside till the ice completely melts. Then filter the product and wash with water until the filtrate is neutral (approximately 170 mL of water) and dry at the  $50^{\circ}\text{C}$ .

Weigh the product and calculate the yield. Measure the melting point of flavone (lit. m.p.  $95 - 97^{\circ}\text{C}$ ). After recrystallization with large volume of petroleum ether pure flavone with m.p.  $98^{\circ}\text{C}$  can be obtained as colorless needles.

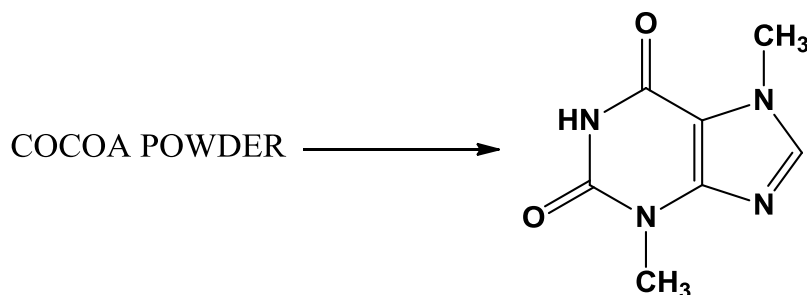
**Thin layer chromatography (TLC):**

on  $\text{SiO}_2$ ,  $\text{CHCl}_3$ /hexane (8:2), the intensity of the spots check under UV light before developing the plate in the mobile phase. Remove the plate and allow the solvent to evaporate. The spots are visible in the UV light. Mark the spots in a pencil.



# NATURAL PRODUCTS

## ISOLATION OF THEOBROMINE FROM COCOA POWDER

**Reagents:**

Cocoa powder	10 g
Magnesium oxide (MgO)	3 g
Methanol	10 mL
Methylene chloride	350 mL
Diethyl ether	65 mL
Iodine (I <sub>2</sub> )	1 g
Potassium iodide (KI)	2 g
Ethanol	100 mL

**Instrumentation and glassware:**

Heating mantle
Round flask 250 mL
Measuring cylinder
Round flask 100 mL
Cooler
Glass rod
filtration kit with Büchner funnel
beaker 100 mL

In a round bottom flask (250 mL) prepare the mixture of cocoa powder (10 g) and methanol (15 mL) then add the solution of MgO (3g) in water (15 mL). The mixture is stirred with a glass rod and heated in heating mantle to dryness. It takes approximately 1 hour. To the dry substance received add 170 mL of methylene chloride and heat under reflux for 30 min. Next, filter the contents on a Büchner funnel.

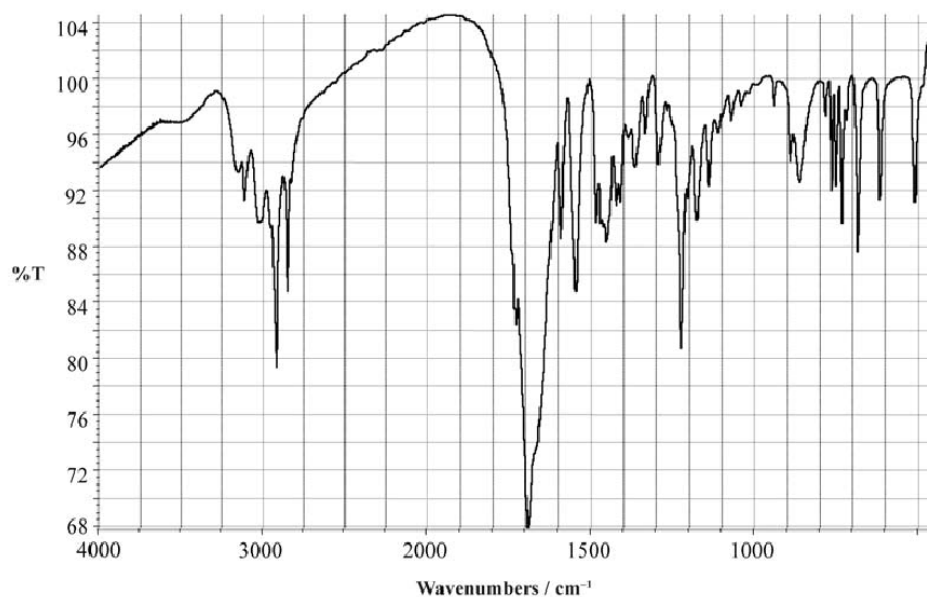
Dry the solution over MgSO<sub>4</sub>. Crush the solid substance and once more put it into a round bottom flask, add 170 mL of methylene chloride. Heat the mixture under reflux for additional 30 min and once more filter on the Büchner funnel.

Dry the extract over MgSO<sub>4</sub>, then filtrate through the funnel with cotton plug to remove MgSO<sub>4</sub>. Transfer the combined fractions into a clean and dry round-bottom flask (100 mL) and concentrate the solution to 10 mL. Move the solution to a beaker (100 mL) wash carefully with chloroform and transfer also to the beaker. Add 45 mL of ether and leave to crystallization to obtain micro-crystals then wash them on a Büchner funnel 5 times with 10 mL of ether. Yield ca. 0.15 g theobromine, mp. 351 °C.

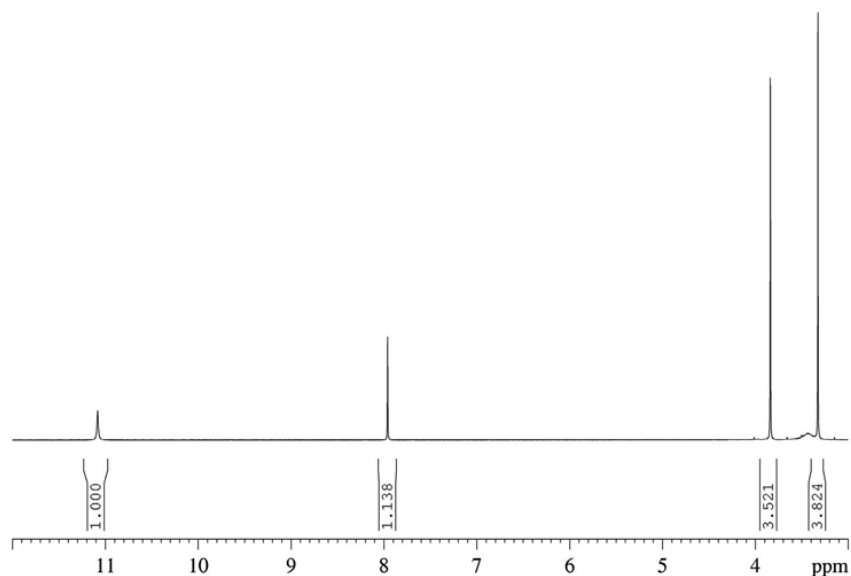
Thin layer chromatography (TLC): on SiO<sub>2</sub>, the spots of product and standard theobromine have to be very intensive (!) - check under UV light before developing the plate in the mobile phase: chloroform-hexane (9:0.5). Remove the plate and allow the solvent to evaporate. The spot of theobromine is visible in the UV light. Mark the spot in pencil. Then dip the plate into the reagent prepared in advance: I<sub>2</sub> (1g), KI (2 g) in EtOH (100 mL). After drying, using forceps dip your plate into the mixture of 25% HCl and ethanol (1:1). The spot of theobromine turns to grey-bluish color and impurities of caffeine to braun-redish color.

**SPECTRA**

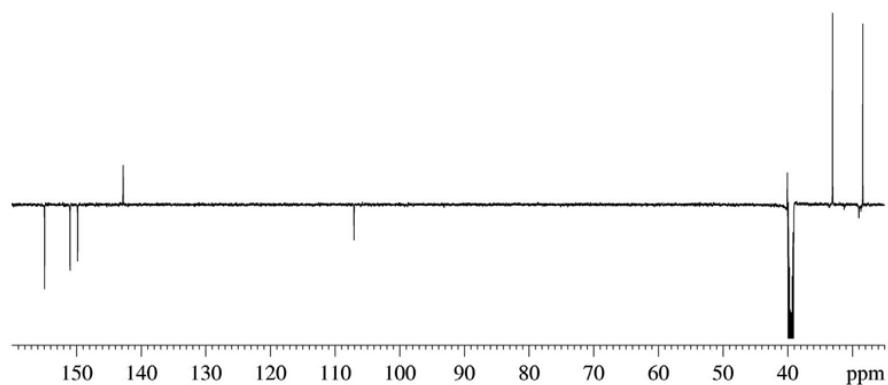
a) FT IR spectrum of theobromine in KBr tablet.

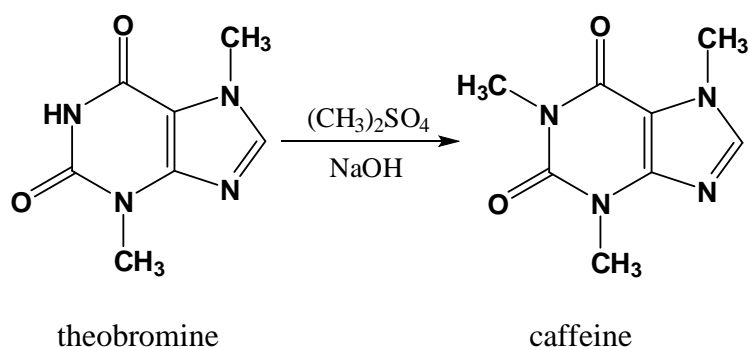


b)  $^1\text{H}$  NMR spectrum of theobromine in DMSO- $\text{d}_6$  (400 MHz).



c) APT  $^{13}\text{C}$  NMR spectrum of theobromine in DMSO- $\text{d}_6$  (400 MHz).





Theobromine	2 g
aq 10% NaOH	3.35 mL
dimethyl sulfate ( <b>strong poison!</b> )	0.7 mL
Chloroform	25 mL
Anh. sodium sulfate	

Magnetic stirrer  
Measuring cylinder  
Round bottom flask 50 mL  
Separating funnel 50 mL  
Conical flask 50 mL

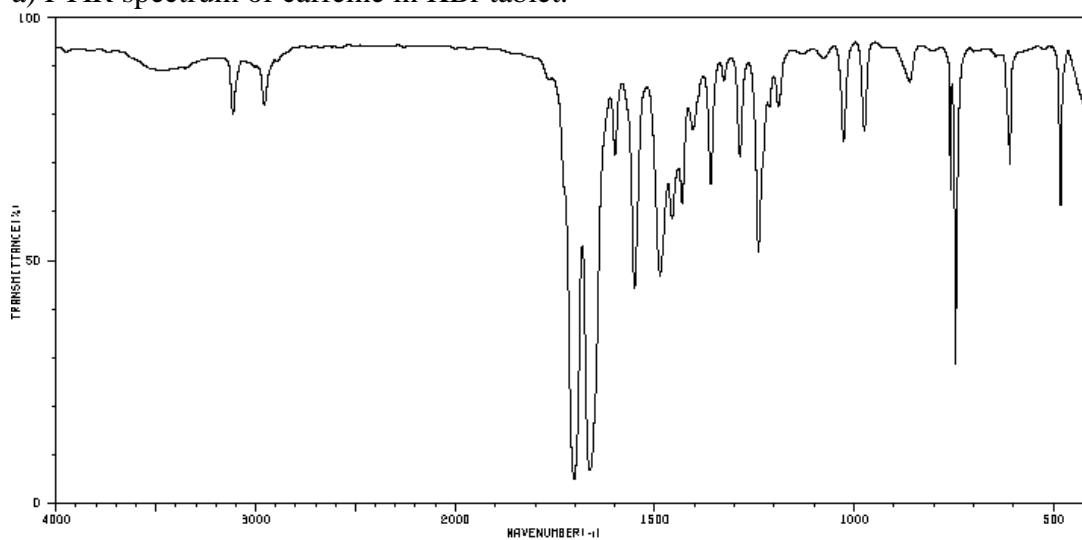
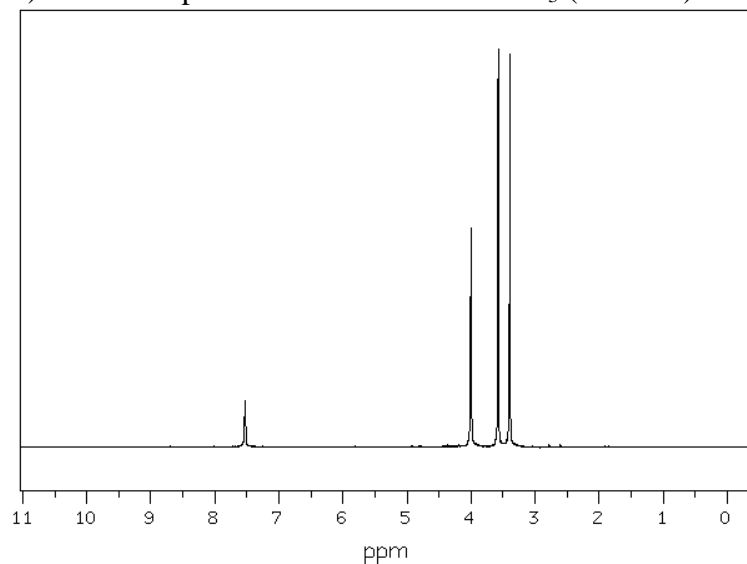
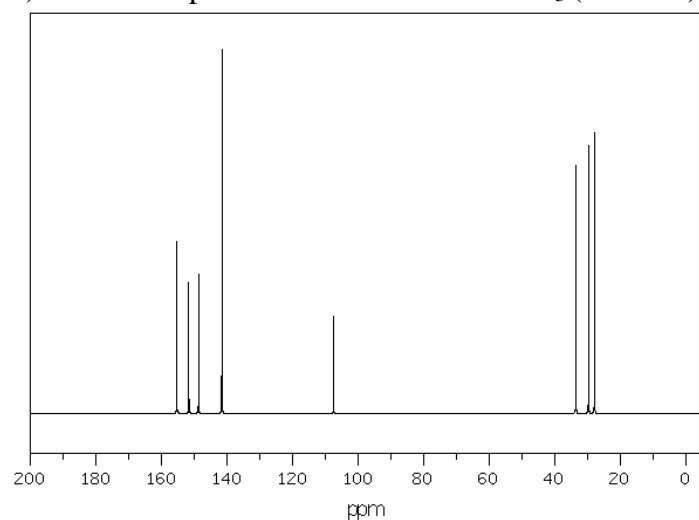
In a round-bottomed flask (50 mL) dissolve 0.2 g crude theobromine in 3.35 mL 10% aqueous sodium hydroxide. To this solution drop carefully 0.7 mL dimethyl sulfate (smelly **strong poison!!!**).

Stir the reaction mixture at room temperature for 20 min. Use a Liebig cooler with a pipe to direct poisonous gases from the reaction system to the hood. Then add 12 mL of chloroform and stir additional 10 min. Next, pour the mixture into separator funnel and isolate the chloroform layer with the product. Extract twice with additional portion of chloroform (2x 12 mL) and the chloroform layers dry above  $\text{MgSO}_4$  and remove the sediment on a regular funnel with cotton plug. Then move the dry extract to a 50 mL round-bottomed flask and concentrate on evaporator. (Warning! The solution can jump rapidly when the vacuum is applied). Dry the crude caffeine in the air. The yield of caffeine ca. 90%, mp. 225-228 °C

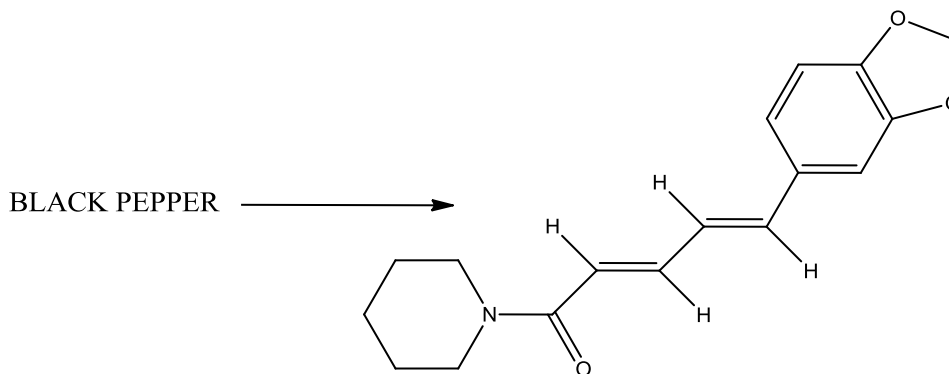
Thin layer chromatography (TLC): on SiO<sub>2</sub>, the spots of the product and standard caffeine have to be very intensive (!) - check under UV light before developing the plate in the mobile phase: chloroform-hexane (9:0.5). Remove the plate and allow the solvent to evaporate. The spot of caffeine, like theobromine, is visible in the UV light. Mark the spots in pencil. Then dip the plate into the prepared reagent: I<sub>2</sub> (1g), KI (2 g) in EtOH (100 mL). After drying, using forceps dip your plate into the mixture of 25% HCl and ethanol (1:1). The spot of caffeine turns to dark brown color.

**SPECTRA**

a) FTIR spectrum of caffeine in KBr tablet.

b)  $^1\text{H}$  NMR spectrum of caffeine in  $\text{CDCl}_3$  (90 MHz).c)  $^{13}\text{C}$  NMR spectrum of caffeine in  $\text{CDCl}_3$  (90 MHz).



**PIPERINE FROM BLACK PEPPER****Reagents:**

black pepper	20 g
chloroform	100 mL
10% KOH in 50% EtOH	
toluene	
cyklohexane	

**Instrumentation and glassware:**

Soxhlet apparatus with condenser  
 conical flask 100 mL  
 crystallizing dish  
 heating mantle  
 round bottom flask 50 mL  
 filtering flask with Büchner funnel  
 beaker 100 mL

Place powdered black pepper (20 g) in the thimble of a Soxhlet apparatus and extract with chloroform for 2 h to obtain the piperine solution. At the end of this operation, the extract obtained is colorless. All of the solvent is removed in vacuo and a brown oil remains. The extract contains all lipophilic constituents of low polarity. In the concentrated extract, triglycerides present are cleaved by saponification with aqueous ethanolic KOH solution, whereas crude piperine crystallizes on standing in the cold.

Add 20 mL of a 10% KOH solution in 50% aqueous ethanol. Stir the mixture for 10 min and filter on the Büchner funnel. Allow to stand the filtrate overnight in a refrigerator at 4 °C. Filter the obtain crystals of crude piperine on the Büchner funnel and wash with 2 mL of cold water to remove the adhering base. Air-dry the crystals and recrystallize from cyclohexane/toluene (4:1, v/v). Use 10 mL of this solvent for each 200 mg of crude piperine (recovery ca. 60%). Piperine crystallizes on standing in a beaker as shiny, pale yellow crystals. Filter the crystals and wash them with a few mL of cyclohexane, mp 130-131 °C

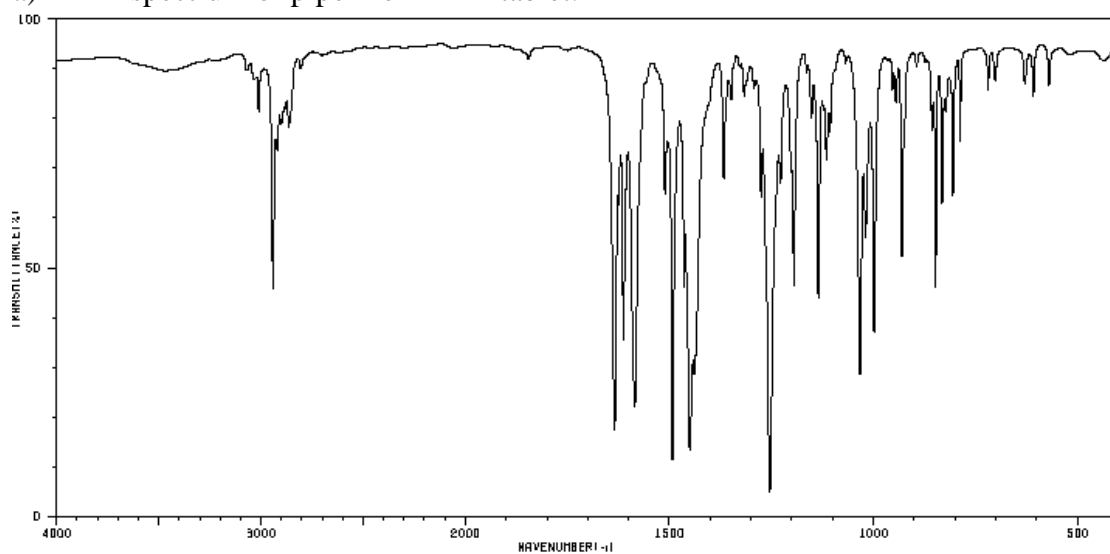
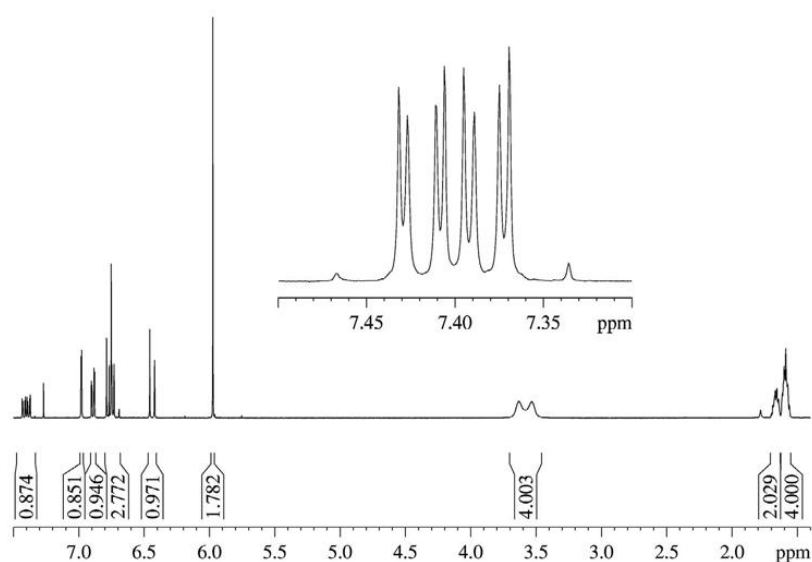
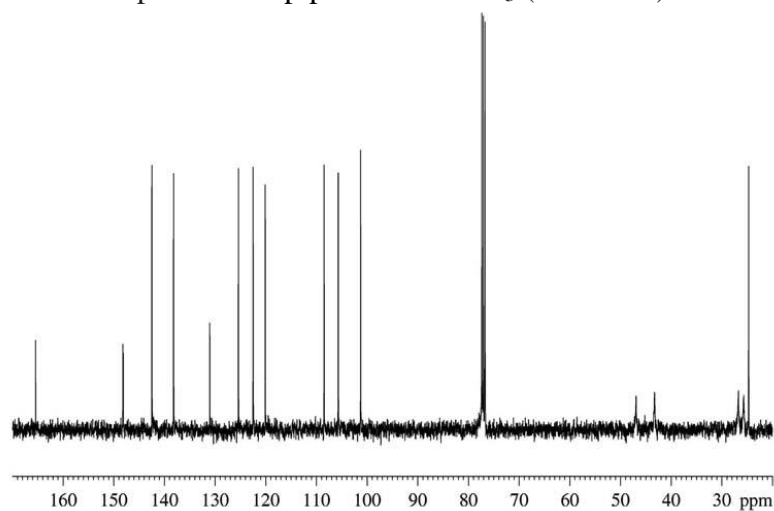
Yield: 200-500 mg depending on the pepper.

**Thin layer chromatography (TLC):**

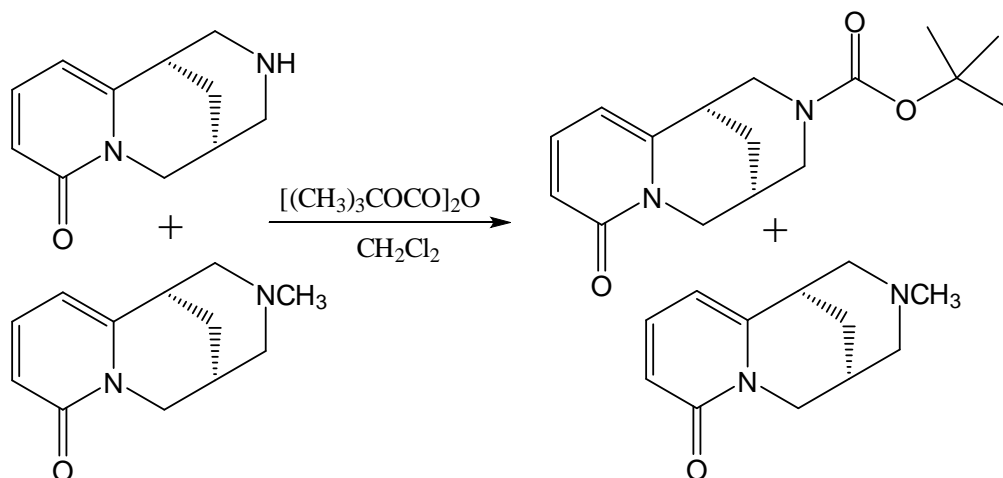
Apply the substrate and product onto SiO<sub>2</sub> plate with capillary, then place the plate vertically into developing tank (small beaker, covered with glass plate). Develop with toluene/ethyl acetate (1:1). Remove the plate and allow the solvent to evaporate. The spot of piperine is visible under the UV light. Mark the spot in pencil. Then, using forceps, dip the plate into a closed jar containing SiO<sub>2</sub> saturated with I<sub>2</sub>. Besides the desired main compound (R<sub>f</sub> = 0.53), a second stereoisomer (R<sub>f</sub> = 0.26) of higher polarity is present.

**SPECTRA**

a) FT IR spectrum of piperine in KBr tablet.

b) <sup>1</sup>H NMR spectrum of piperine in CDCl<sub>3</sub> (400 MHz).c) <sup>13</sup>C NMR spectrum of piperine in CDCl<sub>3</sub> (100 MHz)

## BOC PROTECTION OF AMINE GROUP IN CYTISINE

**Reagents:**

cytosine & <i>N</i> -methylcytosine	1 g (5.26 mmol)
Boc (di- <i>tert</i> -butyl-dicarbonate)	1.37 g (6.3 mmol, 1.2 eq)
methylene chloride	40 mL
aq. conc. NaCl	30 mL
Na <sub>2</sub> CO <sub>3</sub>	0.67 mg in 2 mL H <sub>2</sub> O
anh. MgSO <sub>4</sub>	
diethyl ether	

**Instrumentation and glassware:**

Heating mantle  
 Round flask 250 mL  
 Measuring cylinder  
 Round flask 100 mL  
 Cooler  
 filtrating kit with Büchner funnel

**WARNING! Work under hood! Wear the gloves!**

In a round-bottomed flask (100 mL) dissolve the mixture of cytosine and *N*-methylcytosine in 40 mL CH<sub>2</sub>Cl<sub>2</sub>, add Boc (CAUTION! The compound flammable and toxic by inhalation. Irritating to eyes, respiratory system and skin) and the solution of Na<sub>2</sub>CO<sub>3</sub>. Stir the reaction mixture at 60°C for 1-2 hours. Use a Liebig cooler with a pipe to remove directing poisonous gases from the reaction system to the hood.

Monitor the reaction with TLC. When the reaction is completed cool down the mixture to room temperature and add 30 mL of concentrated solution of NaCl. Check pH of the solution (it has to be below pH < 5). Acidify the mixture with 10% of citric acid to pH=4 and move to separatory funnel. Separate the fractions and wash the organic one with water (3 x 20 ml). Dry organic fraction with anh. MgSO<sub>4</sub> and concentrate it on the rotary evaporator to remove CH<sub>2</sub>Cl<sub>2</sub>. To the crude ***N*-BOC-cytosine** dissolved petroleum ether. Filter the obtained crystals on a Büchner funnel and dry them on air.

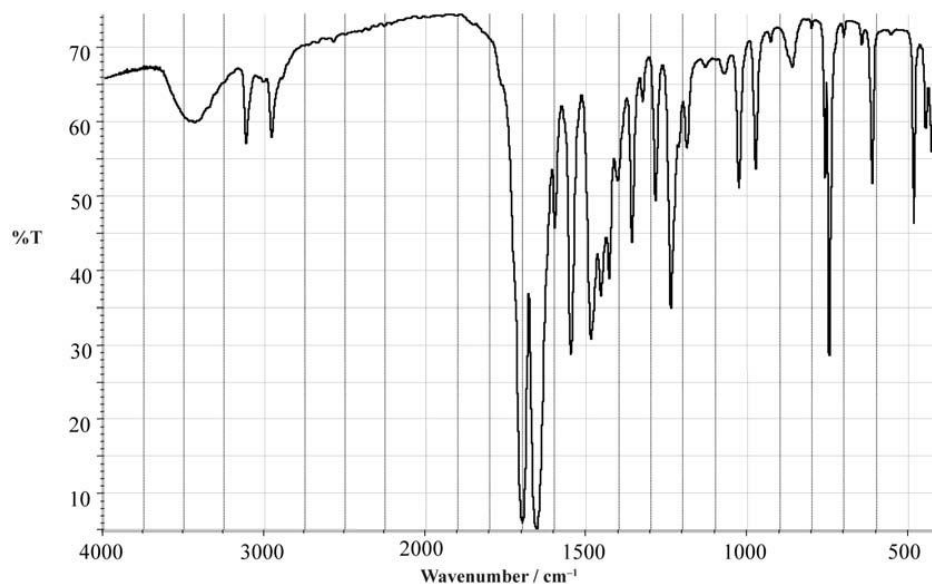
Weigh the product, calculate the percentage yield and measure the m.p.

Thin layer chromatography (TLC): on SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH (10:1:0.1).

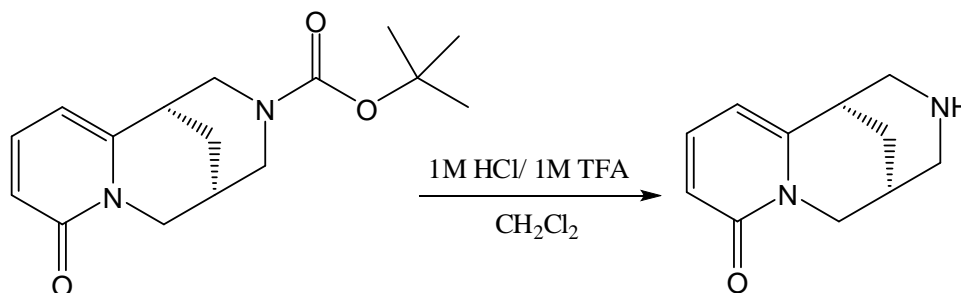
Remove the plate and allow the solvent to evaporate. The spots of alkaloids mixture is visible in the UV light. Mark the spots in pencil. Then dip the plate into the already prepared Dragendorff reagent. The spots of the alkaloids turn to orange color.

## SPECTRA

a) <sup>13</sup>C-NMR spectrum of *N*-BOC-cytisine in CDCl<sub>3</sub> (300 MHz).



$\delta$  (ppm): 163.4; 154.5; 148.7; 138.9; 117.1; 105.8; 80.3; 51.6; 50.5; 48.9; 34.8; 28.0; 27.5; 26.1

DEPROTECTION OF AMINE GROUP IN *N*-BOC-CYTISINE**Reagents:**

<i>N</i> -BOC-cytisine	4 g
1M HCl	5.5 ml
Trifluoroacetic acid (TFA)	5,5 ml
CH <sub>2</sub> Cl <sub>2</sub>	

**Instrumentation and glassware:**

Heating mantle  
 Round flask 100 mL  
 Measuring cylinder  
 Cooler  
 filtrating kit with Büchner funnel  
 beaker 100 mL  
 separatory funnel

Dissolve *N*-BOC-cytisine 4 g in 20 mL of water (in a 100 mL round-bottom flask), add 5.5 mL TFA and 5.5 mL HCl. Attach a reflux condenser and reflux vigorously with stirring for 30 minutes. Monitor the reaction with TLC. When the reaction is completed cool down the mixture to room temperature and alkalize to pH = 14 with KOH.

The mixture move to separatory funnel. Separate the layers and extract the product with CH<sub>2</sub>Cl<sub>2</sub> (4 x 30 mL). Check the alkaloids using Dragendorf test.

Combine the extracts and dry above anh. MgSO<sub>4</sub>. Filter the dried extract through the glass funnel with small plug of cotton and transfer filtrate to a cleaned 250 mL round-bottomed flask. Concentrate the solution to 15 mL and move it by a pipette to smaller weighted flask (50 mL) and evaporate the solvent on evaporator.

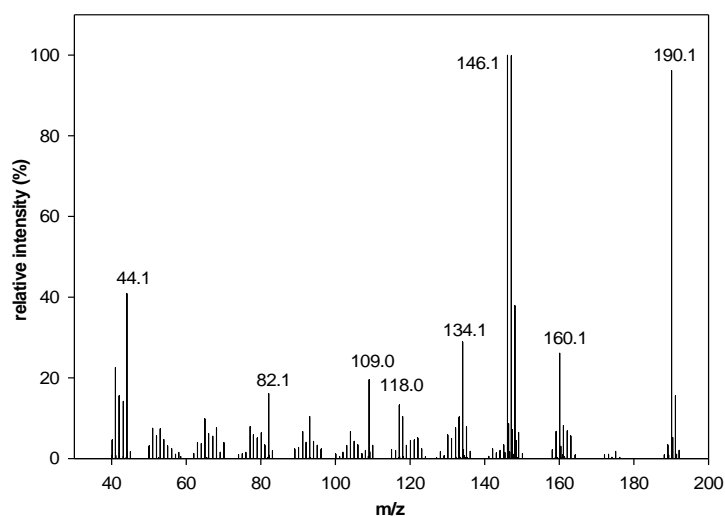
Recrystallize crude product from the mixture of methylene chloride and petroleum ether (1:1 v/v). Cytisine crystallize as light yellow crystals with yield 94%, mp.: 153°C.

Thin layer chromatography (TLC): on SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH (10:1:0.1).

Remove the plate and allow the solvent to evaporate. The spots of alkaloids mixture are visible in the UV light. Mark the spots in pencil. Then dip the plate into the already prepared Dragendorf . The spots of the alkaloids turn to orange color.

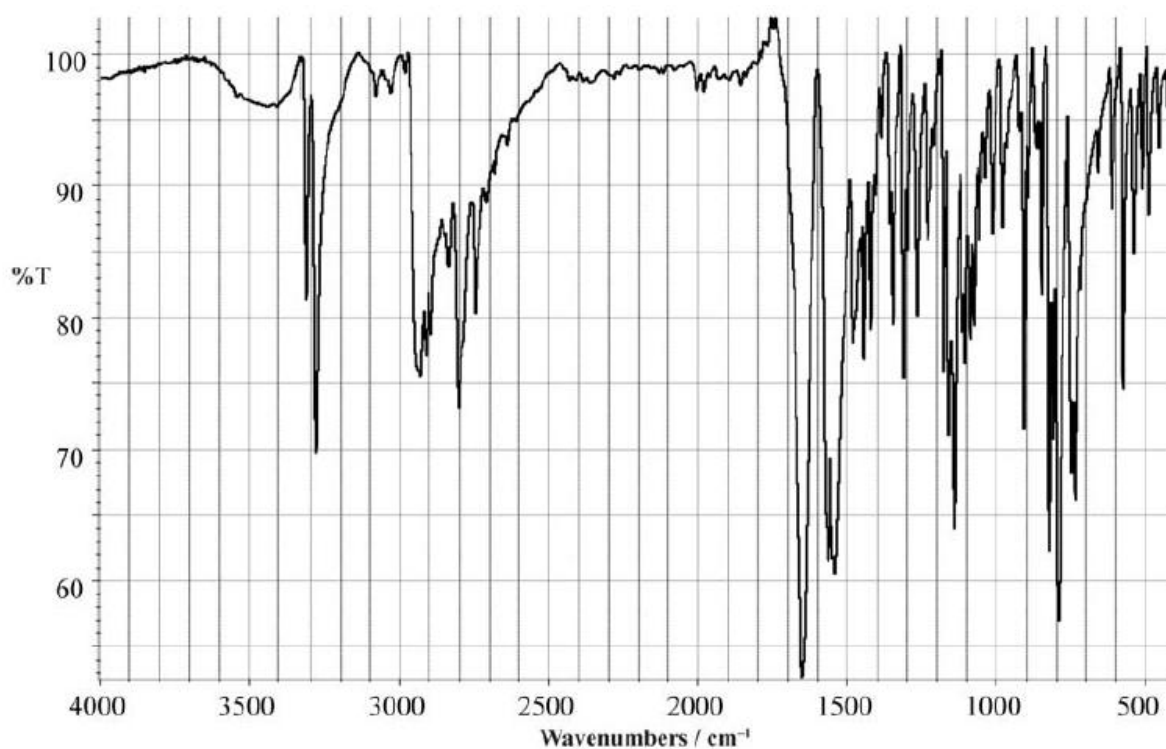
**SPECTRA**

a) MS spectrum of cytosine:



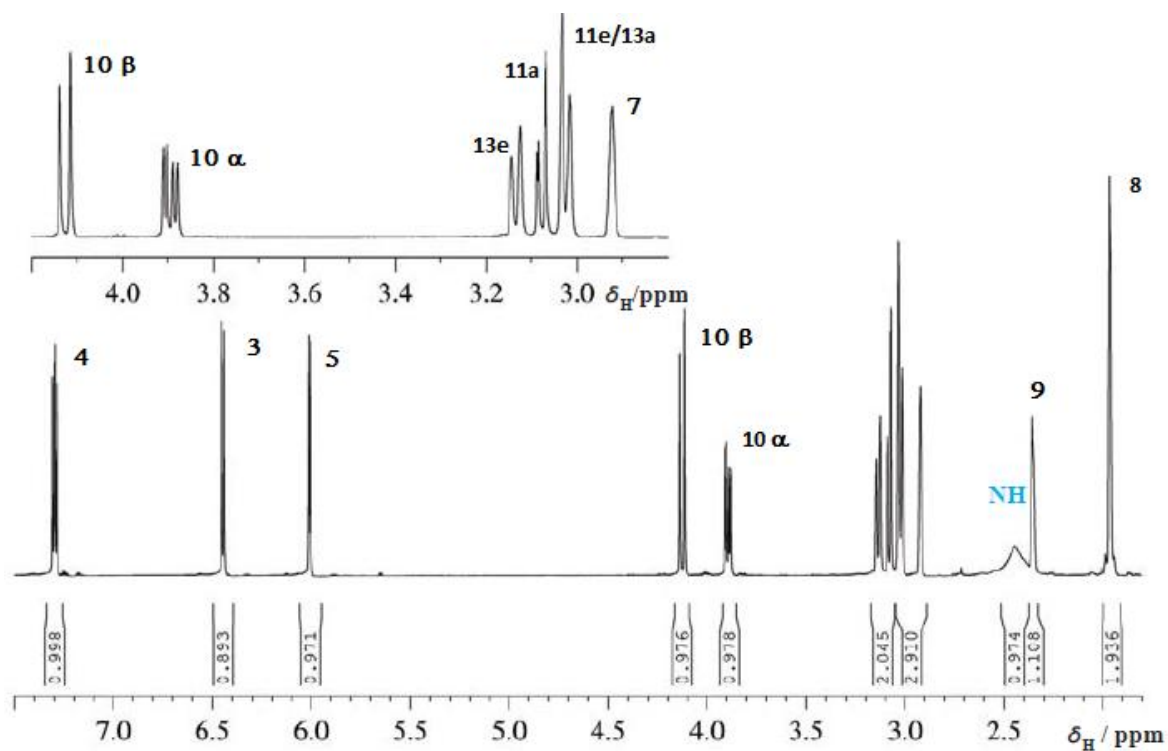
$m/z$  (%): 190 ( $M^+$ , 96), 160 (26), 147 (99), 146 (100), 134 (28), 44 (40).

b) IR spectrum of cytosine in KBr tablet:

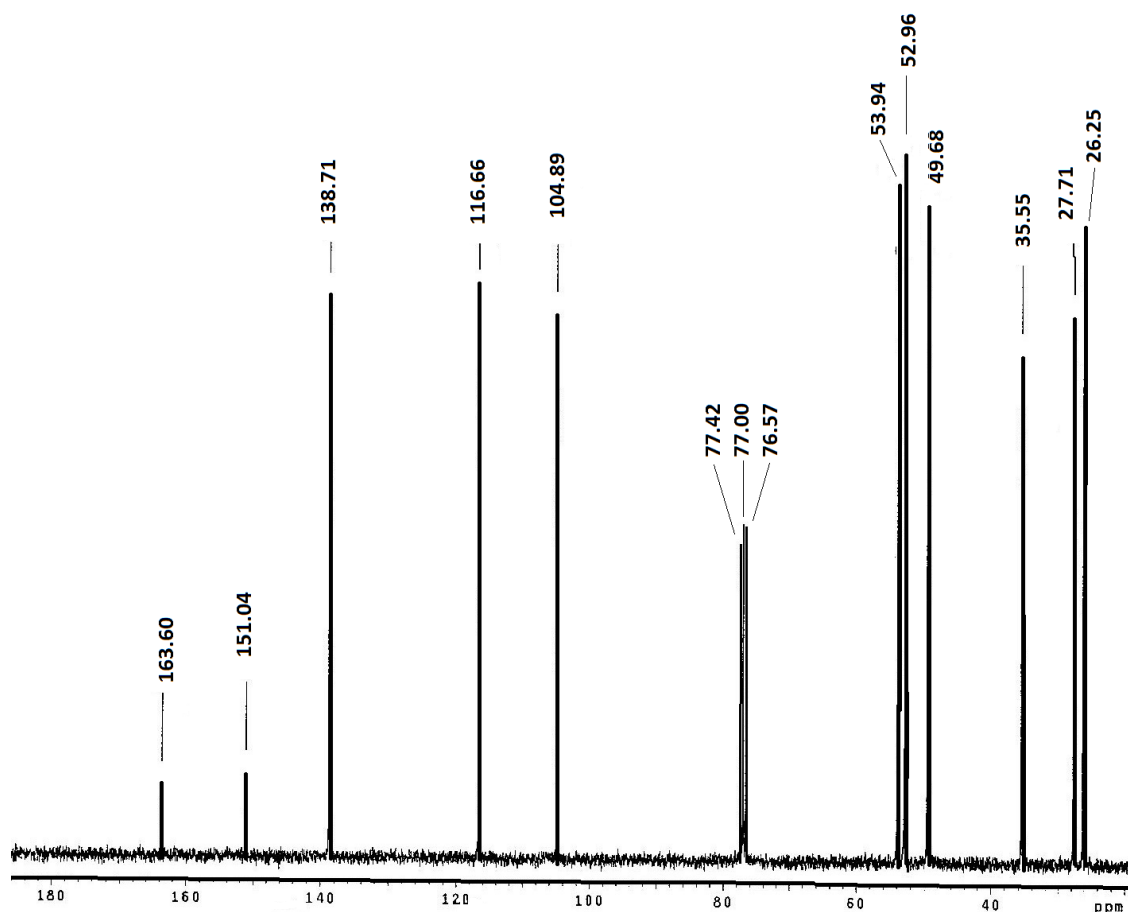


band at  $1649 \text{ cm}^{-1}$  ( $\text{C}=\text{O}$ ), the band at  $3438 \text{ cm}^{-1}$  ( $\text{NH}$ ), and  $1140 \text{ cm}^{-1}$  ( $\text{CN}$ ).

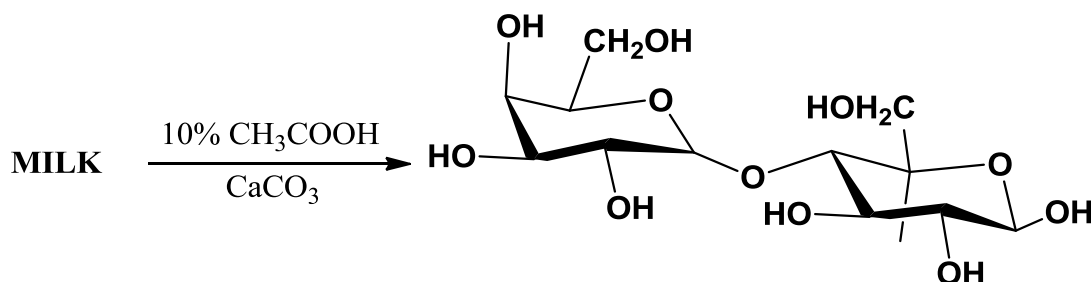
c)  $^1\text{H}$ -NMR spectrum of cytosine in  $\text{CDCl}_3$  (700 MHz).



d)  $^{13}\text{C}$ -NMR spectrum of cytosine in  $\text{CDCl}_3$  (500 MHz).



## LACTOSE FROM SKIMMED MILK

**Reagents:**

Powdered milk	30 g
Acetic acid 10%	18 mL
Calcium carbonate	2.4 g
Ethanol	150 mL
Activated carbon	

**Instrumentation and glassware:**

magnetic stirrer  
 beaker 300 mL  
 measuring cylinder  
 filtering system with Büchner funnel  
 round bottom flask 250 mL

Insert into the beaker 30 g of milk powder and suspend it in 60 mL of warm water (it is possible to use 200 mL skimmed milk (0% or 0.5%)) and stir the mixture at 40-50 °C for 15 min. Then add slowly 10 mL of 20 % acetic acid, stirring the mixture. The coagulation of casein starts. Remove the casein by decantation and pass the rest through the cotton cloth. Weigh it and calculate the yield.

Pour the translucent solution obtained into a beaker and boil it. Mix carefully the hot solution with calcium carbonate (2.4 g) for about 10 min. (Caution! The solution will foam rapidly! Immerse the beaker into a crystallizing dish!) To the warm mixture add a pinch of activated carbon and after stirring filter the warm mixture on Büchner funnel using 3-4 filter papers. Next concentrate the transparent solution to ca 35 mL on a vacuum evaporator. Add ethanol 150 mL and additional pinch of activated carbon. Once more filter the warm mixture on a Büchner funnel using 3-4 filter papers. Concentrate the obtained filtrate on an evaporator to ca. 70 mL and leave for crystallization of lactose in a crystallizing dish with ice and water. Filter the product obtained on a Büchner funnel and wash with cold ethanol. The yield of lactose is 3-5 g.

Thin layer chromatography (TLC): on SiO<sub>2</sub>, toluene - anh. acetic acid - methanol (2:2:6).

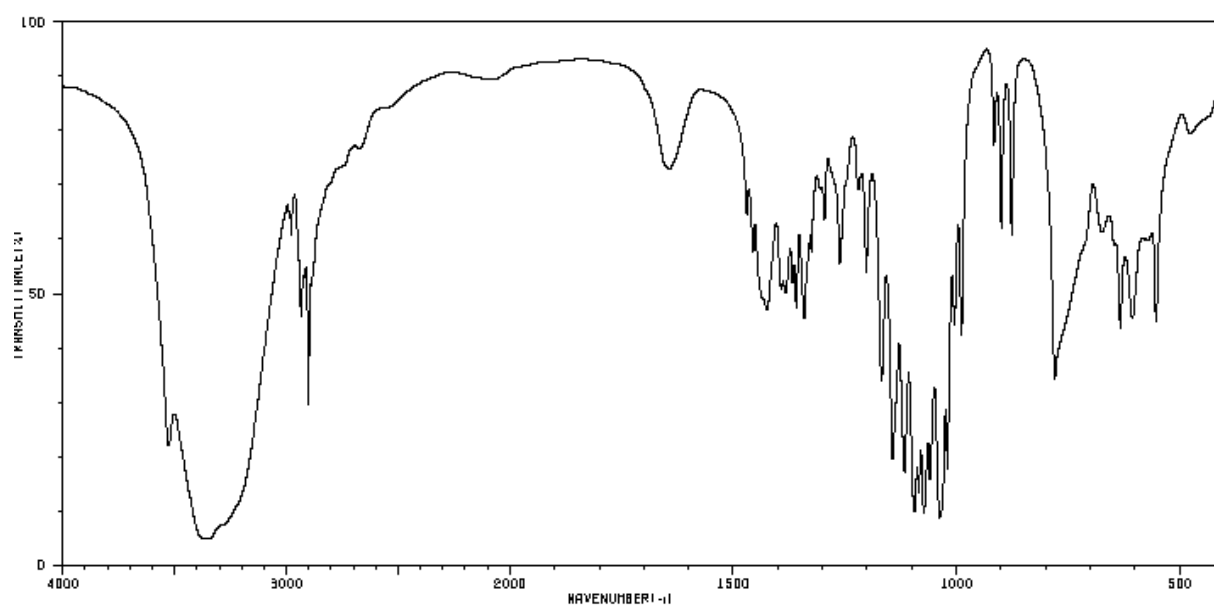
After a few minutes the solvent will reach the upper line, remove the plate using forceps and allow the solvent to evaporate. After drying use a hot-plate and heat the TLC plate carefully and you will see spots developing on the plate. Be sure not to overheat the plate or it may crash!

**Wohlk's test:** to the test tube add solution of the obtained product and boil it with KOH. If any disaccharide sugars are present a red colour appears. The colour will turn yellow-brownish in the presence of reducing sugars (glucose and fructose).

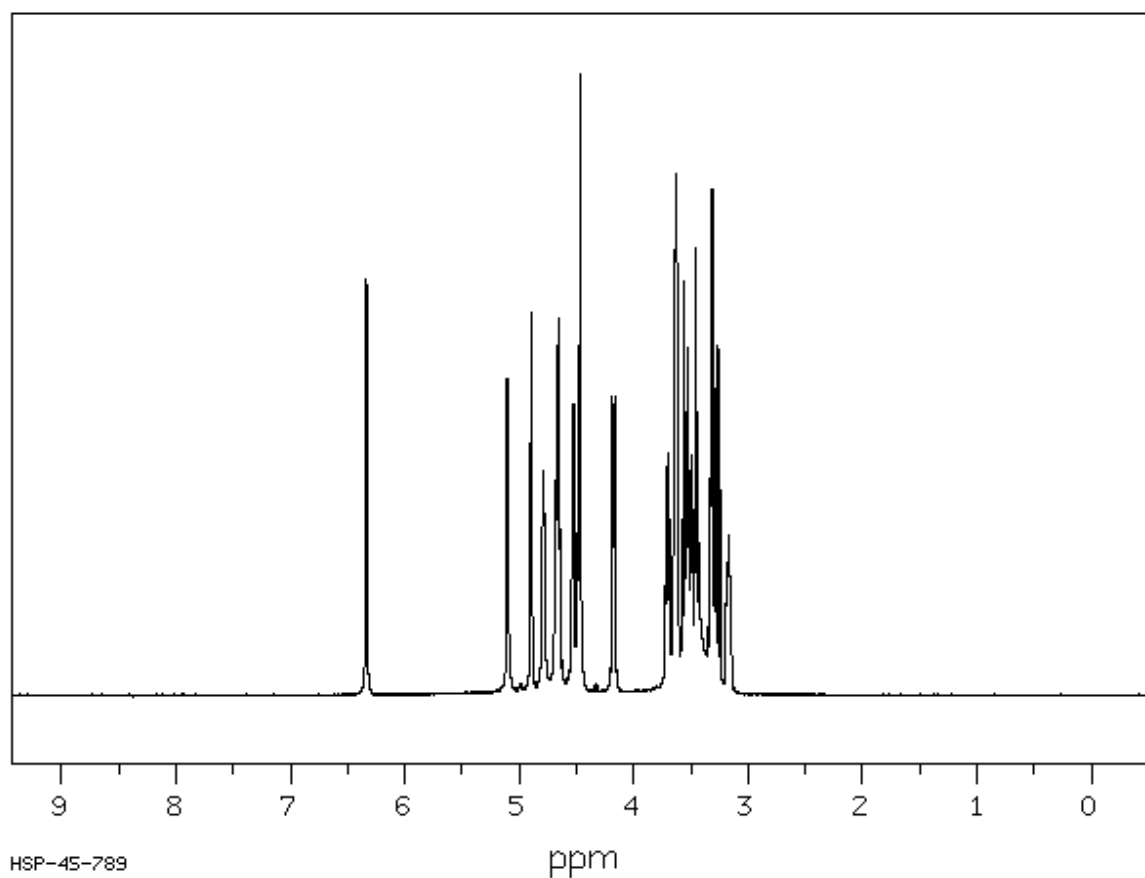


**SPECTRA**

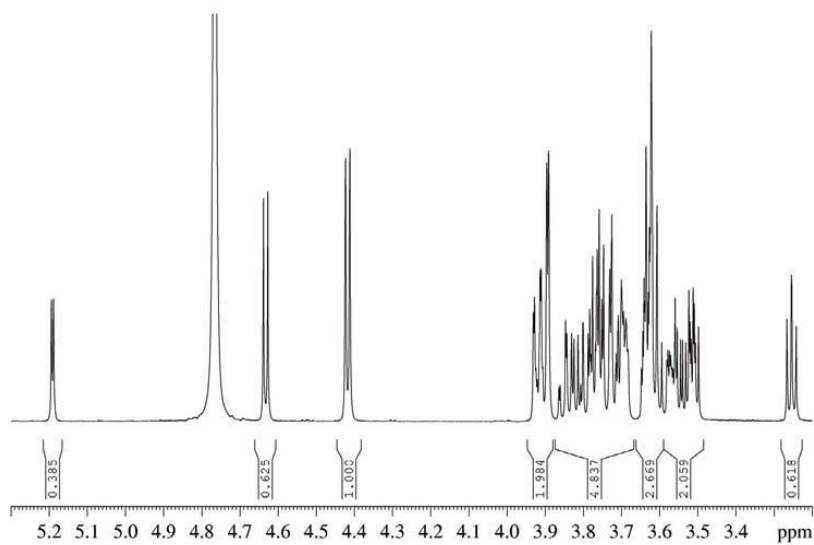
a) FTIR spectrum of lactose in KBr tablet.

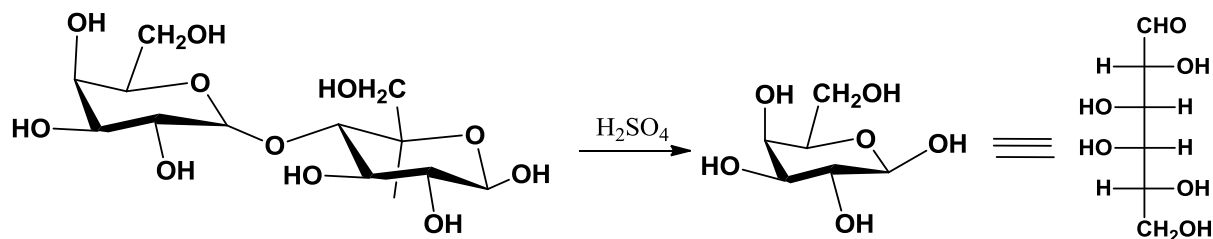


b) <sup>1</sup>H NMR spectrum of lactose in DMSO-d<sub>6</sub> (400 MHz).



c)  $^1\text{H}$  NMR spectrum of lactose in  $\text{D}_2\text{O}$  (700 MHz).



**D-GALACTOSE FROM LACTOSE****Reagents:**

lactose	20 g (0.06 mole)
conc. sulfuric acid	0.6 mL
$\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$	3 g
anh acetic acid	25 mL
methanol	5 mL
diethyl ether	10 mL

**Instrumentation and glassware:**

round-bottomed flask 250 mL  
 Liebig condenser  
 filtering kit with Büchner funnel  
 beaker 50 mL

In a round-bottomed flask (250 mL) place 20 g of lactose, 40 mL water and 0.6 mL of conc. sulfuric acid. Adjust the Liebig cooler and heat under reflux for 2 hours.

To the hot mixture add a pinch of activated carbon and adjust to  $\text{pH} = 7$  by adding barium hydroxide. After stirring and cooling down, filter the mixture on a Büchner funnel using 3-4 filter papers. Concentrate the obtained solution on an evaporator to 10 mL. Pour the obtained translucent filtrate into a beaker and acidify with 0.6 mL of anh. acetic acid and leave for crystallization of D-galactose in a crystallizer with ice and water. Filter the product obtained on a Büchner funnel and wash with acetic acid, then with methanol and next with diethyl ether. The yield 5 g (47%) of D-galactose mp.  $165^\circ\text{C}$ ,  $[\alpha]^{20} = +81.5^\circ$  ( $c=1$ , water)

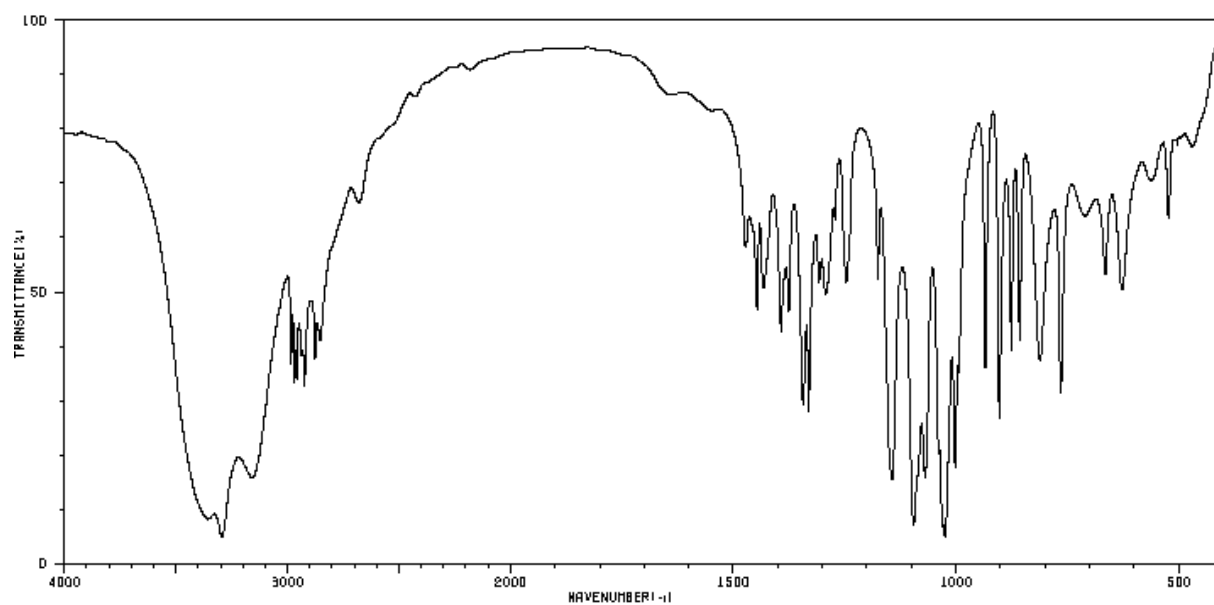
**Thin layer chromatography (TLC):**

on  $\text{SiO}_2$ : propanol – acetic acid-water (4:1:5). Remove the plate and allow the solvent to evaporate. After drying use a hot-plate and heat the TLC plate carefully and spots will develop on the plate.

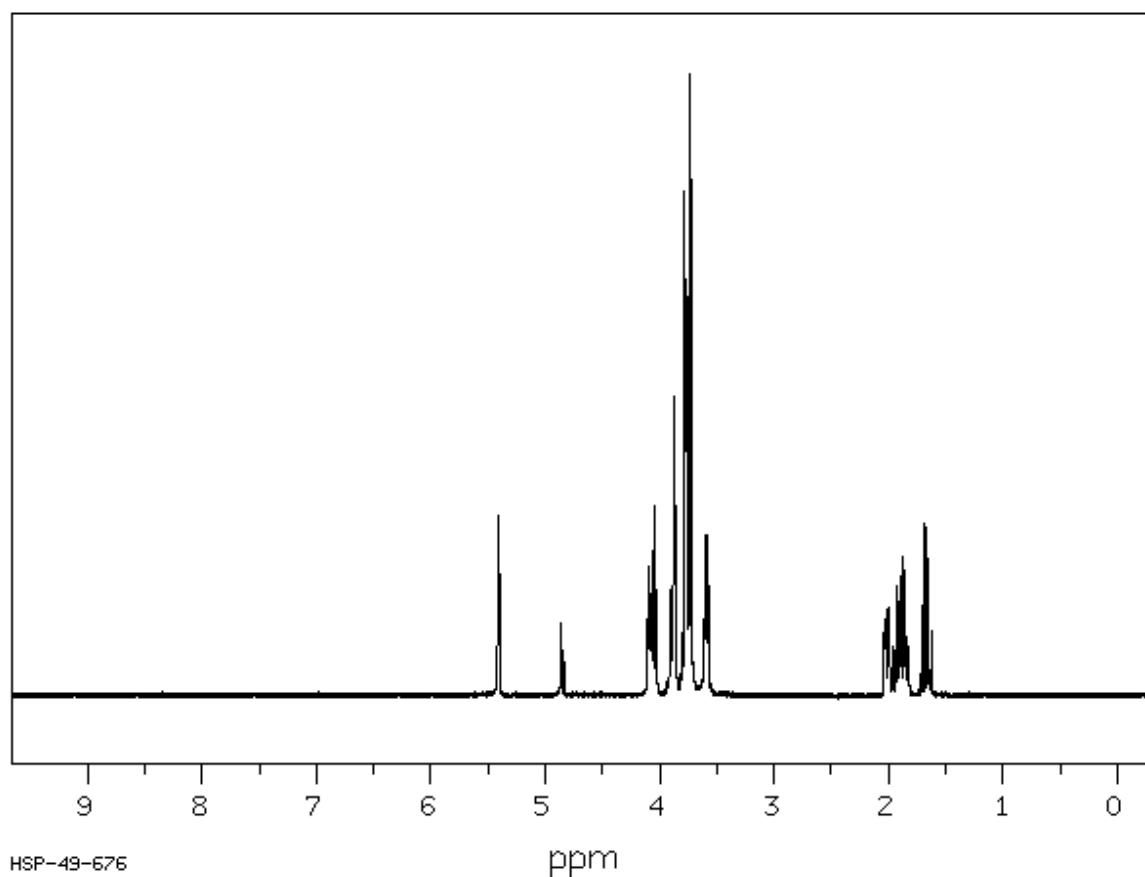
**Barfoed's test:** (in a tube) add a mixture of lactic acid (1 mL of 8,5%) and 1 g of  $\text{Cu}(\text{CH}_3\text{COO})_2$  in 19 mL of water to the test solution of product and boil. If any reducing sugars are present a red precipitate of  $\text{Cu}_2\text{O}$  is formed. The reaction will be negative in the presence of disaccharide sugars as they are weaker reducing agents.

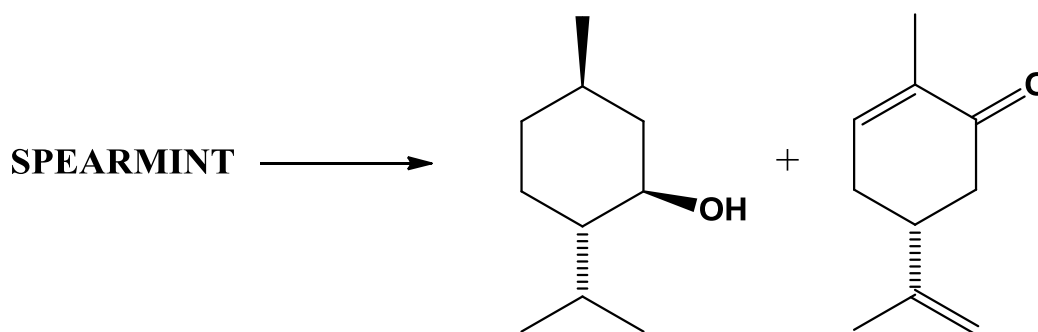
## SPECTRA

a) FTIR of *D*-galactose in KBr tablet.



b) <sup>1</sup>H NMR spectrum of *D*-galactose in D<sub>2</sub>O (400 MHz).



**MENTHOL AND R-(-)-CARAVONE FROM SPEARMINT ( METHOD A)****Reagents:**

spearmint 20 g  
 chloroform 60 mL  
 anh. magnesium sulfate  
 valine 1 g  
 10% sulfuric acid 100 mL

**Instrumentation and glassware:**

steam distillation system with 500 mL flask  
 dropping funnel 250 mL  
 Separating funnel 500 mL  
 Conical flask 250 mL  
 round-bottomed flask 250 mL  
 round-bottomed flask 50 mL

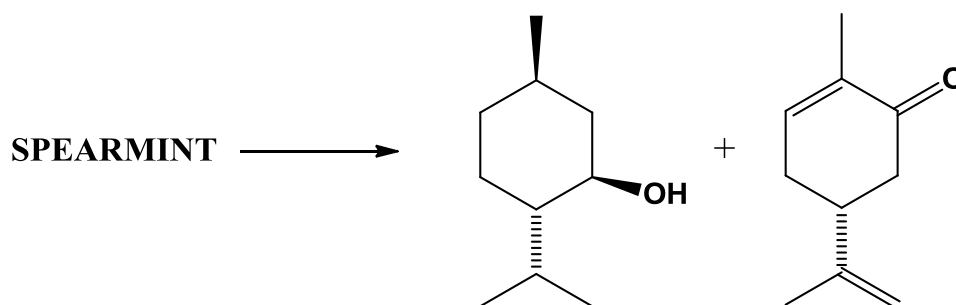
In round-bottomed flask place 20 g of crushed pepper mint and add 150 mL of water. Prepare the set for steam distillation. Collect about 300 mL of the distillate that contains caravone. Extract the product with chloroform (4 x 30 mL) in a separating funnel. Combine the extracts, wash them with distilled water (2x20 mL) and dry above anh.  $\text{MgSO}_4$ . Filter the dry extract through the glass funnel with a small plug of cotton and transfer the filtrate to a clean 250 mL round-bottomed flask. Concentrate the solution to 15 mL and transfer it by a pipette to a smaller weighted flask (50 mL), evaporate the solvent on an evaporator. The yield 300 mg R-(-)- caravone,  $[\alpha]_D^{20} = -61^\circ$  (c=1, EtOH).

Thin layer chromatography (TLC):

on  $\text{SiO}_2$ , hexane/ethyl acetate (9:1). Remove the plate and allow the solvent to evaporate. After drying, using forceps dip your plate into already prepared reagent: valine 1 g in 100 mL 10%  $\text{H}_2\text{SO}_4$ . After drying use a hot-plate and heat the TLC plate carefully and you will see spots develop on the plate. The spot of caravone turns to pinkish color.

Thin layer chromatography (TLC):

on  $\text{SiO}_2$ , hexan-methanol-chloroform (8:2:2). Remove the plate and allow the solvent to evaporate. After drying, using forceps dip your plate into already prepared solution of phosphoromolibdenic acid (5 g) in 25 mL of ethanol. After drying use a hot-plate and heat the TLC plate carefully up to 100 °C and you will see spots develop on the plate. The spot of menthol turns into blue color.

**MENTHOL AND R-(-)-CARAVONE FROM SPEARMINT (METHOD B)****Reagents:**

Spearmint	20 g
diethyl ether	150 mL
valine	1 g
10% sulfuric acid	100 mL
acetone	

**Instrumentation and Glassware:**

Conical flask 500 mL  
 round-bottomed flask 250 mL  
 round-bottomed flask 50 mL  
 filtering system with Büchner funnel

Measure 10 g of crumbled spearmint into a 200 mL conical flask and add 80 mL of diethyl ether. Mix it thoroughly and allow the mixture to stand for 30 minutes. Once the mint has soaked for appropriate time, use the Büchner funnel to filter the mixture (always clamp the filter flask). When you have filtered the solution, wash twice the conical flask with ether (2x10 mL) and wash the mint in the funnel with this solution. After washing the sediment with additional portions of ether, transfer the filtrate to a 250 mL round-bottomed flask. Wash additionally the receiving flask with  $\text{CH}_2\text{Cl}_2$  and concentrate to 15 mL on an evaporator. Then move it by a pipette to smaller weighted flask (50 mL) and evaporate the solvent on evaporator. The yield 300 mg R-(-)-caravone,  $[\alpha]_{\text{D}}^{20} = -61^\circ$  (c=1, EtOH). Calculate the yield in relation to the used spearmint leaves.

**Thin layer chromatography (TLC):**

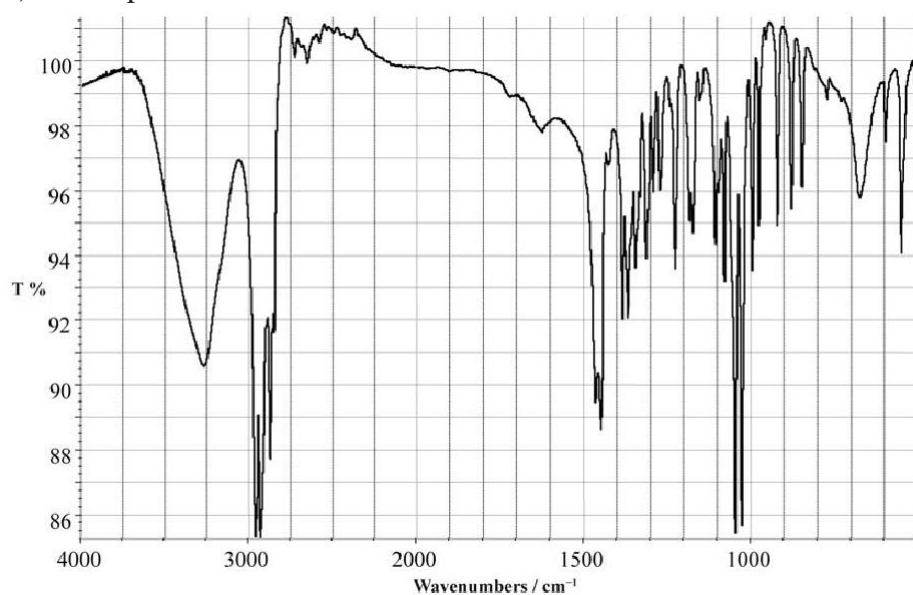
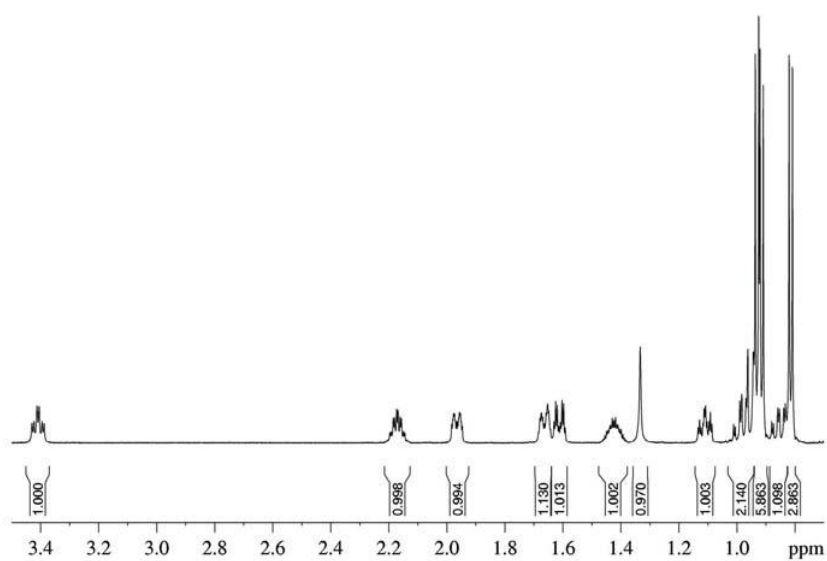
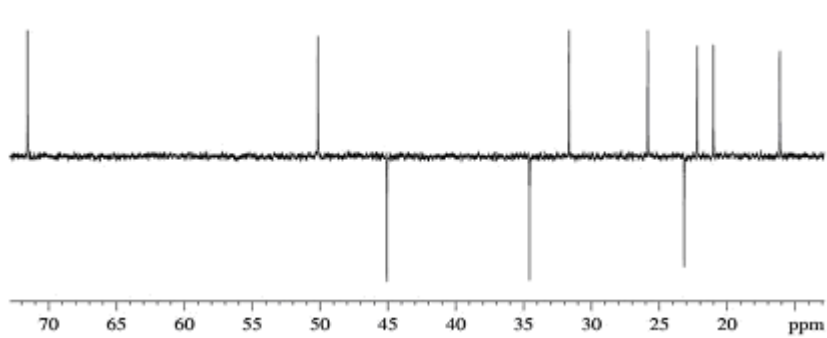
on  $\text{SiO}_2$ , hexane/ethyl acetate (9:1). Remove the plate and allow the solvent to evaporate. After drying, using forceps dip your plate into already prepared reagent: valine 1 g in 100 mL 10%  $\text{H}_2\text{SO}_4$ . After drying use a hot-plate and heat the TLC plate carefully and you will see spots develop on the plate. The spot of caravone turns to pinkish color. The other yellowish and brownish spots come from limonene and leaves pigments (like chlorophylls).

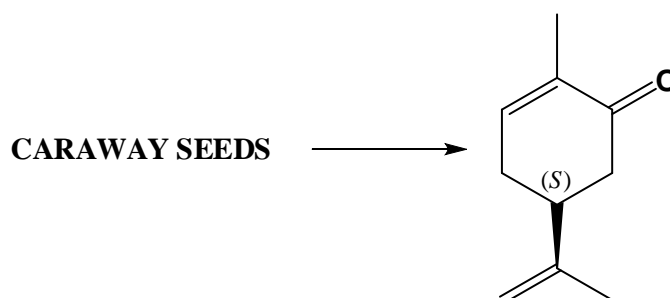
**Thin layer chromatography (TLC):**

on  $\text{SiO}_2$ , hexane-methanol-chloroform (8:2:2). Remove the plate and allow the solvent to evaporate. After drying, using forceps dip your plate into already prepared solution of phosphoromolibdenic acid (5 g) in 25 mL of ethanol. After drying use a hot-plate and heat the TLC plate carefully up to 100 °C and you will see spots develop on the plate. The spot of menthol turns into blue color.

**SPECTRA**

a) FTIR spectrum of menthol in KBr tablet.

b)  $^1\text{H}$  NMR spectrum of menthol in  $\text{CDCl}_3$  (600 MHz)c) APT  $^{13}\text{C}$  NMR spectrum of menthol in  $\text{CDCl}_3$  (150 MHz).

**S-(+)-CARAVONE FROM CARAWAY (METHOD A)****Reagents:**

grounded seeds of caraway	20 g
chloroform	60 mL
anh. magnesium sulfate	
valine	1 g
10% sulfuric acid	100 mL

**Instrumentation and Glassware:**

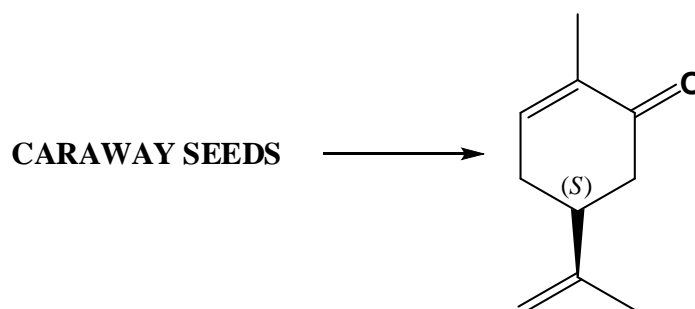
steam distilling system with flask	500 mL
dropping funnel	250 mL
separating funnel	500 mL
conical flask	250 mL
round-bottomed flask	250 mL
round-bottomed flask	50 mL

In round-bottomed flask place 20 g of crushed caraway seeds and add 150 mL of water. Prepare the set for steam distillation. Collect about 300 mL of distillate that contains caravone. Extract the product with chloroform (4 x 30 mL) in separating funnel. Combine the extracts, wash them with distilled water (2x20 mL) and dry above anh.  $\text{MgSO}_4$ . Filter the dry extract through the glass funnel with small plug of cotton and transfer filtrate to a cleaned 250 mL round-bottomed flask. Concentrate the solution to 15 mL and move it by a pipette to smaller weighted flask (50 mL) and evaporate the solvent on evaporator. The yield 300 mg S-(+)-caravone,  $[\alpha]_D^{20} = -61^\circ$  ( $c=1$ , EtOH).

Thin layer chromatography (TLC): on  $\text{SiO}_2$ , hexane/ethyl acetate (9:1).

Remove the plate and allow the solvent to evaporate. After drying, using forceps dip your plate into already prepared reagent: valine 1 g in 100 mL 10%  $\text{H}_2\text{SO}_4$ . After drying use a hot-plate and heat the TLC plate carefully and you will see spots develop on the plate. The spot of caravone turns to pinkish-orange color.



**S-(+)-CARAVONE FROM CARAWAY (METHOD B)****Reagents:**

grounded seeds of caraway	20 g
diethyl ether	80 mL
acetone	
valine	1 g
10% sulfuric acid	100 mL

**Glassware:**

conical flask 200 mL
filtering system with Büchner funnel
round-bottomed flask 250 mL
round-bottomed flask 50 mL

Measure 10 g of crushed caraway seeds into a 200 mL conical flask and add 80 mL of diethyl ether. Mix it thoroughly and allow the mixture to stand for 30 minutes. Once the seeds have soaked for appropriate time, use the Büchner funnel to filter the mixture (always clamp the filter flask). When you have filtered the solution, wash twice the conical flask with ether (2x10 mL) and move it on the seeds in the funnel. After washing the sediment with additional portions of ether, transfer the filtrate to a 250 mL round-bottomed flask. Wash additionally the receiving flask with  $\text{CH}_2\text{Cl}_2$  and concentrate to 15 mL on evaporator.

Then move it by a pipette to smaller weighted flask (50 mL) and evaporate the solvent on evaporator. The yield 300 mg S-(+)-caravone,  $[\alpha]_{\text{D}}^{20} = -61^\circ$  (c=1, EtOH).

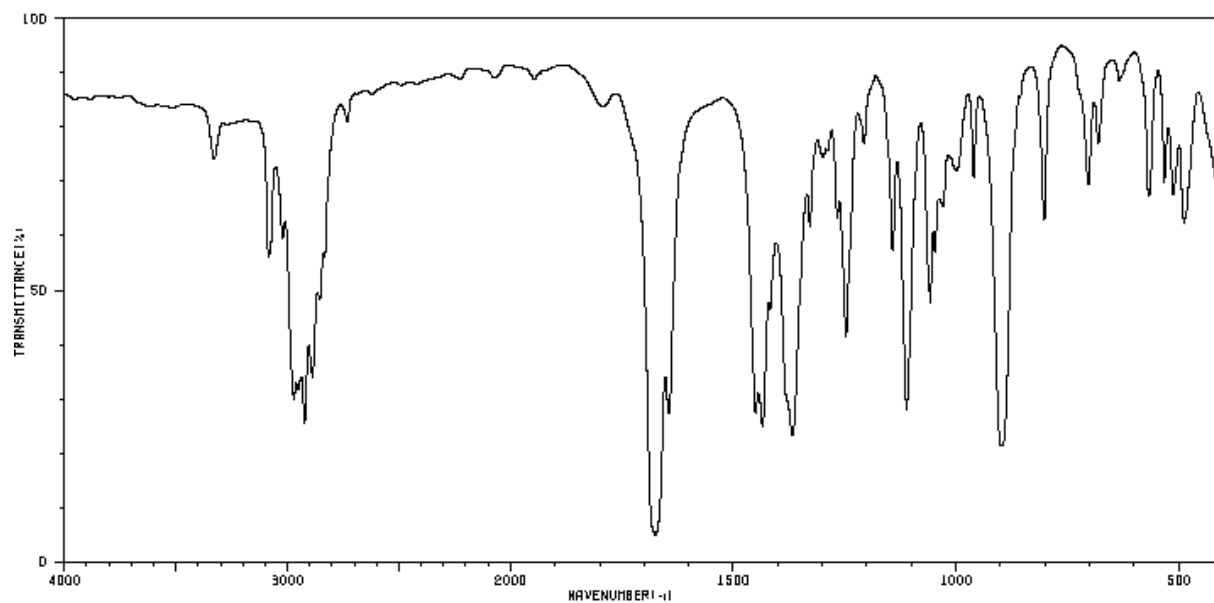
Calculate the yield in relation to the used caraway seeds.

Thin layer chromatography (TLC): on  $\text{SiO}_2$ , hexane/ethyl acetate (9:1).

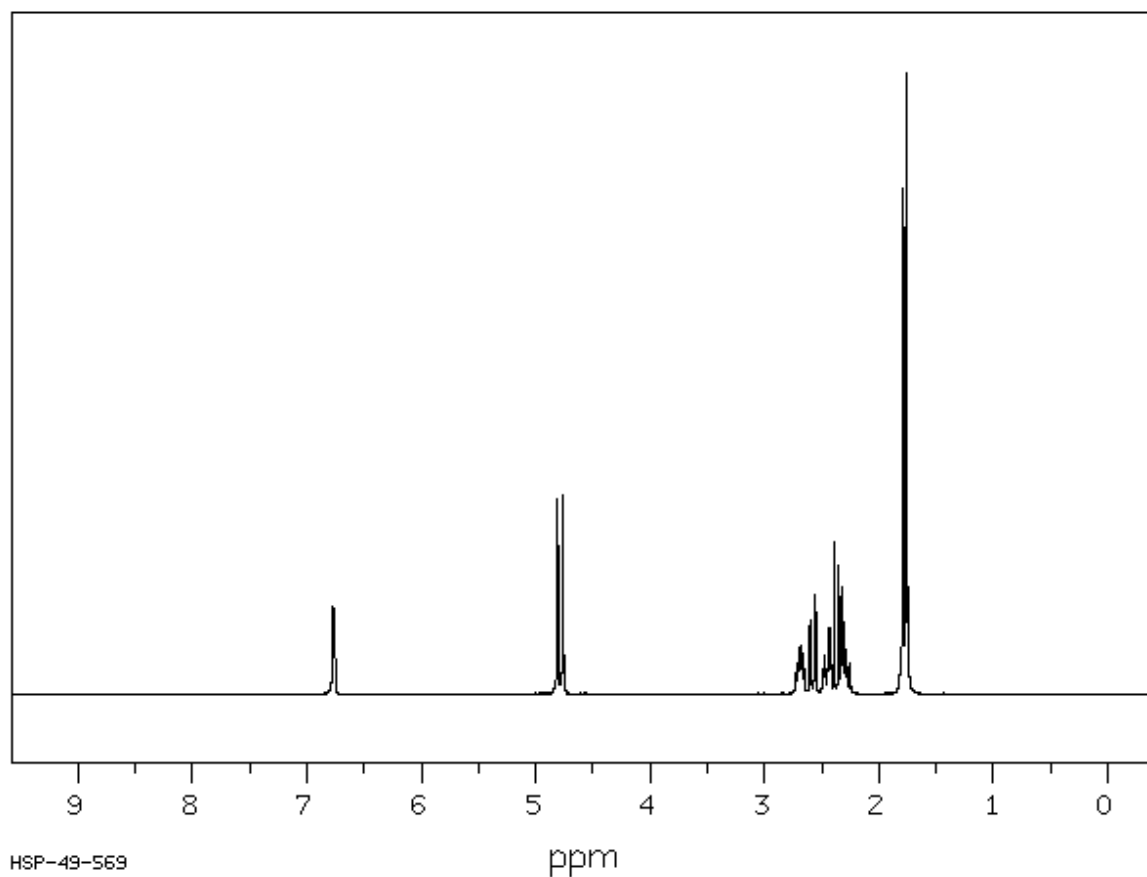
Remove the plate and allow the solvent to evaporate. After drying, using forceps dip your plate into already prepared reagent: valine 1 g in 100 mL 10%  $\text{H}_2\text{SO}_4$ . After drying use a hot-plate and heat the TLC plate carefully and you will see spots develop on the plate. The spot of caravone turns to pinkish-orange color. The other yellowish and brownish spots come from limonene and leaves pigments (like chlorophylls).

## SPECTRA

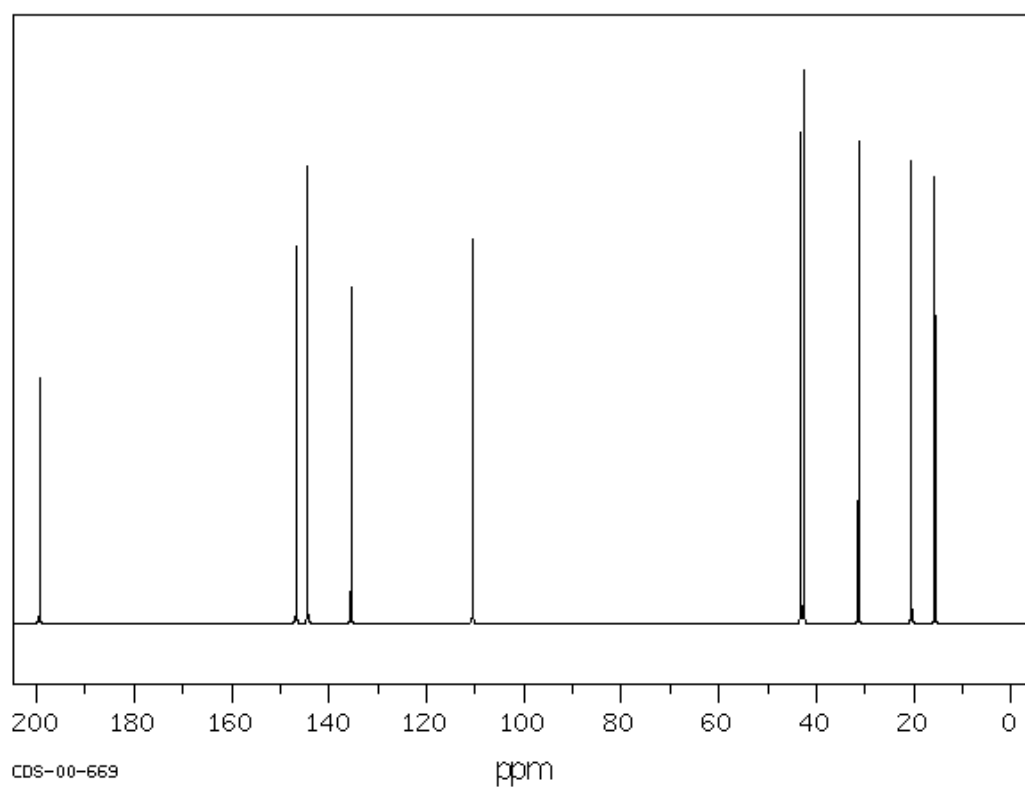
a) FTIR spectrum of caravone, liquid film.



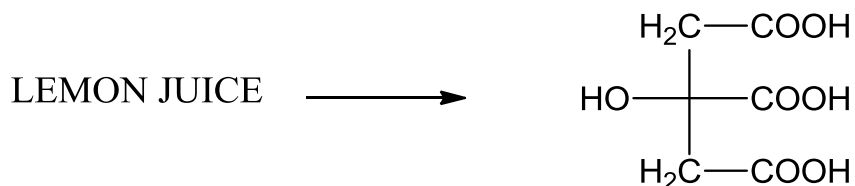
b)  $^1\text{H}$  NMR spectrum of caravone in  $\text{CDCl}_3$  (400 Hz)



c)  $^{13}\text{C}$  NMR spectrum of caravone in  $\text{CDCl}_3$



## ISOLATION OF CITRIC ACID

**Reagents:**

Lemon juice (ca. 100 mL – 3 lemons)  
 $\text{CaCl}_2$  5 g  
10% NaOH  
2M  $\text{H}_2\text{SO}_4$   
2M HCl  
2M NaOH

**Glassware:**

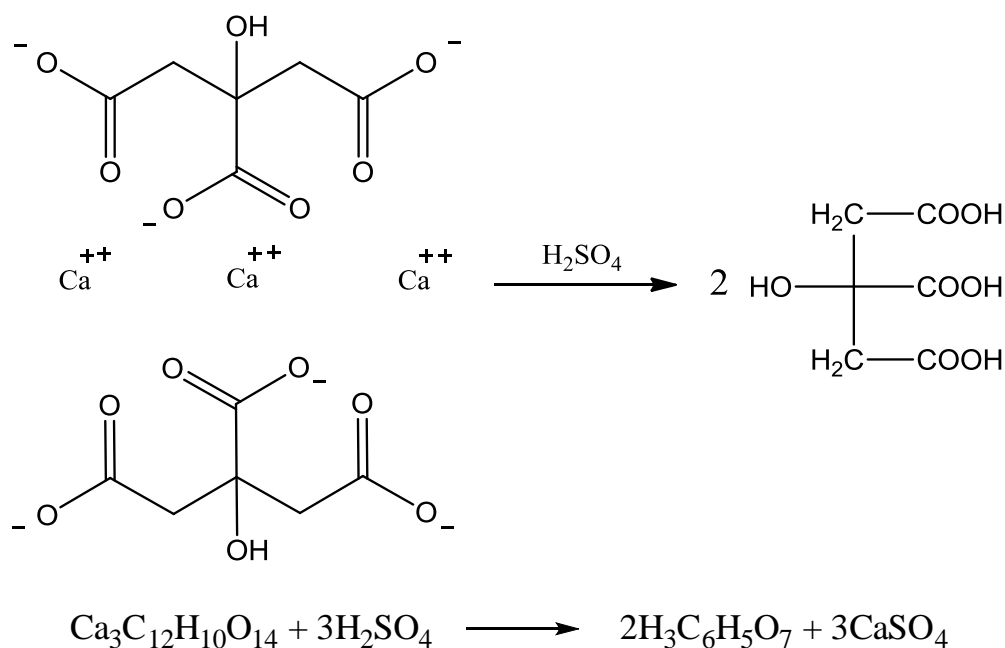
Magnetic stirrer  
beakers 250 mL (3)  
Measuring cylinders (2)  
filtering system with Büchner funnel  
flask 100 mL  
Pipettes (2)  
Pasteur's pipettes  
beaker 50 mL  
glass rod

Place a beaker (v. 250 mL) on a stirrer and pour into it 100 mL of fresh squeezed lemon juice (weight it!). Add dropwise slowly and carefully 10% aqueous NaOH basifying the mixture up to pH = 8. You will recognize this moment by changing of the colour from light yellow to light orange.

Filter the obtained mixture on a Büchner funnel. (Caution! Change from time to time the filter papers. The pores of filter paper become blocked, so it is necessary to replace the filter paper with a new one. Repeat it as often as necessary. A clog paper can cause an explosion of the filter flask!).

Move the obtained transparent layer to the beaker, place it on a stirrer and add 50 mL of 10% aqueous  $\text{CaCl}_2$ . Stir for 15 minutes, then heat it to boiling and filter calcium citrate ( $\text{Ca}_3\text{C}_{12}\text{H}_{10}\text{O}_{14}$ ) from the hot mixture on a Büchner funnel. Wash the obtained product on funnel with hot water. Dissolve the obtained crude product in small amount of 2M HCl (5 mL). Then neutralize the solution with 2M NaOH to approximately pH = 7.5 and boil the mixture. Separate the sediment on Büchner funnel and dry on the air.

Weight the product and calculate the yield relative to the amount of the fresh lemon juice.

**TRANSFORMATION OF CALCIUM CITRATE TO CITRIC ACID**

To transform calcium salt into citric acid add to it the proper amount of 2 M aqueous  $\text{H}_2\text{SO}_4$  (calculate it according to the reaction).

Stir with a glass rod and leave to stand for 10 min. Then filtrate the sediment of  $\text{CaSO}_4$  on a Büchner funnel, move to the beaker and concentrate the aqueous layer to 10 mL by boiling on a hot-plate. Cool down concentrated solution and after a few minutes the crystals of citric acid should appear. Filter the product obtained, dry it in the air and weigh. Measure the melting point (152-154 °C). Calculate the yield of citric acid relative to lemon juice used.

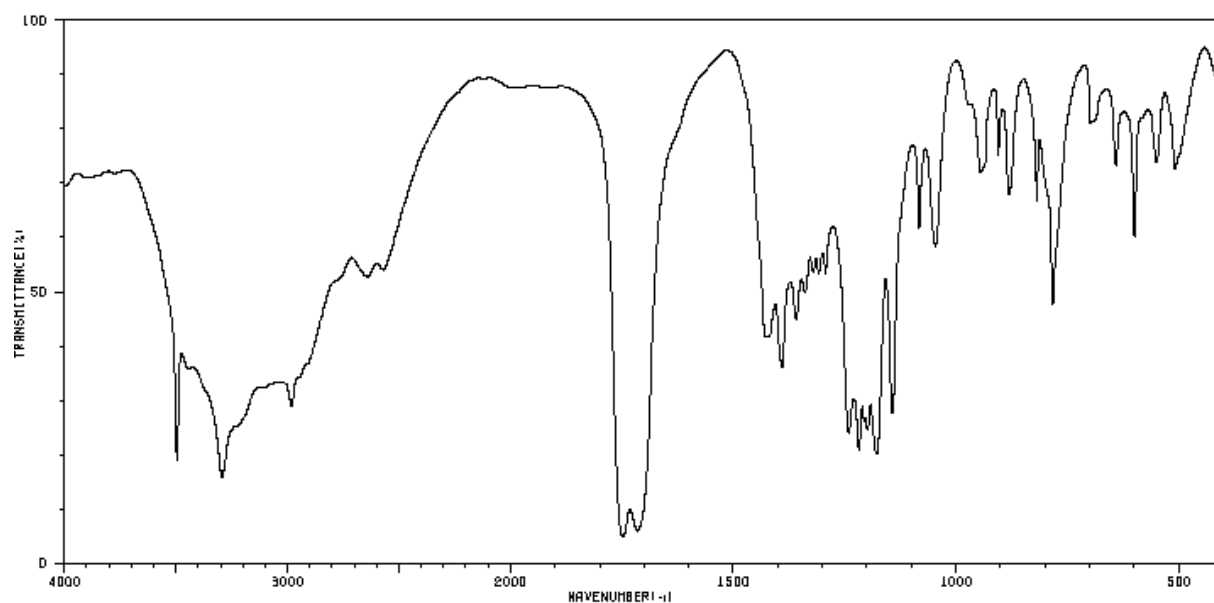
Thin layer chromatography (TLC):

on  $\text{SiO}_2$ , methanol - aq. ammonia (5:2).

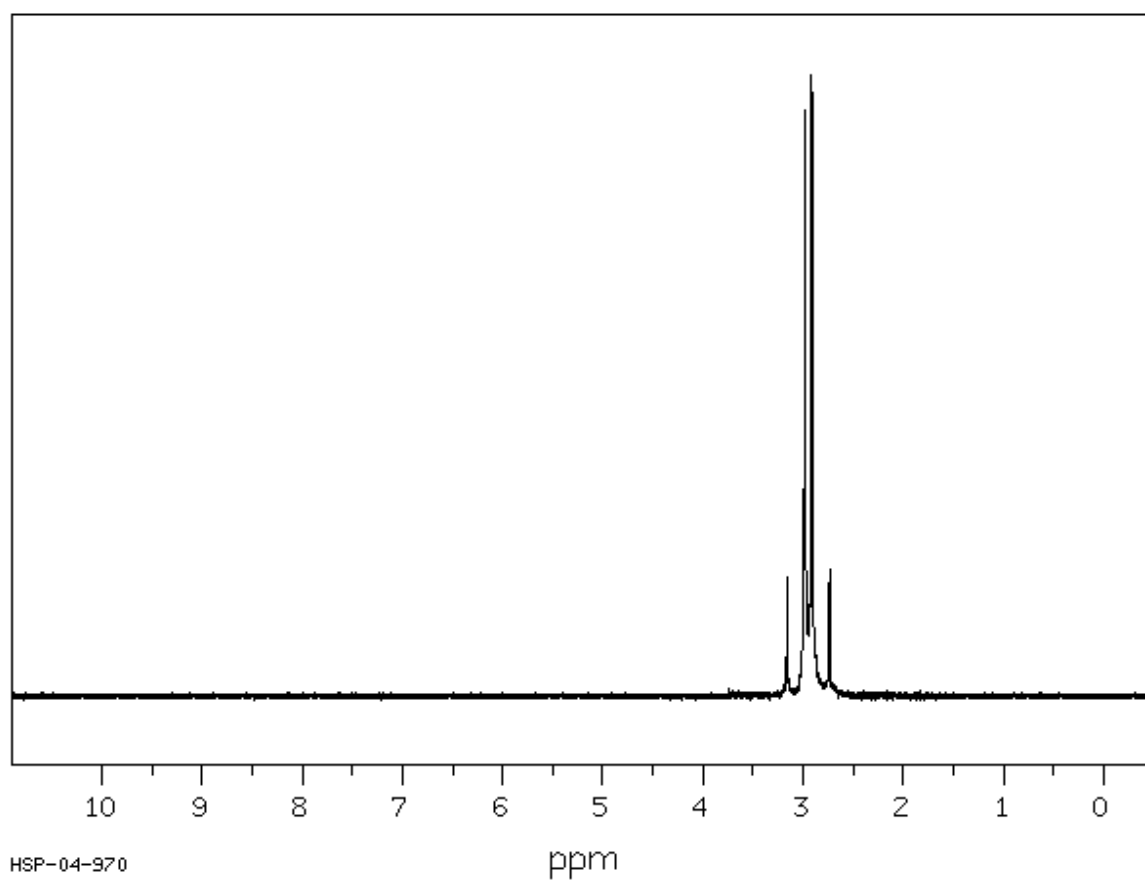
Remove the plate and allow the solvent to evaporate. After drying use a hot-plate and heat the TLC plate carefully and you will see spots develop on the plate.

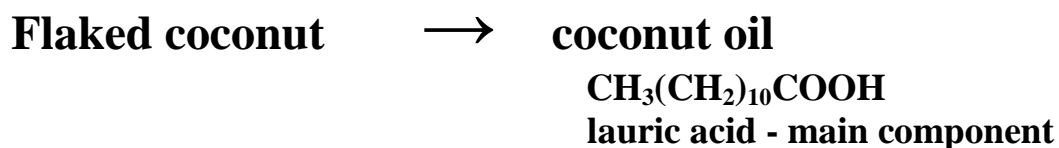
**SPECTRA**

a) IR spectrum of citric acid in KBr.



b) <sup>1</sup>H NMR of citric acid in D<sub>2</sub>O (90 MHz).



**FATTY ACIDS AND LIPIDS IN COCONUT OIL FROM COCONUT****Reagents:**

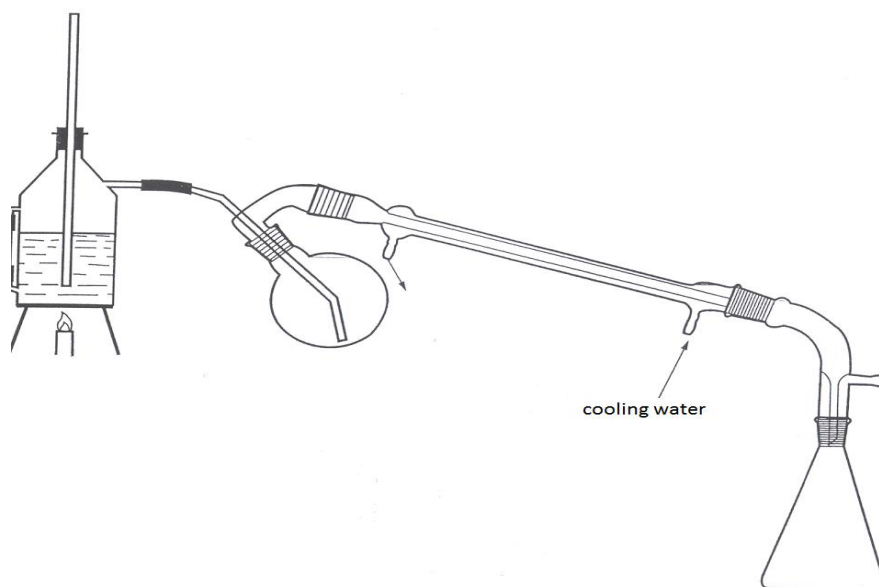
coconut turnings      2 x 15g  
petroleum ether      100 mL  
chloroform      250 mL  
anhydrous magnesium sulfate

**Instrumentation and glassware:**

steam distillation set (boiler, condenser, flask 500 mL, distillation head, heating mantle, conical flask 2 x 250 mL, plastic joints, rubber pipe)  
conical flask 300 mL  
Round-bottomed flask 250 mL  
reflux condenser

**Part A**

Maceration with petroleum ether. Place 15 g of flaked coconut in round-bottomed flask (250 mL) and add 100 mL of petroleum ether and heat under reflux for 1 h. Then cool down the mixture and filter coconut turnings from the solution on a funnel with filter paper. Concentrate obtained solution under reduced pressure and weigh.

**Part B**

Distillation using water steam distillation. Coconut oil can be also obtained by using water steam distillation.

Combine distillation set as it is shown in the picture above.

Place 15 g of flaked coconut in round bottomed flask (500 mL) and add 100 - 150 mL of distilled water. Run process until 300 mL of distillate was obtained. Received distillate should have milky-white color. Chloroform can be added to a receiver what cause better separation of coconut oil from water layer to chloroform layer. Transfer obtained solution into a separatory funnel and extract efficiently using chloroform (5 x 50 mL). Combine obtained extracts and dry them over anhydrous magnesium sulfate. Then concentrate it under reduced pressure. Weigh and calculate the yield of received oil.

Coconuts contain about 40 % of lauric acid.

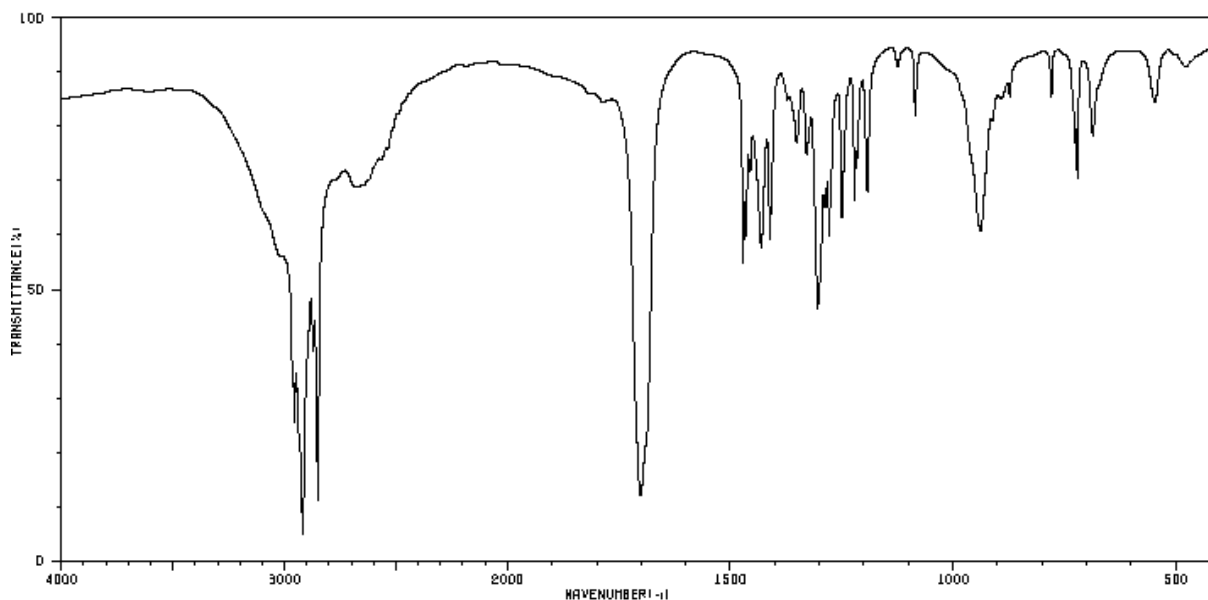
#### Thin layer chromatography (TLC):

on  $\text{SiO}_2$ ,  $\text{CHCl}_3/\text{MeOH}$  (5:5). Remove the plate and allow the solvent to evaporate. After drying use a hot-plate and heat the TLC plate carefully and you will see spots develop on the plate.

In a summary compare the amounts and smell of obtained coconut oils in method A and B. Draw some conclusions.

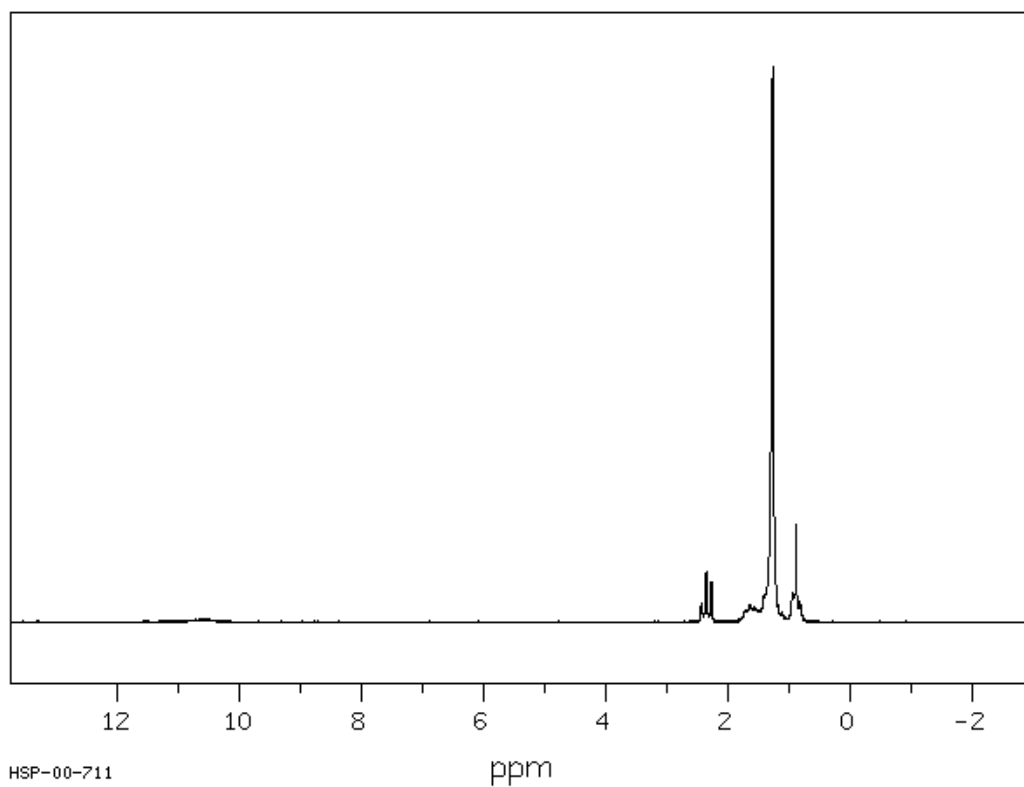
### SPECTRA

a) FTIR of lauric acid in KBr tablet.

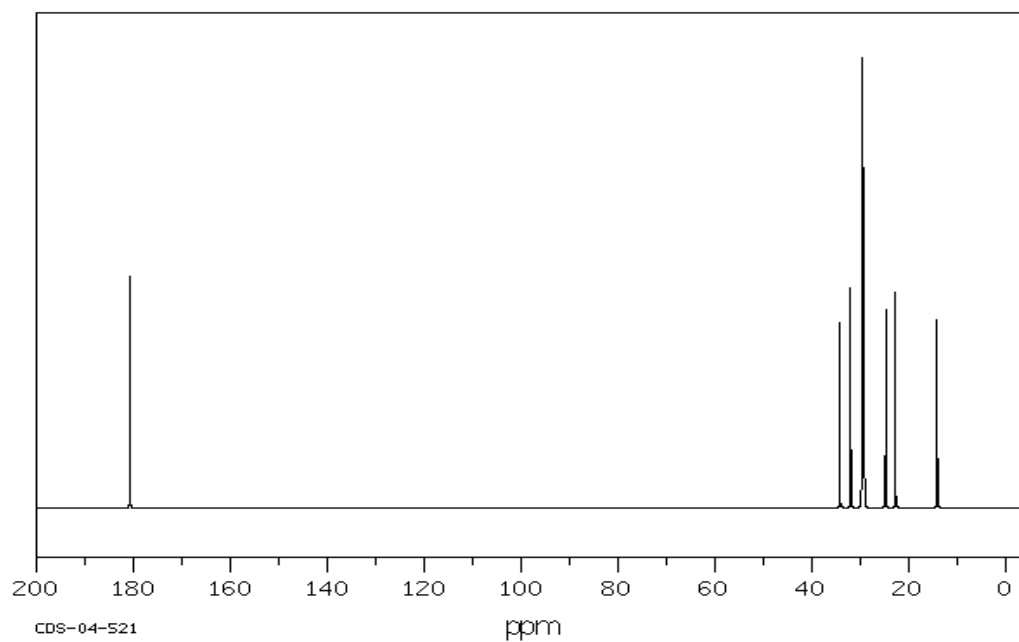




b)  $^1\text{H}$  NMR spectrum of lauric acid in  $\text{CDCl}_3$  (90 MHz).



c)  $^{13}\text{C}$  NMR spectrum of lauric acid in  $\text{CDCl}_3$  (90 MHz).



powdered cinnamon bark	30 g
ethyl acetate or chloroform	250 mL
MgSO <sub>4</sub> (anhydrous)	

steam distillation set (boiler, condenser, flask 500 mL, distillation head, heating mantle, conical flask 2 x 250 mL, plastic joints, rubber pipe)  
separatory funnel 250 mL  
round bottom flask 250 mL  
beaker 250 mL

1. *Journal of the American Medical Association*, 2000; 284: 2689-2695.

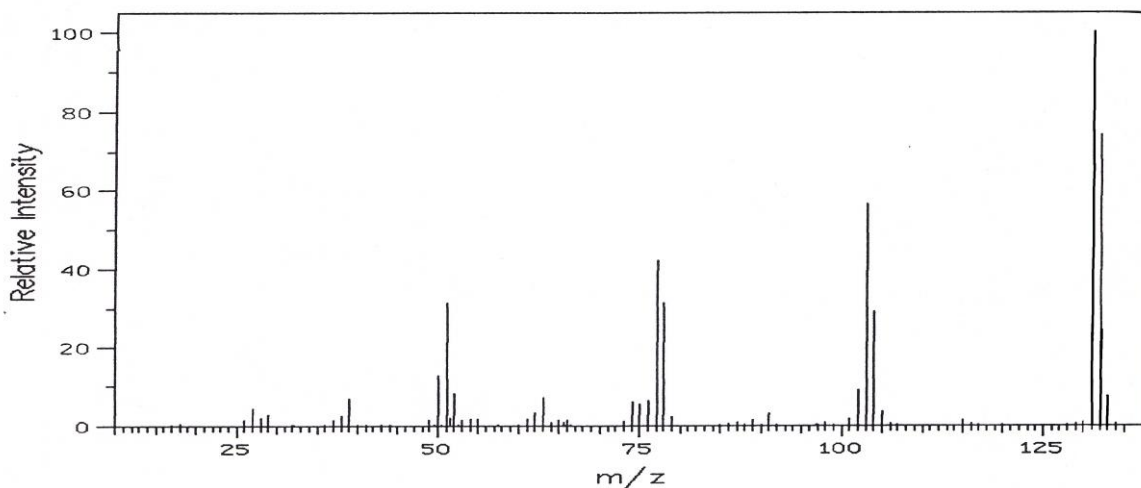
distillate into separatory funnel and extract efficiently using chloroform or ethyl acetate (5 x 50 mL). Combine obtained extracts and dry them over anhydrous magnesium sulfate. Then concentrate it under reduced pressure. Weigh and calculate the yield of received oil. Cinnamic aldehyde is a yellow liquid with boiling point 248°C.

#### Thin layer chromatography (TLC):

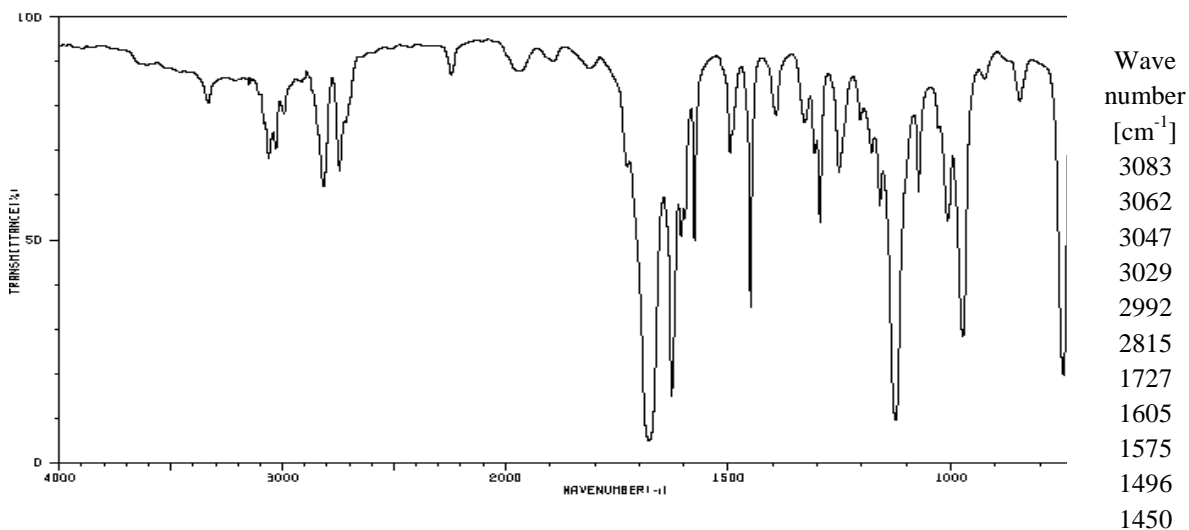
Small amounts of standard samples of benzoic acid, benzaldehyde and cinnamic acid should be dissolved in methanol. Place the solutions of standard samples on silica gel plate with capillary and diluted solution of given cinnamic oil. Eluent: dichloromethane. After drying the plate check the result under UV lamp and mark the spots using pencil. Comparing the spots of standards with spots of cinnamic oil indicate which compounds are present in given cinnamic oil.

### SPECTRA

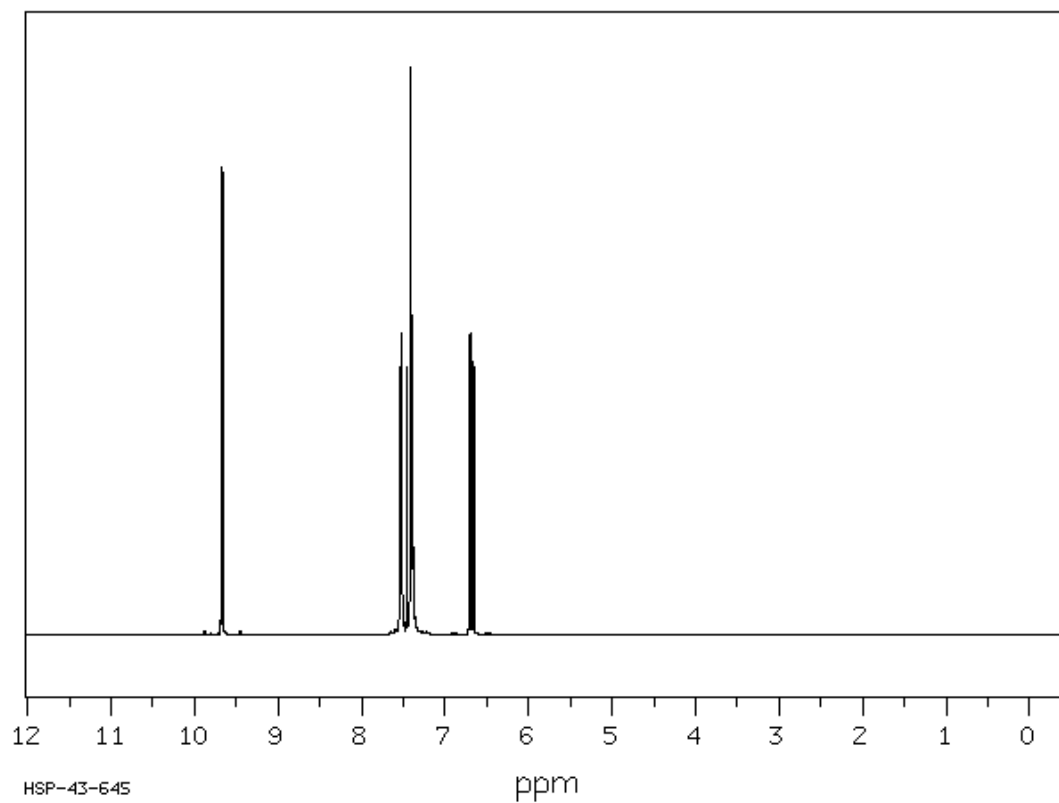
#### a) EIMS spectra of cinnamic aldehyde



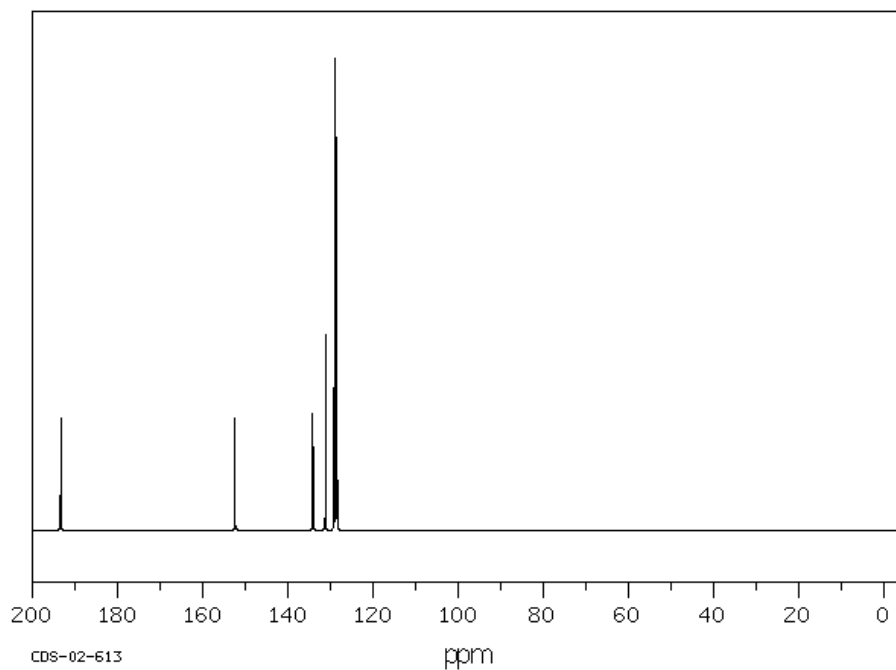
#### b) FTIR Spectra of cinnamic aldehyde in liquid film

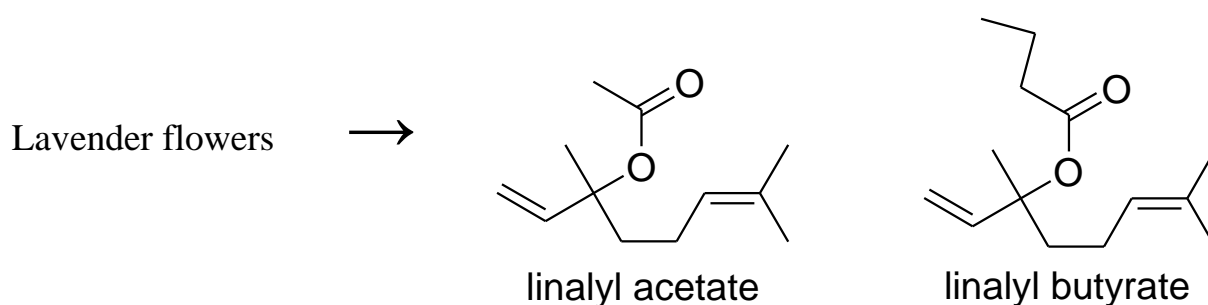


c)  $^1\text{H}$  NMR spectrum of cinnamic aldehyde in  $\text{CDCl}_3$



d)  $^{13}\text{C}$  NMR spectrum cinnamic aldehyde in  $\text{CDCl}_3$



**ESTERS IN LAVENDER OIL FROM LAVENDER FLOWERS****Reagents:****Part A**

lavender flowers            15 g  
dichloromethane        450 mL  
magnesium sulfate anhydrous

**Part B**

lavender flowers            15 g  
dichloromethane        300 mL  
magnesium sulfate anhydrous

**Instrumentation and glassware:**

Round-bottom flask 250 mL  
condenser  
heating mantle  
funnel  
separatory funnel 250 mL  
steam distillation set (boiler, condenser, flask 500 mL, distillation head, heating mantle, conical flask 2 x 250 mL, plastic joints, rubber pipe)

steam distillation set (boiler, condenser, flask 500 mL, distillation head, heating mantle, conical flask 2 x 250 mL, plastic joints, rubber pipe)  
separatory funnel 250 mL

The main aim of this experiment is isolation of lavender oil from lavender flowers with two methods (Part A and Part B) and in the next step comparison of the yield of these two methods. Linalyl acetate and linalyl butyrate are the main components of lavender oil, which gives lavender flowers their characteristic smell.

**Part A**

Place 15 g of lavender flowers into a round-bottom flask (250 mL) and pour 100 mL of dichloromethane (add boiling chips!). Heat mixture for 1h then cool down to the room temperature and separate flowers from solution by filtering on a funnel with a paper filter. Concentrate the obtained greenish solution under reduced pressure and weigh.

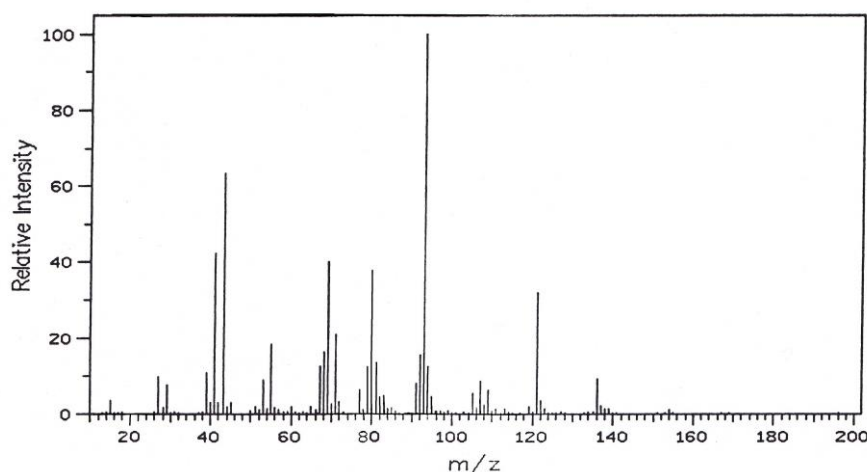
**Part B**

Assemble set for steam distillation as it is showed in the picture, page 64. Place 15 g of lavender flowers into round-bottom flask (500 mL) and add 100 - 150 mL of distilled water. Then, run distillation till 300 mL of distillate is obtained. Next, carry the distillate into separatory funnel and extract efficiently using chloroform or ethyl acetate (5 x 50 mL). Combine obtained extracts and dry them over anhydrous magnesium sulfate. Then concentrate it under reduced pressure. Weigh and calculate the yield of received oil.

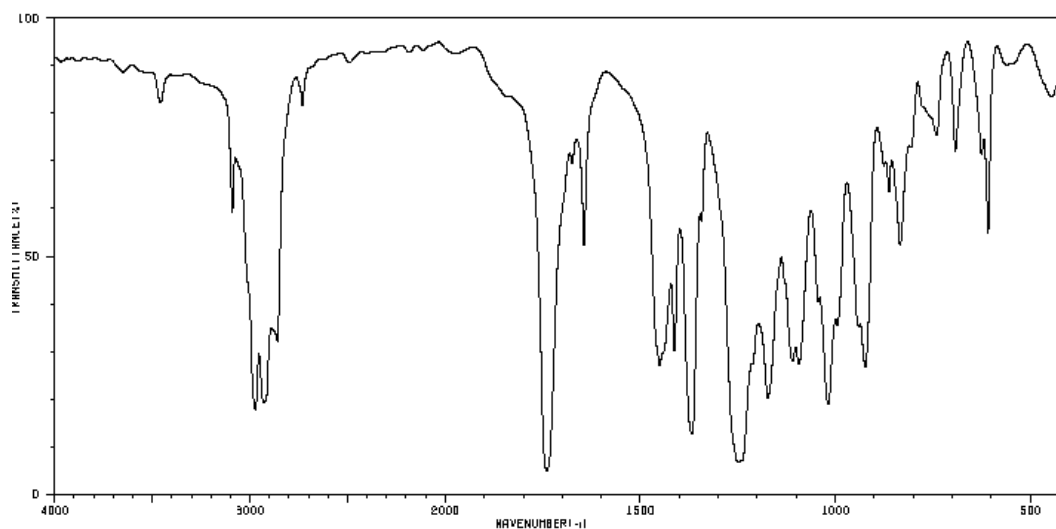
In a summary compare the amounts, smell and color of obtained lavender oils in method A (two steps) and B. Compare the efficiency of both procedures.

**SPECTRA**

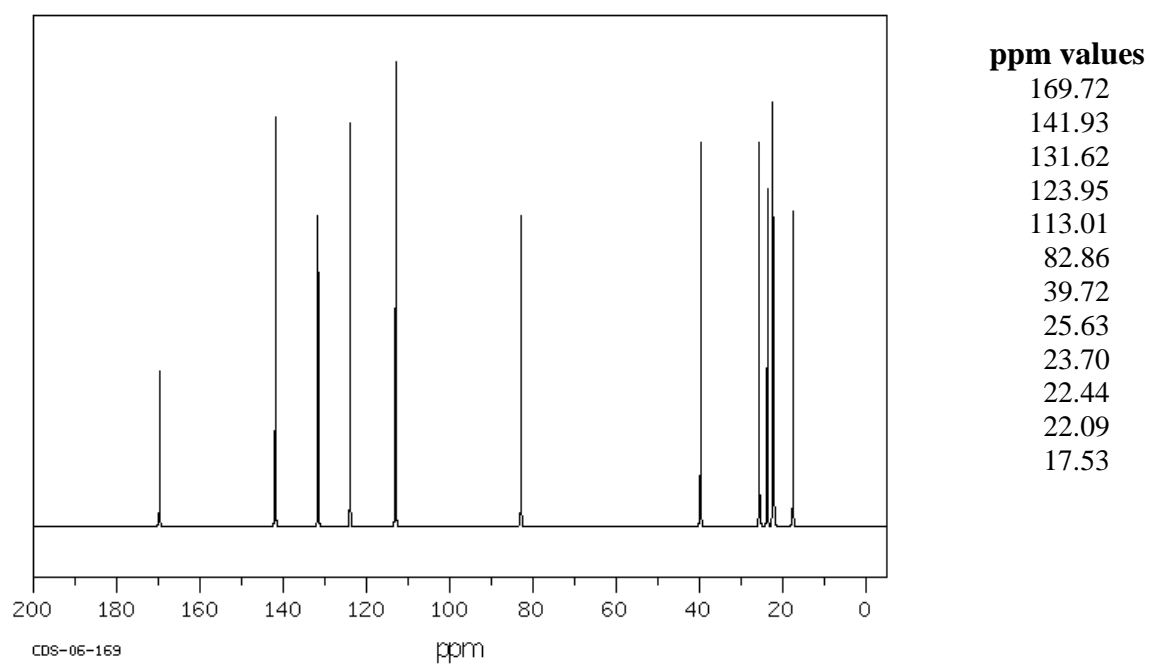
a) EI MS spectrum of linalic acetate



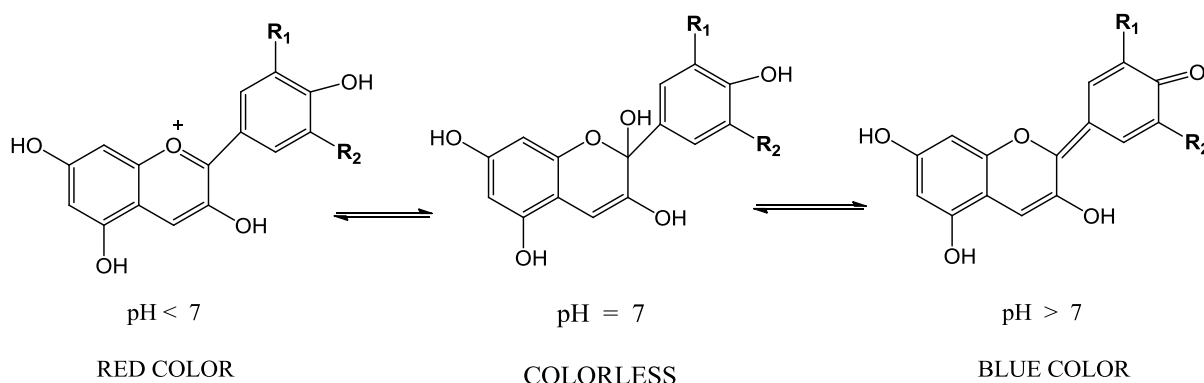
b) FT IR spectrum of linalyl acetate



c)  $^{13}\text{C}$  NMR spectrum of linalyl acetate



## ANTHOCYANINS

**Reagents:**

plant material: 10 g hawtorn fruits  
                     5 g hibiscus flowers  
                     10 g dog rose fruits  
                     5 g black hollyhock flowers  
 methanol  
 inorganic salts (see table)

**Instrumentation and glassware:**

beaker 400mL and 200mL  
 30 tubes  
 funnel  
 glass rod  
 Pasteur pipettes

Start maceration process by adding chosen plant material (10 g hawtorn fruits, 5 g hibiscus flowers, 10 g dog rose fruits, 5 g black hollyhock flowers) into a beaker (400 mL) and add 200 mL of methanol. Keep plant material in the solvent for half an hour and stir by glass rod from time to time. Then received macerate should be filtered on funnel and concentrated under reduced pressure. Weigh and calculate the percentage content of received anthocyanins oil in plant material.

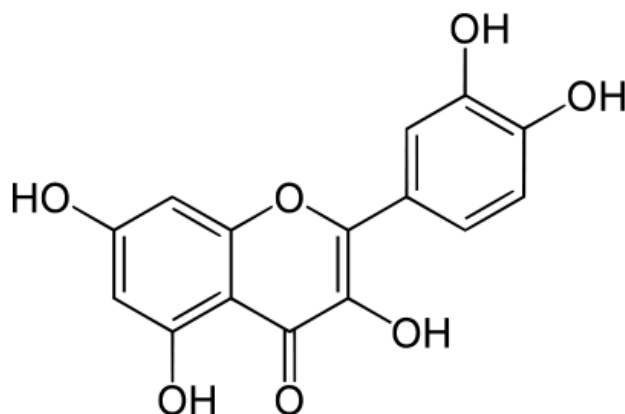
Dissolve anthocyanins oil in methanol/water (1:1). In one tube prepare solution which will be color standard by adding 6-10 drops of anthocyanins solution to 5 mL of distilled water. In 15 tubes prepare aqueous solution of respective inorganic salts (table below) by dissolving in about 5 mL of distilled water. Determine the pH of each solution of inorganic salts from the appropriate ionic hydrolysis reaction. Using Pasteur pipette add solution of anthocyanins dropwise to the respective tubes. Note the solution color immediately after mixing both solution. Check the change of colors after half an hour have and write down observations in the table.



In the conclusions compare the colors of the solutions with monovalent, divalent and trivalent metal ions, how the color of the solutions depends on pH of the solutions.

Tube number	Salt	pH	Color of the solution directly after adding salt solution	Color of the solution after half an hour from adding salt solution
1.	LiClO <sub>4</sub>			
2.	Li <sub>2</sub> CO <sub>3</sub>			
3.	lithium citrate			
4.	NaCl			
5.	sodium citrate			
6.	NaNO <sub>2</sub>			
7.	Na <sub>2</sub> SO <sub>3</sub>			
8.	KBr			
9.	K <sub>2</sub> CO <sub>3</sub>			
10.	MgSO <sub>4</sub>			
11.	CaCl <sub>2</sub>			
12.	NH <sub>4</sub> Cl			
13.	FeCl <sub>3</sub>			
14.	FeSO <sub>4</sub>			
15.	Al(NO <sub>3</sub> ) <sub>3</sub>			

## FLAVONOIDS



Quercetin one of flavonoids in onion skins

**Reagents:**

plant material: 5 g onion skins  
                  5 g yellow flowers  
                  5 g willow bark  
                  5 g oak bark  
                  5 g birch bark

methanol

inorganic salts (table)

**Instrumentation and glassware:**

beaker 400mL and 200mL

30 tubes

funnel

glass rod

Pasteur pipettes

Start maceration process by adding chosen plant material (5 g onion skins, 5 g yellow flowers, 5 g willow bark, 5 g oak bark or 5 g birch bark ) into a beaker (400 mL) and add 200 mL of methanol. Keep plant material in the solvent for half an hour and stir by glass rod from time to time. Then received macerate should be filtered on funnel and concentrate under reduced pressure. Weigh and calculate the percentage of received flavonoids oil in plant material.

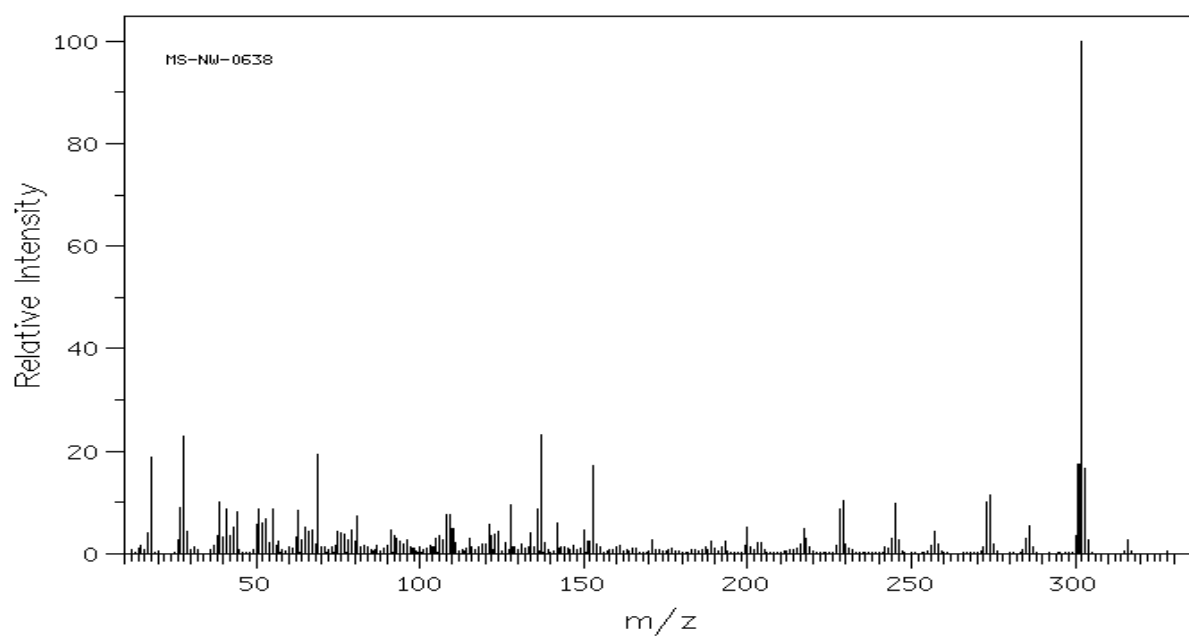
Dissolve flavonoids oil in methanol : water (1:1 v/v). Prepare in tube solution which will be color standard by adding 6-10 drops of flavonoids solution to 5 mL of distilled water. In 15 tubes prepare aqueous solution of respective inorganic salts (table below) by dissolving in about 5 mL of distilled water. Determine the pH of each solution of inorganic salts by writing the ionic hydrolysis reactions. Add dropwise by using Pasteur pipette solution of flavonoids to the respective tubes. Note the solution color directly after mixing both solution. Then wait and after half an hour check if colors of the solutions have changed and write down observations in the table.

In the conclusions compare the colors of the solutions with monovalent, divalent and trivalent metal ions, how the color of the solutions depends on pH of the solutions.

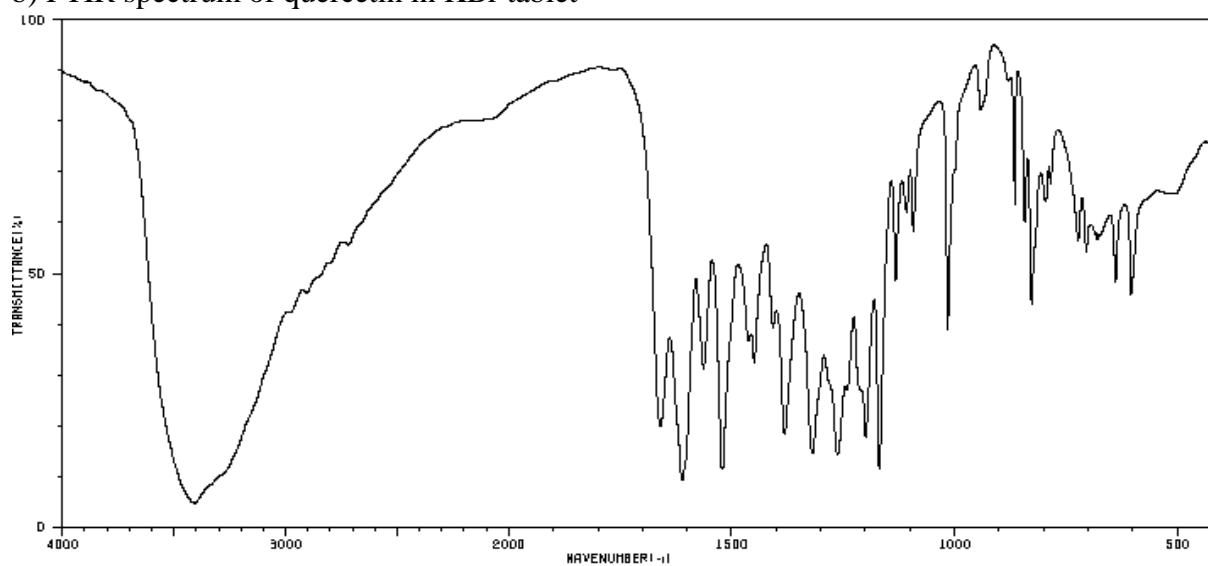
Tube number	Salt	pH	Color of the solution directly after adding salt solution	Color of the solution after half an hour from adding salt solution
1.	LiClO <sub>4</sub>			
2.	Li <sub>2</sub> CO <sub>3</sub>			
3.	lithium citrate			
4.	NaCl			
5.	sodium citrate			
6.	NaNO <sub>2</sub>			
7.	Na <sub>2</sub> SO <sub>3</sub>			
8.	KBr			
9.	K <sub>2</sub> CO <sub>3</sub>			
10.	MgSO <sub>4</sub>			
11.	CaCl <sub>2</sub>			
12.	NH <sub>4</sub> Cl			
13.	FeCl <sub>3</sub>			
14.	FeSO <sub>4</sub>			
15.	Al(NO <sub>3</sub> ) <sub>3</sub>			

**SPECTRA**

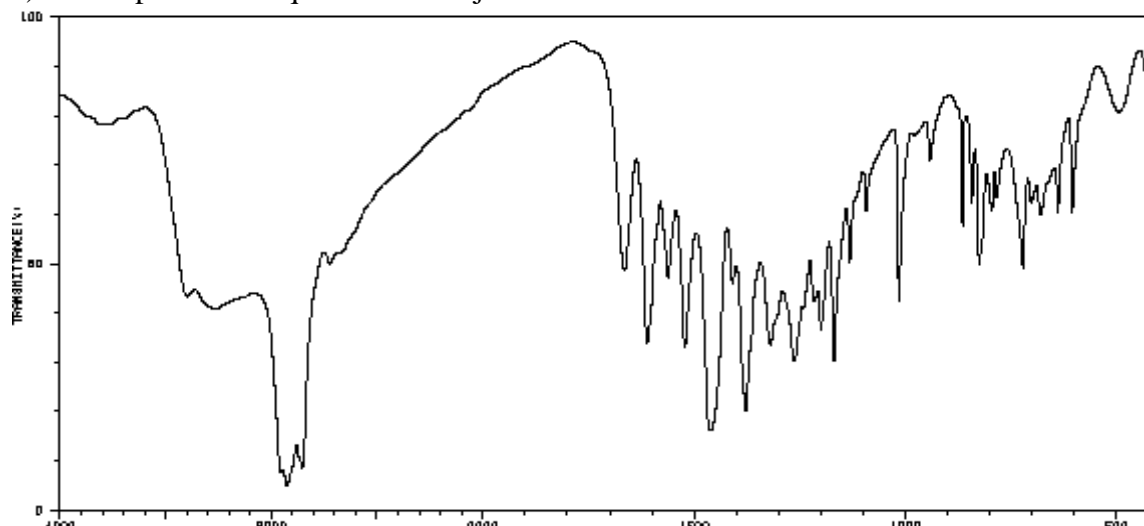
## a) EI MS mass spectrum of quercetin



## b) FTIR spectrum of quercetin in KBr tablet



c) FTIR spectrum of quercetin in nujol



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