PROGRAM STUDI KIMIA
UNIVERSITAS MA CHUNG
Villa Puncak Tidar N-01, Malang 65151, Jawa Timur

Organic Chemistry Laboratory

Module

KI 222

NAME : NIM :	
Experimental Course Schedule: • In Organic Chemistry at MRCPP Laboratory. • Every Monday. • Must check in at the latest 15 minute before the course begins, otherwise could not attend the course.	
Morning 08:00 – 12:00	Afternoon 13:00 – 17:00
• Check in, Briefing, Small Quiz • Experimental Course	• Experimental Course • Closing Remark

Halaman Pengesahan

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"Organic Chemistry Laboratory"

Penyusun:

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ORGANIC CHEMISTRY
LABORATORY MANUAL

Identification Reaction, Qualitative Analysis
And
The Synthesis

A COLLECTION OF STANDARD EXPERIMENTAL PROCEDURES

UNIVERSITAS
MA CHUNG

Compiled and Edited
by
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Universitas Ma Chung
2016

Experiments in Organic Chemistry, Ma Chung University, 2016

This book includes laboratory works that are compiled and edited from internationally recognized courses such as *Laboratory Methods of Organic Chemistry* by L. Gattermann, *Elementary Practical Organic Chemistry* by A.I. Vogel, *Basic Organic Chemistry Laboratory Course* by the University of Helsinki, and *Microscale Organic Laboratory with multistep and mutiscale synthesis* by D.W. Mayo, R.M. Pike, and D.C. Forbes.

The laboratory works consist of three parts, i.e. identification reaction of organic compound, two-component qualitative analysis and synthesis of eight organic compounds.

Compiler and Editor:

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CHECK IN PROCEDURE

Check In Procedure for Organic Chemistry Laboratory Course, Universitas Ma Chung:

1. Prior to the laboratory work the student has to come to the laboratory **30 minutes** before the course begin. After having contact with an assistant, the student has to fill out the logbook and prepare the equipment, chemicals and materials needed for the experiment.
2. Pre-work examination will be done **15 minutes** before the laboratory work begins. The examination consists of understanding of safety and security combined with the understanding of reaction mechanisms and theoretical knowledge of the protocol. The student only has **10 minutes** to finish the examination. The examination **has to be passed** before the laboratory work can be continued and the pass mark influences the final mark.
3. Failure in doing the procedure will result to the cancelation of the laboratory experiment.

1 Safety and Security Regulation

The organic chemistry laboratory provides you with a unique opportunity to do the reactions which you can only read about in your lecture course. However, before you begin your laboratory experience you must learn some basic safety procedures, because chemistry laboratories are **potentially dangerous**. You must learn how to protect yourself from chemicals, fire, broken glassware, and electrical shock. You must know what to do in the event of a fire or other emergency in the labroom or building.

The Ma Chung organic chemistry teaching laboratories have minimized laboratory hazards by converting experiments to semi-microscale, substituting toxic chemicals with less harmful ones whenever possible and eliminating the use of Bunsen burners. Know the hazards of the chemicals you use so that you will know what level of caution to use when handling them. If you do this, you will not be exposed to a harmful amount of any chemical during your year in organic chemistry laboratory. Use common sense and work carefully to avoid chemical spills, broken glassware, electrical shock, and fires. Ultimately, the responsibility for safety rests with each and every student in the laboratory.

Accidents do happen. When they do, you need to know what steps to take to prevent further injury. Most accidents are minor and methods of dealing with them are detailed in the sections below. In the event of a serious accident, remember that injured people are often in shock and are unable to help themselves. **You should be prepared to help your neighbor if a serious accident occurs. A matter of seconds can be critical.**

1.1 Special Health Problem

If you are aware that you have allergies to specific chemicals or drugs, or to UV light, or if you have asthma or other health problems, you may want to consult you doctor before taking organic chemistry lab. Feel free to discuss any questions you may have with the Laboratory Coordinator.

SAFETY RULES

Wear safety goggles. You may only remove you goggles if no one in the lab room is using chemicals or washing glassware.

Contact lenses must not be worn.

Wear protective clothing and closed-toed shoes. Gloves are highly recommended at all times.

Food and drink are not allowed in the lab room.

No smoking.

Prevent chemical spill by clamping flask containing chemicals whenever possible.

Work in you hood at all times. Cover containers of compounds during transport through the lab.

Prevent breakage by handling glassware with care at all times. Securely clamp reaction and vacuum flasks.

Place broken glassware in the proper receptacle.

Use a Bunsen burner only when directed by your

TA. Place hazardous waste in the proper container.

When the fire alarm sounds, leave the building immediately.

No unauthorized experiments.

The Safety Rules list summarizes the rules students must follow in the lab to ensure the safety of themselves and other students in the lab. The reasons for these rules are detailed in the section that follows. Strict adherences to all safety rules is essential in the organic chemistry laboratory: Anyone consciously violating safety rules will be asked to leave the laboratory immediately, no make-up session will be allowed and will be graded as fail in the laboratory course.

1.2 Eye Safety

1.2.1 Goggles

Laboratory safety goggles **must be worn** whenever anyone in the room is handling chemicals or washing glassware. Even if you are finished with your experiment, another student may not be and could splash chemicals in your direction. Your eyes are particularly sensitive to chemicals and a tiny splash in your eye has the potential to cause a lot of harm, possible even blindness. The vapors from some chemicals cause eye irritation. Simply put, you must wear goggles to prevent eye damage. Think of it as wearing a seat belt: Get used to it!

1.2.2 Contact Lenses

Contact lenses **must not be worn** since they prevent efficient washing of the eye in case of contact with chemicals. Also, soft lenses absorb chemical vapors, causing lens damage and possible focusing the contact to the eye.

1.2.3 Eye Wash Locations

Know the location of the eye wash in your lab. They are appropriately marked.

EMERGENCY RESPONSE CHEMICALS IN THE EYE>> Immediately go to the eye wash and begin flushing your eyes. Hold the affected eye open to assure proper rinsing. Continue flushing for 10-15 minutes. Medical treatment is strongly advised after the rinsing period is complete.

1.3 Chemical Safety

The following sections overview the hazards and handling advisories for the common solvents and corrosives used in the organic chemistry lab. Specific hazard information for all chemicals used in the laboratories is given in the procedure sections of each experiment of this *Handbook*. **Please, always advised yourself through attentive reading of the hazards of chemicals in the laboratory, Material Safety Data Sheet (MSDS) provided by Sigma Aldrich or any chemical companies, and bottle labels.**

Organic chemicals have the potential to be more dangerous than most of the chemical used in the first year chemistry labs because the chemicals are often flammable, volatile, and/or toxic. Organic chemicals also use strong acids and bases. The list of hazards for a particular chemical is sometimes long and alarming. This might lead you to believe that the organic chemistry lab is always a dangerous place in which to work. This is not the case! The hazards are real by only *potential*. You need to know what the hazards are so that you will know when to take extra precaution to minimize your exposure. Organic chemists have an average life span as long as anybody else because they have learned how to handle chemicals.

REMEMBER: If handled properly, even a dangerous chemical will cause no harm.

1.3.1 Hazards of Solvents

Flammable. When working with flammable solvents, make sure that there is not an open flame anywhere in the lab room. At any time, only use a Bunsen

burner only when specifically told to do so by your TA. Steam baths and heating mantles are to be used as heat sources whenever possible. Use care not to spill flammable solvents on the hot surface of a heating mantle.

Volatile. Volatile solvents are those that vaporize easily; they are a potential hazard because if you breathe in a large volume of the vapors, you might experience irritation of the respiratory tract, intoxication, central nervous system depression, drowsiness, or nausea. Prevent accidental vapor inhalation by always working with these solvents in the fumehood and always cover containers of them if you have to carry them through the lab.

Health hazard. Methanol, hexanes, methylene chloride, and to a lesser extent ethanol, acetone, and ether could cause serious harm if you have a one-time over-exposure to them. If you ingested any of them in sufficient quantity, you would definitely get sick. Do not eat them! Do not bring food or drink into the lab, because someone might spill a chemical on it before you eat it. Each has a slight contact hazard and could cause an irritation if you got some on your skin. Avoid spilling solvents on yourself, and especially on open wounds or in your eyes. Protect yourself at all times by wearing goggles, gloves, and protective clothing, either a lab coat or long pants. Always wear close-toed shoes in the lab to protect your feet. Keep all of these solvents in the hood as much as possible and never sniff a container of them.

Some solvents also show a cumulative effect when the body is exposed to small amounts repeatedly over a long period of time. Halogenated solvents such as methylene chloride are not detoxified by the body and build up to level, which can cause serious organ damage and even perhaps cancer. Ethanol on repeated use can cause cirrhosis of liver. Minimize your exposure by always wearing protective gloves and clothing, working in your hood, avoiding spills.

In the past 15 year, the organic chemistry community has been very cognizant of the environmental impact of organic solvents and thus green solvents are growing in importance. Green solvents are defined as solvents that have

minimal toxicity to humans and the environment where their toxicities are well understood. Green solvents that are growing in importance include ionic liquid, fluorous solvents, supercritical carbon dioxide, water, ethanol and aqueous micelles and polymers. Although green solvents are nontraditional, there are traditional solvents that are considered green, including acetic acid, benzyl benzoate, diethyl glycol dimethyl ether, DMSO, ethyl acetate, glycerol, hexane, methanol, *t*-butanol and THF. In addition to green solvents, organic chemists have identified alternatives (or drop-in) solvents which are considered safer alternatives for a variety reason.

Traditional Solvent	Issue with Solvent	Drop-in Solvent
Benzene	Carcinogen	Toluene
Carbon tetrachloride	Carcinogen; Depletion of ozone	Cyclohexane
Chloroform	Toxicity; Stability	Dimethoxyethane
Dichloromethane	Volatile; Possible carcinogen	Benzotrifluoride
Diethyl ether	Low flash point	Methyl *t*-butyl ether
Hexane, Pentane	Volatility	Heptane
THF	Miscibility with H_2O	2-MeTHF
Dioxane, Dimethoxyethane	Toxicity	2-MeTHF
HMPA	Carcinogen	DMPU

DMPU: *N*,*N*-Dimethylpropylene urea or 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone; HMPA: hexamethylphosphoric triamide; THF: tetrahydrofuran

1.3.2 Hazards of Corrosive

Strong acids and bases are used frequently in the organic chemistry teaching labs. The acids used include acetic, hydrochloric, sulfuric, nitric, and phosphoric. In concentrated form, each of these can cause chemical burns on your skin and is extremely harmful to your eyes. Sulfuric and nitric acids cause severe burns than do the other acids. If you spill them on your skin, you will feel an immediate painful stinging. Do not inhale any of these acids, as they are extremely irritating to the nasal passage and respiratory tract. The more dilute the acid, the less likely it is to cause harm.

The concentrated bases used in the organic labs are sodium and ammonium hydroxide. Like concentrated acids, they will cause chemical burns on your skin and tissue damage, although they do not cause an immediate stinging. Often you will not known you have spilled them on you until the damage is already done. Ammonium hydroxide is a severe bronchial irritant: avoid breathing these vapors.

When handling acids and bases, wear goggles, gloves, and protective clothing. Work with them in the hood and cover containers of them during transport.

1.3.3 Hazards of All Chemicals

In general, you should treat all chemical as if they are harmful. Do not eat them, prevent them from contacting your skin and eyes, and avoid breathing the vapors. Always look up the hazards of the chemicals which you will be using in an experiment *before* you come to lab.

1.3.4 Hazardous Wastes

Any chemical which you will no longer use is considered "Hazardous Waste". All such waste must be placed in the proper container in the main hood.

1.3.5 Personal Protection Equipment (PPE)

PPE includes safety goggles, face shields, clothing, gloves, and respirators. In most organic teaching laboratory situations, safety goggles, clothing and gloves are sufficient PPE. Lab coats are certainly strongly advised. An MSDS or bottle label for a compound might recommend a specific type of PPE. For instance, full suits or overalls or even boots might be required. Respirators are rarely used in teaching laboratories, although they can be used if a hood is not available, especially if you need to handle a very toxic chemical.

Gloves are highly recommended when handling chemicals and glassware. After reading about the hazards of chemicals and broken glassware, if you still do not want to wear gloves, it is your choice. Thick Playtex gloves may be

purchased at any local supermarket. Thick gloves work the best to protect your hands from spill, cuts, and burns; however, they can make it difficult to handle small pieces of glassware. Students complain that thick gloves lead to glassware breakage, and prefer thin Latex gloves, purchased at any stores. These gloves do not protect you from solvents or corrosives; these chemicals actually degrade the glove on contact. Latex gloves do provide a small level of protection from solid chemicals and solvents, and they make it easier to handle glassware without breaking it.

"Nitrile" gloves are a new type of glove which combine the best attribute of both thick Playtex and thin Latex gloves. These gloves are thin enough to allow easy glassware manipulation, while they are resistant to solvents such as methylene chloride for up to 4 minutes.

EMERGENCY RESPONSE: CHEMICAL SPILLS AND CHEMICAL VAPOR INHALATION >>

Spill. Wash the affected area with lots of water, especially if the chemical was a strong acid or base. If the affected area is large, use hand-held wash which is located by the eye wash in each lab. Soap helps remove the chemical, but never try to "treat" the spill with another solvent or with a neutralizing solution unless directed to do so by your TA. If the affected area remains more than slightly red after the rinsing period, seek medical attention. Know what chemicals you have been exposed to so that you can tell the attending physician.

Inhalation. If you inhale to many chemical vapors, tell the TA or the Lab Coordinator and leave the area immediately, preferable to an outside location in the fresh air. If you continue to feel the effects of the vapors, you will need medical attention.

1.4 Glassware and Equipment Safety

In the chemistry laboratory, broken glassware has the potential to do serious harm. Cuts from glassware pose not only a bleeding hazard, but they increase the risk of chemical exposure. Hot glassware can cause burns.

Use common sense when handling glassware. Keep glassware away from the edge of the benchtop. Always clamp your reaction flask and the suction flask securely to a ring stand to prevent them from falling over. Check each piece of glassware for hairline or star cracks before using it. When doing a distillation, clamp each piece of glassware securely. Wear heavy gloves to protect your hands while handling glassware. If you do break a piece of glassware, wear thick gloves and use a brush and dustpan to sweep up the broken glass: do not leave broken glass in the sink or on the benchtopbecause some- one may inadvertently get cut. Place the broken glassware in one of the "Broken Clean Glassware" containers located in the labs.

Protect your feet by wearing closed-toed shoes, not only to protect your feet from dropped glassware, but to protect them from broken pieces of glass which may be on the floor from a previous lab section. Always wear your goggles to protect your eyes from flying broken glassware.

If your reaction is heated with a heating mantle or steam bath, remember that the glass- ware or the clamps used to hold glassware can become hot enough to cause a thermal burn on your skin. Wear heavy gloves to prevent this.

Use electrical equipment (heating mantles, Variacs, stir motors) properly to prevent electrical shock. Check the cord or plug to make sure that it is not damaged. Always disconnected the plug from the socket by pulling firmly on the plug: Do not yank it out by the cord! Keep water away from all electrical equipment.

EMERGENCY RESPONSE: CUTS AND BURNS>>

If you cut yourself, wash the wound immediately with large amount of cool water. If it is your neighbor who has been hurt, be prepared to help them if they are unable to help themselves. Apply direct pressure to stop the bleeding as necessary. If the bleeding is profuse, elevate the affected limb. Watch for evidence of shock and contact your TA and the Lab Coordinator as necessary.

Thermal burns are treated by covering the affected area with cool water or ice. After a while, you can apply a pain-relieving cream. If the burn looks like it is more than just are red-dening of the skin, seek medical attention.

1.5 Fire Safety

The chances of a fire occurring in the organic chemistry teaching labs have been minimized both by eliminating Bunsen burners and by using only small quantities of flammable solvents. If you are directed by your TA to use a Bunsen burner, remove all solvents from the immediate area and ask your TA for any other directions.

Fires can occur from sources other than Bunsen burners. If a solvent falls onto a hot surface such as a heating mantle, it might ignite into flame. Electrical fires are also possible. Or, the fire could originate outside the lab. Once a fire has started, the presence of flammable solvents makes the situation quite dangerous.

1.5.1 Fire Extinguisher

There are two types of fire extinguishers in the labs, CO_2 and dry chemical. Both of these are suitable for type B and C fires:

Type B fires: flammable liquids

Type C fires: electrical equipment

The best choice is usually a CO_2 extinguisher; these can be quickly differentiated from the dry-chemical type because they have a large nozzle. Dry chemical extinguishers can create a messy cleanup problem. In the case of an electrical fire, dry chemical extinguishers might work better, since the dry chemical will act as an insulator to stop the flow of electricity and prevent the fire from re-starting. CO_2 extinguishers can be used on electrical fires if the burning electrical equipment can be unplugged.

1.5.2 If the Fire Alarm Sounds

When the fire alarm sounds, all students should immediately walk out the lab. Do not take time to gather belongings! You may shut off bunsen burners and electrical equipment if it can be done quickly. Do not use elevator! Use the emergency stairway that leads to outside of the building or the stairway that leads to the hallway. Upon exiting the building, assemble at the designated assembly point until the Police or Fire Department officers announce that it is safe to re-enter.

EMERGENCY RESPONSE: FIRE IN THE LABORATORY >>

If your clothing catches fire, immediately drop to the floor and roll to smother the flames and call for help.

If a compound or solvent catches on fire, *if you can* quickly cover the flames with a piece of glassware. If feasible, use a fire extinguisher to put the fire out.

Do not put water on an organic chemical fire because it will only spread the fire

If the fire is too large, do not take chances: evacuate the lab and the building immediately, and tell your TA or Coordinator.

2 IDENTIFICATION REACTIONS

2.1 The Lassaigne test

2.1.1 The identification of nitrogen, sulfur and halogens

Study the handling of sodium before starting the work. Place 25•30 mg of fresh sodium metal into a dry Pyrex test tube (**make sure that the test tube is undamaged**). Add approximately 10 mg of the (anhydrous) compound to be analyzed. Let the mixture rest for a few minutes. Heat the test tube over a small flame, just enough to melt the sodium. Then heat more strongly so that the test tube becomes glowing red. Keep it glowing for 1•2 minutes and then let it cool down to room temperature. Carefully add 1 mL of methanol and heat the test tube softly. When all of the sodium has reacted, add 6•7 mL of water, heat the mixture until it boils and filter it. The filtrate possibly contains sodium cyanide (originates from the elements of the sample), sulfide and halogens as an alkaline solution. Use the filtrate (A) in the following tests.

2.1.2 Detection of nitrogen in the form of cyanide ions

To 1 mL of filtrate A, add a few drops of iron(II)sulfate (ferrosulfate) solution, and boil the mixture (to oxidize a part of the Fe^{2+}•ions to Fe^{3+}•ions by oxygen in air). Acidify the solution by adding 2 M sulfuric acid. A blue•green colour or blue precipitate (Berlin blue, $Fe_4[Fe(CN)_6]_3$) confirms the presence of cyanide ions.

$$FeSO_4 + 6\ NaCN \rightarrow Na_4[Fe(CN)_6] + Na_2SO_4$$

$$3\ Na_4[Fe(CN)_6] + 2\ Fe_2(SO_4)_3 \rightarrow Fe_4[Fe(CN)_6]_3 + 6\ Na_2SO_4$$

2.1.3 Detection of sulfur (sulfide) as sodium nitroprusside

Add one drop of a fresh, aqueous solution of sodium nitroprusside $Na_2[Fe(CN)_5NO]$ to about 1 mL of the filtrate A. A pink or purple colour verifies the presence of sulfide ion.

2.1.4 Determination of halogens

Place 2•3 mL of the filtrate A into a test tube and acidify the liquid by adding 2 M sulfuric acid. Boil the solution in a fume cupboard (for example in a beaker) for five minutes to remove HCN and H_2S.

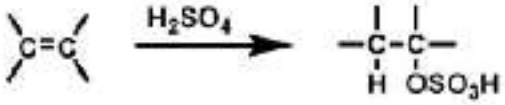
Boiling is unnecessary if there is no nitrogen or sulfur in the sample. Take a sample of the cool acidic solution and add one drop of a silver nitrate solution. The appearance of a white or yellowish precipitate which is insoluble in nitric acid confirms the presence of halogen. If this test is positive, the halogens present are identified according to the following tests. Place a second sample of the sulfuric acid containing solution into a test tube; add chloroform (1 mL) and chlorine water or 1% sodium hypochlorite solution (2 drops). Mix well and let the chloroform phase separate. The colour of the chloroform solution gives a hint on the identity of the halogen present. Brown means that there is bromine, a violet colour points at iodine while chlorine is present if the colour of the solution does not change at all. If the iodine or the bromine test is positive, chlorine can be detected as follows: Boil a third sample of the original sulfuric acid solution with the same amount of concentrated nitric acid for 5 minutes, then add silver nitrate solution (this way Br_2 and I_2 are removed from the solution by distillation). An AgCl precipitate shows that the sample also contains chlorine.

2.2 Alkenes

Alkenes are easily identified through addition reactions to their double bonds.

2.2.1 Reaction with concentrated sulfuric acid

Place one drop of an alkene (eg. cyclohexene) into a test tube and add 5•10 drops of cold, concentrated sulfuric acid. Note that the mixture gets warm, the alkene dissolves and the colour of the solution darkens. This reaction is not specific to alkenes, as for example oxygen containing compounds form soluble oxonium salts.



2.2.2 Bayer test

The reaction is based on the ability of potassium permanganate ($KMnO_4$) to oxidize double bonds whereby diols are formed. Depending on the structure of the diols, they can be oxidized further either to carbon dioxide, ketones or carboxylic acids.

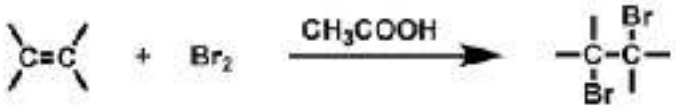
Procedure: Place a few (three) drops of cyclohexene or olefins into a test tube and dissolve it into a small amount of acetone. Add dilute (ca 0.5 %) aqueous potassium permanganate solution, drop by drop. Observe the quickly f a d i n g violet colour of the permanganate ion.

For comparison, perform the test on cyclohexane. It cannot be oxidized. Besides the disappearance of the permanganate colour, evidence that the reaction occurs can be obtained also from the formation of a brown MnO_2 precipitate. Also other compounds such as alkynes, aldehydes, hydroxy and keto acids and various alcohols react with potassium permanganate.

With the exception of alkynes, the reaction is slower with these.

2.2.3 Reaction with bromine in acetic acid

Alkenes react rapidly with bromine in an addition reaction. The dibromo derivatives of most hydrocarbons are colourless which facilitates the observation of the progress of the addition reaction as the red•brown colour of bromine disappears.



Place a few drops of cyclohexene (or 0.1 g of a solid material) into a test tube and dissolve it into 1•2 mL of acetic acid. Add a few drops of 5 % bromine solution in acetic acid and mix well. If the colour of bromine disappears, continue with a drop by drop addition of bromine solution until the colour persists. Bromine does not add to all double bonds. If electronegative groups are bound to the double bond (eg. •COOH) the reaction will be slower or will not occur at all.

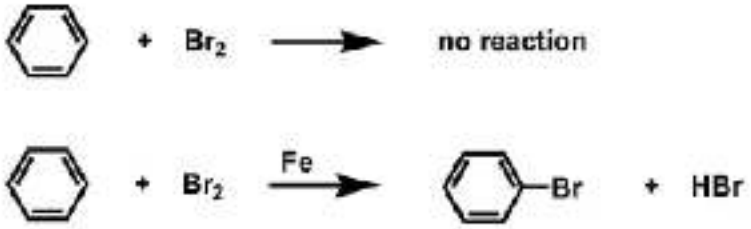
2.3 Aromatic hydrocarbons

2.3.1 Flame test

Aliphatic compounds burn in air with a yellow, nearly smokeless flame, while aromatic compounds show a yellow, strongly soothing flame. In general one can say that the larger the degree of unsaturation of a certain compound, the sootier its flame. The test is carried out by burning a small amount of the examined compound on the tip of a glass rod. The test is not suitable for highly volatile compounds.

2.3.2 Substitution by halogens

In contrast to alkenes, aromatic hydrocarbons do not react with bromine in an addition reaction. If iron powder is used as a catalyst, though they react by substitution whereby the colour of bromine vanishes. Very strongly activated benzene rings react by substitution, even without the use of a catalyst.

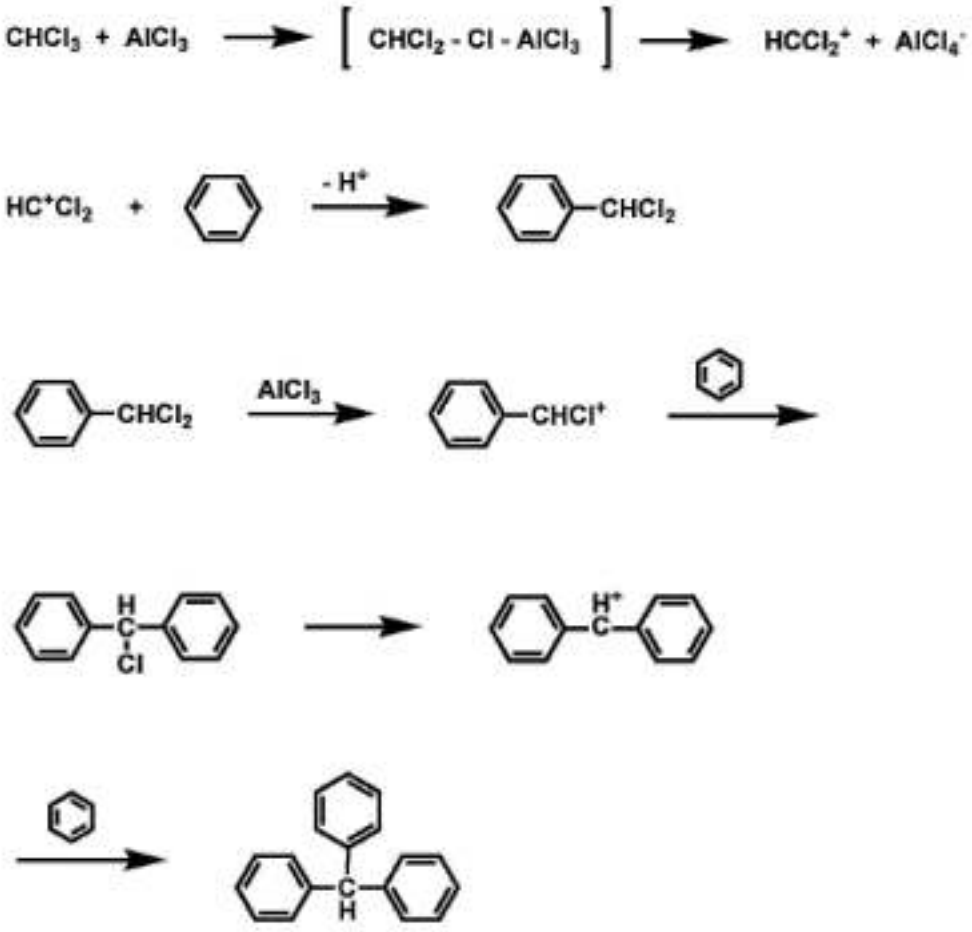


Procedure: Place about three drops of an aromatic hydrocarbon compound (eg. toluene) into a test tube and add two drops of bromine in acetic acid (5 %). Notice that the colour of bromine persists. Add some iron powder and a drop of water. Observe that the colour of bromine now vanishes.

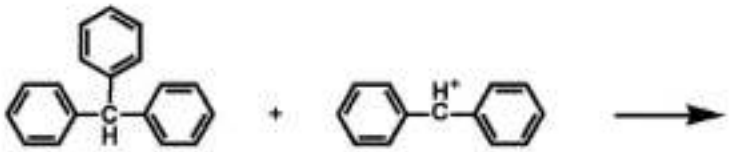
2.3.3 Friedel•Crafts reaction

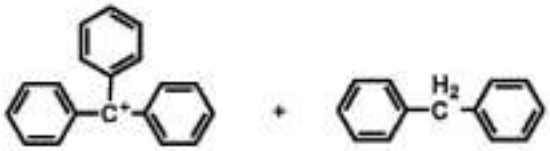
The Friedel•Crafts reaction is an electrophilic aromatic substitution reaction where a hydrogen atom on the aromatic ring (monocyclic or condensed) is substituted by an alkyl or acyl group in the presence of anhydrous

aluminium chloride. The progress of the reaction can be observed through the formation of a coloured complex. Simple aromatic compounds such as benzene and its homologs show an orange•yellow or red colouring, those with several rings a blue (naphthalene) or purple (biphenyl, phenanthrene), sometimes even a green hue (anthracene). The reaction does not take place if the ring contains substituents that strongly deactivate the ring towards electrophilic substitution (i.e., meta directing substituents). In addition, the reaction does not work with polysubstituted derivatives.



The triarylmethane that forms in the reaction can react with carbocations that are present as intermediates. As a result a stable and stronglycoloured triarylmethyl cation forms.





coloured

Procedure: Place 2 mL of chloroform (=trichloromethane) and three drops of toluene (or 0.1 g of a solid compound) into a test tube. Add 0.5 g of anhydrous aluminium chloride. The latter forms a coloured complex when it comes into contact with the solution.

Exercise: Three test tubes hold a colourless liquid each. One holds heptane, the second cyclohexene and the third benzene. By which simple chemical reaction can you show which text tube contains which of the above mentioned compound? Write the reaction equations.

2.4 Halogenated hydrocarbons

Halogenated hydrocarbons are usually unreactive and are therefore widely used as solvents in organic chemistry.

2.4.1 Reaction with silver nitrate at room temperature

Add a drop (= 0.03 mL) (or 30 mg) of the compound to 0.5 mL of a saturated solution of $AgNO_3$ in ethanol. If the test is positive, a precipitate forms within two minutes. Compounds that react positively are alkyl iodides and bromides, tertiary alkyl chlorides, alicyclic iodides and bromides, allylic halides and 1,2•dibromoalkanes.

2.4.2 Reaction with silver nitrate under boiling

If no precipitate forms in the previous test after two minutes, the sample is boiled for half a minute. A positive reaction (the formation of a precipitate) now occurs in case the sample contains a primary or secondary alkyl chloride. Aryl and vinyl halides as well as polyhalogenated compounds (eg. chloroform) give a negative result.

2.4.3 Hydrolysis

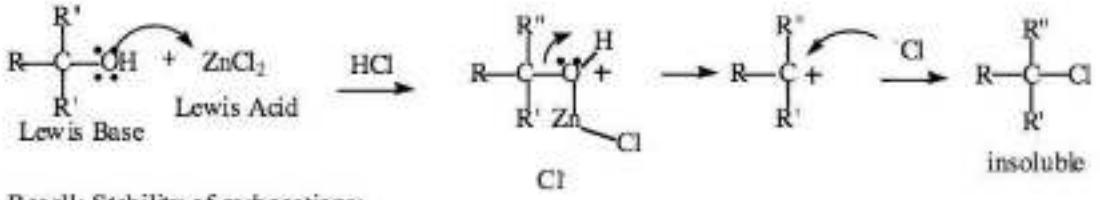
Mix 100 mg of the compound to be examined with 5 mL of a 5 % solution of KOH in alcohol and boil the mixture for 5 min. Add 10 mL of water to the cool mixture, acidify with HNO_3 and filter if necessary until the solution is clear. Add two drops of a 5 % silver nitrate solution. A positive reaction (precipitate) is obtained with all alkyl and cycloalkyl iodides, bromides and chlorides. Fluorides and aryl halides give a negative result (with the latter a cloudy solution might appear).

2.5 Alcohols

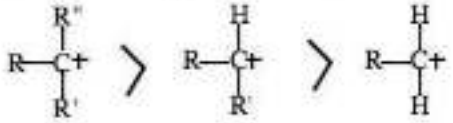
2.5.1 Reaction with hydrochloric acid and zinc chloride (Lucas test)*

This test can be used to find out if a compound is a primary, secondary or tertiary alcohol. The test is based on the reaction of alcohols with concentrated hydrochloric acid in the presence of anhydrous zinc chloride (Lucas Reagent) whereby alkyl chlorides are formed.

Tertiary alcohol



Recall: Stability of carbocations:



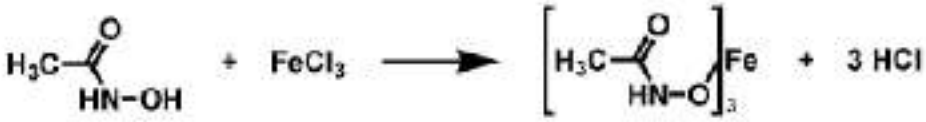
The alcohols that are soluble in Lucas reagent are changed into insoluble alkyl chlorides. The nature of the alcohol can be determined from the relative speed of the reaction.

Procedure: Place 1 mL of n•butanol, sec•butanol and tert•butanol, each in a different test tube. To each test tube add 6 mL of Lucas reagent. Mix the solutions well and leave them to rest. In the test tube containing the tertiary alcohol, an emulsion or two separate layers form almost immediately. The secondary alcohol reacts more slowly. An emulsion or different layers appear after 5•10 minutes. The solution containing the primary alcohol stays clear for at least 15 minutes.

Alcohols with a carbon skeleton of 7 or more carbon atoms are not soluble in the Lucas reagent. The test is therefore unsuitable for such alcohols. As the test is dependent on the formation of alkyl chlorides and the development of a new liquid phase, the compound under investigation has to be soluble in the reagent.

2.5.2 Esterification and the hydroxamic acid test*

The test is based on the reactions shown below. An ester is prepared from the examined alcohol. When the ester reacts with hydroxylamine, a hydroxamic acid is formed. The latter reacts in its turn with ferric ion to produce a brown•red hydroxamate.



Procedure: To a mixture of 0.1 mL of acetyl chloride and 0.2 mL of alcohol, add 0.1 mL of dimethylaniline (to bind the HCl). Stir the mixture for approximately 5 min. Add 1 mL of cold water dropwise in order to hydrolyze excess acetyl chloride. With the help of a clean Pasteur pipette collect a few drops of the separated ester layer (if no layer has formed, collect from the solution). Add 0.5 mL of a 5 % solution of hydroxylamine hydrochloride in methanol. Make the mixture basic by adding 2 M NaOH solution. Warm the mixture until it boils and then cool it immediately. Acidify the cool solution with the help of dilute (2 M) HCl and then add a drop of 10 % iron(III)chloride solution. Observe a wine•red colour, which indicates the (initial) presence of alcohol.

2.5.3 The iodoform reaction

The presence of secondary alcohols of the type CH_3•CH(OH)•R that can be oxidized to the corresponding methyl ketones CH_3•C(=O)•R can be established through the iodoform test. Also ethanol and acetaldehyde give

positive results in this reaction. The test uses an alkaline solution of iodine (sodium hypoiodite) as reagent.

Procedure: Place 2 drops of the examined alcohol into a test tube; add 5 mL of water and 1 mL of a 10% NaOH solution. Add a potassium iodide•iodine solution (contains KI, I_2 and H_2O in the ratio 2:1:8) dropwise to the obtained solution while shaking it continuously, until the colour of the solution turns to light yellow. If a precipitate does not form after a few minutes, warm the mixture in a water bath (60°C) for about 5 minutes. If necessary, add iodine solution dropwise in such a way that the colour of the solution stays light yellow. In a positive reaction, a yellow iodoform precipitate forms. The reaction is also positive for amongst others the following compounds:

On the other hand, the test is negative for compounds of the type CH_3COCH_2X, if X=CN, NO_2 or COOR. Especial y easily hydrolyzable ethyl and isopropyl esters also give a positive result (why?)

2.5.4 Reaction with chromotropic acid

The identification of methanol through the chromotropic acid test: To a test tube containing a drop of a 10 % aqueous solution of methanol add a drop of 5 % phosphoric acid and a drop of 5 % potassium permanganate solution. After a minute, while shaking add a saturated $NaHSO_3$ solution until the colour disappears. If a brown MnO_2 precipitate forms, add an additional 7 drops of phosphoric acid and a very small amount of $NaHSO_3$ solution. While shaking, add 4 mL of concentrated sulfuric acid and 4 drops of a 2% aqueous solution of chemotropic acid (stored in a dark bottle) to the colourless solution. The violet colour, which deepens while the solution cools down, indicates the presence of formaldehyde which was formed from methanol when oxidized by $KMnO_4$ in a phosphoric acid solution.

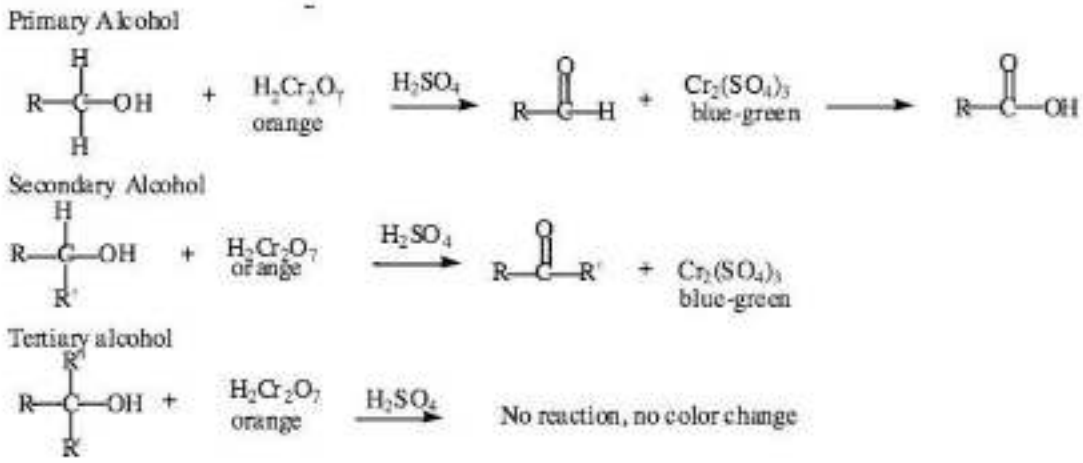
Exercises:

1. Give an example of a primary, secondary and tertiary alcohol.

2. Which of these is not oxidized by potassium chromate?

2.5.5 Chromic acid oxidation

This test distinguished primary and secondary alcohols from tertiary. Chromic acid will oxidize a primary alcohol first to an aldehyde and then to a carboxylic acid and it will oxidize a secondary alcohol to a ketone. Tertiary alcohols do not react. The OH-bearing carbon must have a hydrogen atom attached. Recall, that a carbon is oxidized when it loses a hydrogen or hydrogens or gains a more electronegative atom. Since a carbon atom is being oxidized in primary and secondary, the orange chromium Cr^{6+} ion is being reduced to a blue-green Cr^{3+} ion.



Procedure: Set-up three small test tubes in your test tube rack. The tubes do not need to be dry. Label the first test tube as a primary alcohol, the next as a secondary alcohol and the third as a tertiary alcohol. Write don in your logbook which alcohol you are going to be using. Add 2 mL of acetone to each test tube and then add 3-4 drops of your test alcohol. Be sure that the drops fall into the acetone and do not remain on the sides of the tube. Add 2 drops of the Chromic Acid Test Reagent (also called the Bordwell-Wellman Reagent, a mixture of $K_2Cr_2O_7$ and concentrated sulphuric acid). Shake vigorously using a small, tight fitting cork. You should see a colour change to a blue or blue-green or similarly colored precipitate within a few seconds to indicate a positive test. Record you result in your logbook.

2.6 Ethers

Ethers are unreactive like alkanes but **form easily explosive peroxides as impurities.**

2.6.1 The solubility of ether

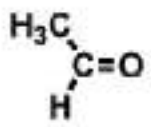
Mix 1 mL of ether and 2 mL of conc. HCl in a test tube. A homogeneous, viscous solution forms. Add water so that the ether separates from the solution. The solvation of ether is caused by the formation of oxonium ions:

diethyl ether	hydrochloric acid	diethyloxonium chloride

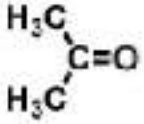
Alcohols are protonated to oxonium ions in the same way but usually react further (by substitution or elimination reactions).

2.7 Aldehydes and ketones

Aldehydes and ketones, functional group: carbonyl group C=O.



aldehyde



ketone

Aldehydes and ketones share the same functional group. Therefore they usually react with the same reagents resulting in similar reaction products.

2.7.1 The reaction with 2,4•dinitrophenylhydrazine*

Reagent: Dissolve 3 g of 2,4•dinitrophenylhydrazine in 15 mL of concentrated sulfuric acid (the solution might be ready), and add this solution with stirring to 20 mL of water and 70 mL of 95% ethanol. After thorough mixing, filter the mixture. 2,4•Dinitrophenylhydrazine is a common reagent used to indicate the presence of carbonyl compounds. Both aldehydes and ketones react with it to produce 2,4•dinitrophenylhydrazones.

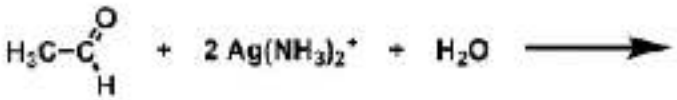
Procedure: Place 2 drops of a ketone in a test tube and add 3 mL of the reagent solution to it. Warm the mixture for 1 minute. While cooling, the 2,4•dinitrophenylhydrazone of the carbonyl compound precipitates. The precipitate is usually yellow if the carbonyl group is not conjugated. The conjugation with a carbon•carbon double bond or an aromatic ring causes the colour to change to orange•red. Strongly sterically hindered compounds do not react.

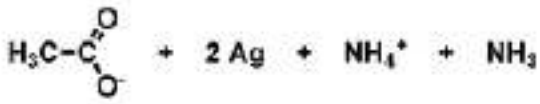
2.7.2 The iodoform test*

This test can be used for the detection of a certain type of carbonyl compound (see the reactions of alcohols).

2.7.3 The silver mirror test*

Silver ion oxidizes the aldehyde group to a carboxyl group in alkaline solution, and is itself reduced at the same time to metallic silver. The reagent used is the diamine silver(I)ion (Tollens' reagent).





The finely divided metallic silver formed adheres to the wall of the test tube as a silver mirror or precipitates as a black silver deposit.

Procedure: Place 1 mL of a 5 % $AgNO_3$ solution and one drop of 2 M sodium hydroxide into a clean, unscratched test tube (the silver mirror only forms on a clean and smooth surface). While shaking the test tube, add dropwise just enough concentrated ammonia to the solution that the precipitate formed in the previous step (grey silver oxide) dissolves. Be careful not to add ammonia in excess. Add a drop of aldehyde to the mixture and dip the test tube into a 60°C water bath. Within half an hour a silver mirror or a silver precipitate should appear. Avoid direct daylight as light easily leads to the decomposition of silver compounds. Formic acid also gives a positive result for this reaction.

<u>Exercises:</u>

The examined sample is

a) pentanal or 3•pentanone

b) 2•pentanone or 2•pentanol

c) cyclopentanone or 2•cyclopentenone

Which simple analytical reaction can be used to identify each of the compounds of the above-mentioned compound pairs? Write the reaction equations.

2.8 Esters

Esters are derivatives of carboxylic acids where the hydroxyl group of the acid has been replaced by a RO• or ArO•group.

2.8.1 The hydroxamic acid test

Esters react with hydroxylamines to yield hydroxamic acids which in their turn form a wine red ferric hydroxamate with ferric ions. Hydrochloric acid and acid anhydrides also form hydroxamic acids under the described conditions. They can be converted into salts, though, by the action of NaOH and slight warming. As salts they cannot react to hydroxamic acids.

hydroxamic acid

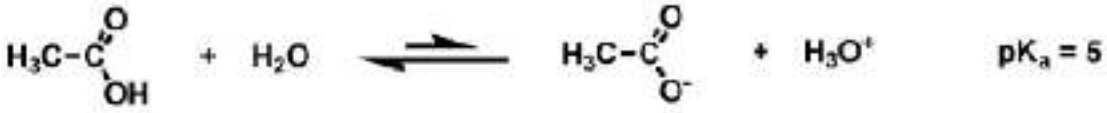
ferric hydroxamate

Procedure: Place a drop of an ester to be examined into a test tube. Add 0.5 mL of a 1 *M* solution of hydroxylamine hydrochloride in methanol and a 5 *M* solution of potassium hydroxide in a 80 % mixture of methanol and water. Add enough of the latter so that the solution turns alkaline. Heat the mixture until it boils, then cool it down rapidly and add 2 *M* HCl until the solution becomes acidic. With the addition of one drop of 10 % iron(III)chloride solution, brown•red iron(III)hydroxamate should form.

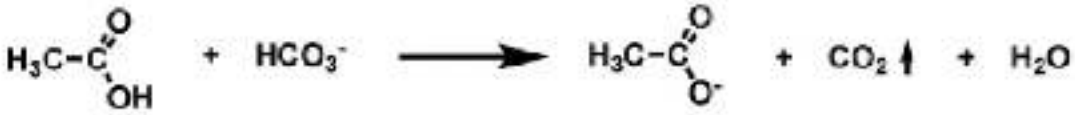
2.9 Carboxylic acids

2.9.1 Reaction with sodium bicarbonate

Carboxylic acids are weak acids. Their protolysis in water is far from complete.



The degree of protolysis depends on the structure of the acid. Carboxylic acids are nevertheless stronger acids than carbonic acid H_2CO_3 (pK_a = 6.35). The former will therefore cause the release of the latter from bicarbonate or carbonate solutions. Being unstable, carbonic acid is instantly decomposed into carbon dioxide and water.

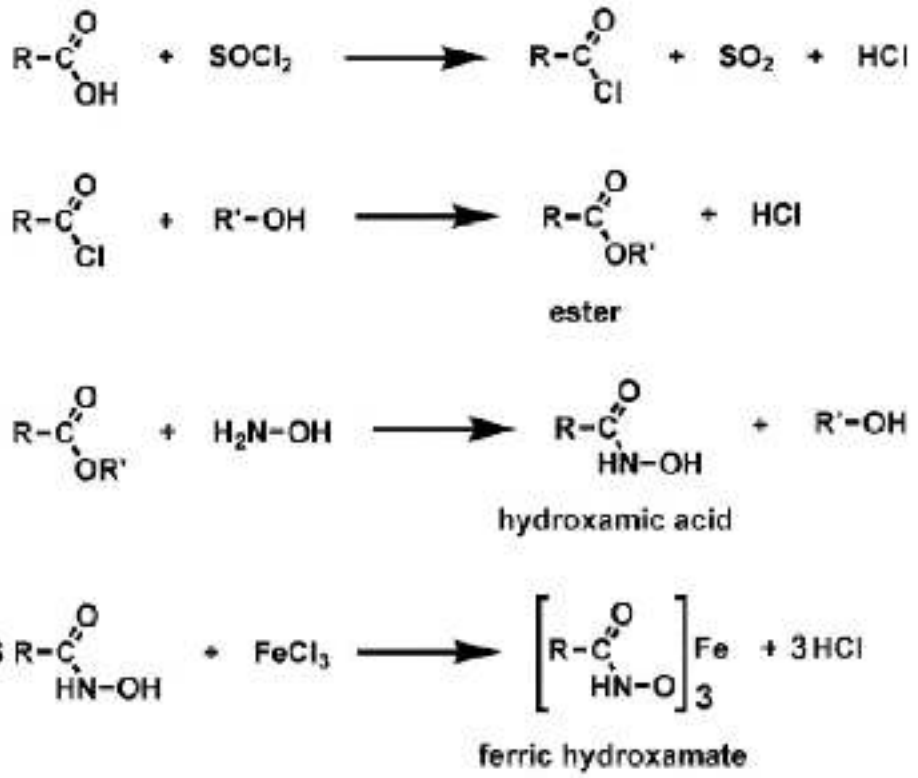


Procedure: Place 4•5 drops (or about 0.2 g of a solid • eg. benzoic acid) of a carboxylic acid into a test tube and dissolve it into water. In case the acid is insoluble in water, dissolve it first into a few milliliters of ethanol and then into the same amount of water. Add several drops of saturated $NaHCO_3$ solution. Notice the development of carbon dioxide (bubbling). No bubbles can be observed if the solvent used (eg. acetone) can dissolve large amounts of carbon dioxide.

2.9.2 Esterification of carboxylic acids and the hydroxamate test*

Place one drop of carboxylic acid into a dry test tube and add 2•3 drops of thionyl chloride. Heat the mixture in a boiling water bath for half a minute. Then add a few drops of butanol or pentanol and continue with heating for one minute. In a fume cupboard, add 0.5 mL of water in order to hydrolyze excess thionyl chloride. Continue with the addition of 0.5 mL of a 1 M solution of hydroxylamine hydrochloride in methanol and enough of a 5 M

solution of potassium hydroxide in an 80 % mixture of methanol and water that the mixture becomes basic. Heat the mixture to boil, cool it immediately and add enough 2 M HCl so that the solution turns acid. Finally add one drop of a 10 % iron(III)chloride solution whereupon wine•red iron(III)hydroxamate appears.



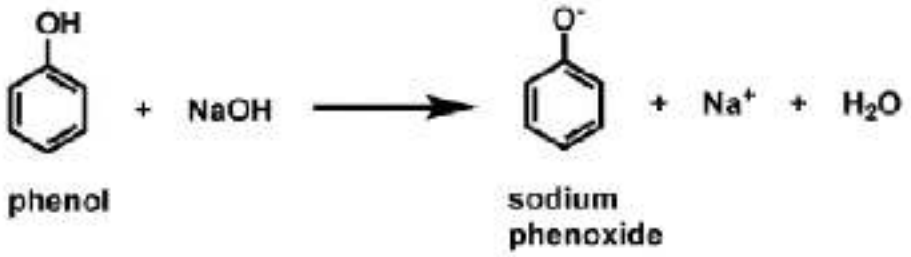
Exercises:

1. Given two test tubes, one containing propanoic acid and the other cyclopentanoic acid, choose simple chemical tests to distinguish the acids. Write an equation for the reaction.

2. In the reaction between an acid A and an alcohol B, the ester $C_6H_{12}O_2$ is produced. On the other hand, oxidation of the alcohol B gives the acid A. Draw the structure of the ester.

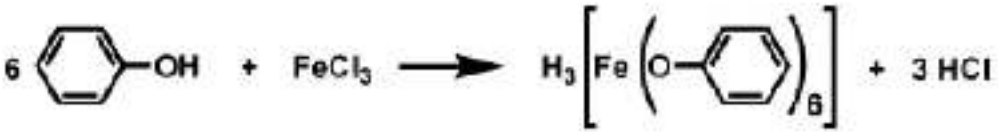
2.10 Phenols

Phenols are weaker acids that carbonic acid (pK_a11) which means that they do not react with $NaHCO_3$, but do so with NaOH.



2.10.1 Reaction with ferric chloride

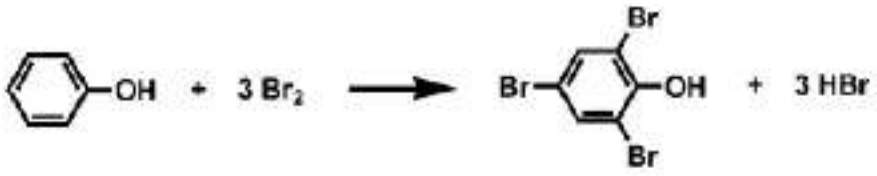
To a 1 % aqueous solution of phenol (or a 10 % ethanol solution) add a drop of a 5 % ferric chloride solution. The colour that arises originates from a three•valent complex anion:



The reaction is typical for most phenols and enolizable (over 5 % of enol) compounds. Hydroquinone does not give a colour reaction as it is oxidized to quinone when it comes into contact with ferric ions. The colour of the Fe^{3+} complex depends on the phenol in question: violet, blue, purple or green.

2.10.2 Reaction with bromine water (reaction of the aromatic ring)

A hydroxyl group connected to an aromatic ring activates the ring towards electrophilic aromatic substitution and is *ortho/para*•directing. Phenol reacts rapidly with bromine water whereby 2,4,6• tribromophenol precipitates. It is a white compound that is only slightly soluble in water.

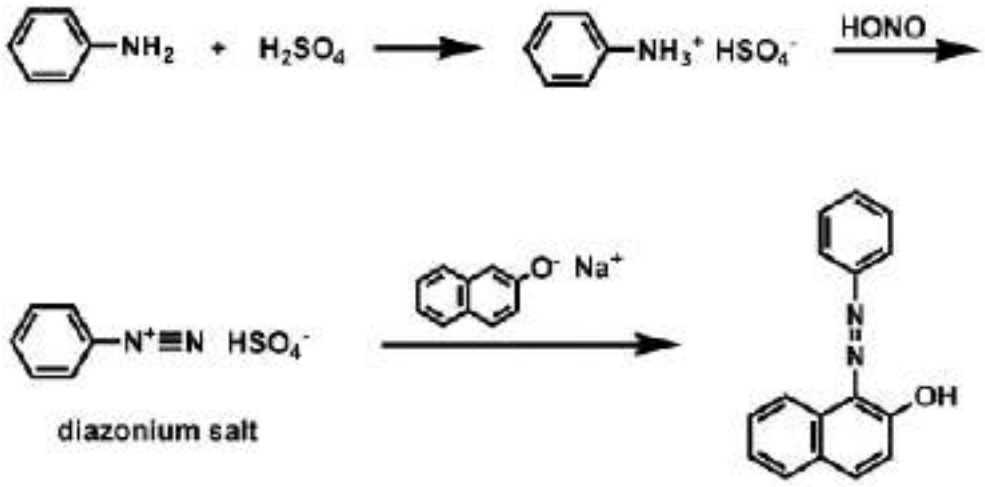


The reaction is not specific for phenols. Also other compounds, such as aniline and a few enols, can form similar precipitates.

Procedure: Add bromine water to a dilute aqueous phenol solution. A yellowish•white precipitate appears almost immediately.

2.10.3 Reaction with diazonium salt

The reaction can be used for the detection of several kinds of phenols (another phenol in place of β•naphthol in the scheme above).



diazonium salt

1-phenylazo-2-naphthol

Procedure: In a test tube mix 1 mL of water, 5 drops of concentrated sulfuric acid and 100 mg of a primary arylamine. Cool the test tube in an ice bath. Little by little, add 1 mL of a cold 10% sodium nitrite solution (the temperature of the mixture should be about 0•5°C) while at the same time shaking the test tube. Finally add 200 mg of β•naphthol that has been dissolved in 2 mL of 10 % NaOH. An azo dye forms (usually red).

2.11 Amines

Amines are basic by nature and dissolve in acids under the formation of ammonium salts. Eg.:

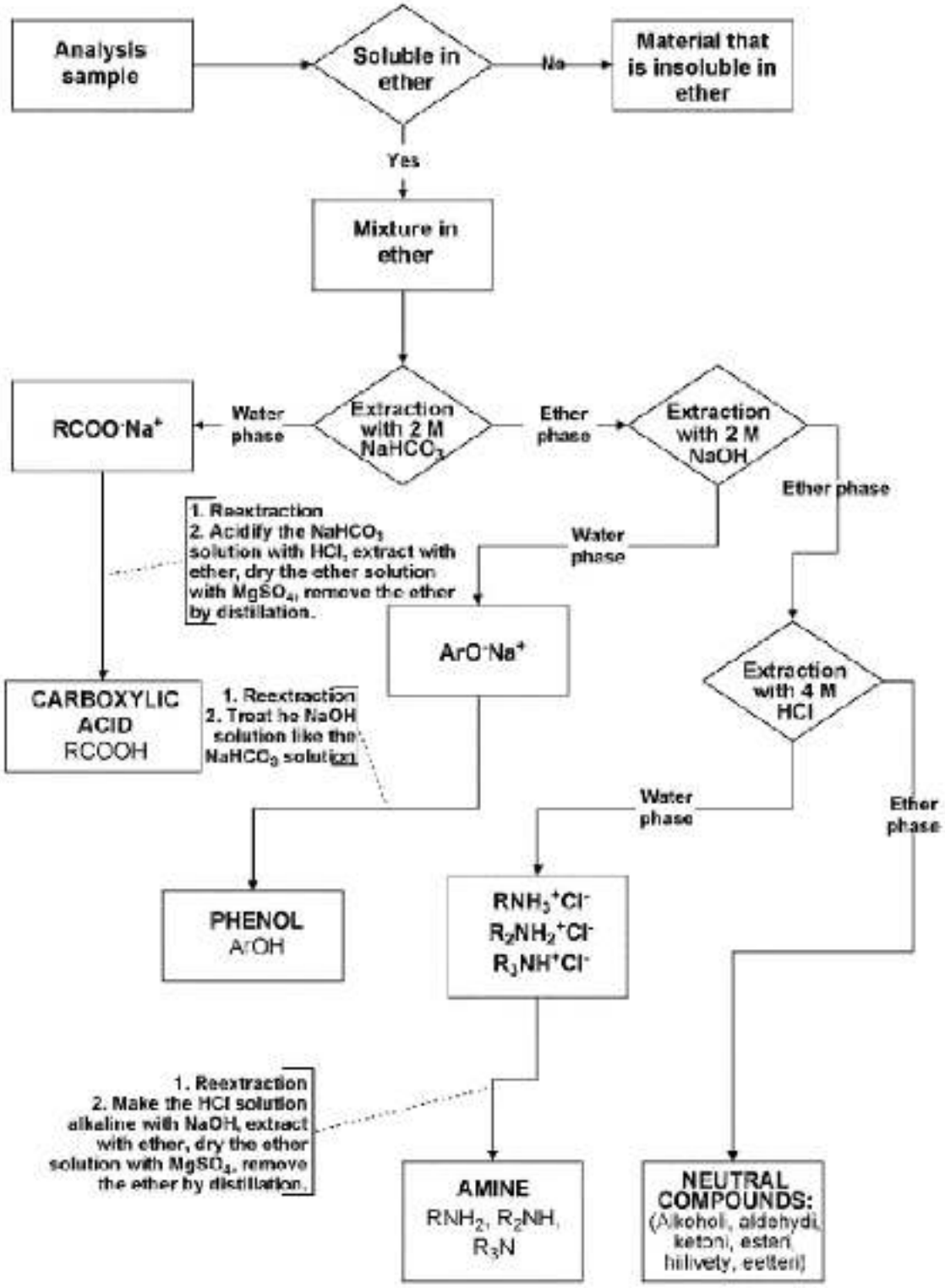
aniline **anilinium hydrochloride**

2.11.1 Colour reaction with copper ion

Mix a 3 % aqueous solution of an amine with the corresponding amount of 10 % copper sulfate solution. A blue or green colour or precipitate appears.

2.11.2 The diazo reaction of primary aryl amines

See the diazo reaction in Phenols section


Analysis sample
Soluble in ether
No
Material that is insoluble in ether
Yes
Mixture in ether
RCOO⁻Na⁺
Water phase
Extraction with 2 M NaHCO3
Ether phase
Extraction with 2 M NaOH
Ether phase
1. Reextraction
2. Acidify the NaHCO3 solution with HCl, extract with ether, dry the ether solution with MgSO4, remove the ether by distillation.
Water phase
ArO⁻Na⁺
Extraction with 4 M HCl
CARBOXYLIC ACID
RCOOH
1. Reextraction
2. Treat he NaOH solution like the NaHCO3 solution
Water phase
Ether phase
PHENOL
ArOH
RNH3⁺Cl⁻
R2NH2⁺Cl⁻
R3NH⁺Cl⁻
1. Reextraction
2. Make the HCl solution alkaline with NaOH, extract with ether, dry the ether solution with MgSO4, remove the ether by distillation.
AMINE
RNH2, R2NH, R3N
NEUTRAL COMPOUNDS:
(Alkoholi, aldehydi, ketoni, esteri, halivety, eeiteri)

3 QUALITATIVE ANALYSIS

3.1 The pre-examination of the analysis mixture

The information obtained from the pre-examination of a mixture can be used to determine which separation techniques should be used. The testing can include an infrared (IR) spectrum of the mixture, solubility tests, the determination of elements present (the Lassaigne test) and identification reactions that detect functional groups. You can use about a third of the mixture to be analysed.

3.2 Solubility test

Test the solubility of the mixture to determine which types of compounds are present:

water	
Aqueous $NaHCO_3$ solution	Identification of acids
Aqueous Na_2CO_3 solution	Identification of acids
Aqueous NaOH solution	Identification of phenols
Aqueous HCl solution	Identification of amines
Ether	

In addition to solubility tests, more evidence has to be collected to prove that an unknown component belongs to a certain group of compounds. This information can be obtained by IR spectrometry and by reactions characteristic of specific functional groups.

3.3 IR spectrum of the mixture

Try to identify the following:

•functional groups (COOH, OH, C=O, CHO, NH_2)

•aliphatic/aromatic nature of compounds

3.4 The separation of the components

The components are separated by extracting them with solvents, w h i c h react with acid and basic components of the analysis mixture. The mixture is dissolved in ether (ca. 30 mL) (original ether solution). Any undissolved material is filtered off and examined separately (physical constants, solubility tests, identification reactions, IR spectrum). Note that this component may be partly dissolved in ether. A suitable extraction solution is chosen on the basis of the pre-examination results.

Carboxylic acids are extracted from the ether solution by using 2 M aqueaous $NaHCO_3$ (3x50 mL or more, until the generation of carbon dioxide has ended). The basic water solution is extracted with ether (2•3 x 30 mL) and the ether phases obtained are combined with the original ether solution. This so-called re-extraction with ether assures the complete removal of the second component, which might be soluble in water to a certain degree. The alkaline water solution that contains the sodium salt of the carboxylic acid is acidified (pH 2•3) with concentrated aqueous hydrochloric acid (the vessel has to be cooled in an ice bath), which leads to the release of the carboxylic acid from its salt. If the carboxylic acid forms a precipitate, it is filtered off by suction filtration. If no precipitate forms, the acidic aqueous solution is saturated with NaCl (salting out procedure) and is then extracted with ether (4x30 mL). The ether solution is dried with $MgSO_4$, the drying agent filtered off and the ether removed in a rotavapor or by distillation.

Phenols are extracted from the ether solution with 2 M aqueous NaOH (2x50 mL). The basic water solution that contains the sodium salt of the phenol is

treated the same way as was described for carboxylic acids: re-extraction followed by the release of the phenol from its salt.

Amines are extracted from the ether solution with 4 M aqueous HCl (3x40 mL). The acidic aqueous solution is extracted with ether (3x30 mL) and the ether solutions obtained are combined with the original ether solution (re-extraction). The acidic solution, which contains the hydrochloride of the amine, is made basic (pH ca. 11) with concentrated (40 %) NaOH, whereby the amine is liberated from its salt. If the amine forms a precipitate, it is filtered by suction filtration. If no precipitate forms, the solution is saturated with NaCl and then extracted with ether (4x30 mL). The ether solution is dried with $MgSO_4$, the drying agent is filtered off and the ether removed in a rotavapor or by distillation. The original ether solution may contain neutral compounds after it has been extracted with $NaHCO_3$, NaOH and HCl. It is dried with $MgSO_4$, the drying agent is filtered off and the ether removed in a rotavapor or by distillation.

3.5 The purification of the separated compounds

Liquid compounds are purified by distillation either under atmospheric pressure or under reduced pressure. Determine the boiling point!

Solids are recrystallized. Examples for suitable recrystallization solvents can be found in the table below:

Compound	Solvent	bp. °C	Added solvent
dicarboxylic acid*	ether•toluene	35•111	
phenol	water	100	ethanol,
amine	water	100	ethanol
unpolar	carbon tetrachloride*	76	
unpolar	propanol•water	97•100	

In search for a suitable solvent, a small amount of the solid is first tested. The compound to be recrystallized should dissolve well in the boiling solvent but poorly when the solvent is cold. If a suitable solvent cannot be found, a second solvent can be added to it in a certain stage. The detailed crystallization procedure will be explained later in this *Handbook*.

3.6 The identification of the separated compounds

Once the functional groups of an unknown compound have been determined, the compound is identified by its physical properties (melting point/boiling point, refractive index). With the help of these, search for suitable candidates in the following books:

A. I. Vogel, *Vogel's Textbook of Practical Organic Chemistry*, corrected Furniss, B. S. et al., 4. edt., Longman, London 1978, p. 1155.

A. I. Vogel, *Vogel's Textbook of Practical Organic Chemistry*, corrected Furniss, B. S. et al., 5. edt ., Longman, Harlow 1989, p. 1155.

A. I. Vogel, *Elementary Practical Organic Chemistry*, Part 2, 2. edt., Longman, Green and Co., London 1966, p. 313.

CRC Handbook of Tables for Organic Compound Identification, Z. Rapoport, toim., 3. edt., The Chemical Rubber Company, Cleveland 1976.

Besides the identification methods mentioned above you can also use the determination of mixed melting points, the preparation of crystalline derivatives (see the above references) and the comparison of infraredspectra (*The Aldrich Library of Infrared Spectra*, 3. edt., Laboratory of MRCPP).

3.7 The determination of mixed melting points

Mix the same amount of an unknown and a reference compound. Grind the mixture in a mortar until it is homogenous. Measure the melting point. If the melting point stays between that of the reference and the unknown

compound so that there is no lowering of the melting point, the two compounds that were mixed are identical.

3.8 Infrared (IR) spectroscopy*

In the course Organic Chemistry Laboratory you will be introduced to infrared spectroscopy. In IR spectroscopy a compound's absorption spectrum in the intermediate IR region is measured. Usually the spectrum is presented with transmittance as a function of the wave number (= the inverse of wavelength). The infrared radiation in the intermediate area (2.5•50 µm; 4000 • 200 cm^{-1}) has energy suitable to cause resonance in the vibrational energy levels of the molecules. Different bond types usually absorb at different specific IR wavelengths. IR spectroscopy is used for the determination of the structure and the identification of mainly organic and organometallic compounds.

Preparation of the sample:

Solid samples. Mix and grind 1• 3 mg of the solid with 100•200 mg of dry potassium bromide. Press this mixture into a transparent tablet in a hydraulic press. Alternatively, dissolve the solid in dry carbon tetrachloride (ca. 10 % solution). Transfer the obtained solution into a IR cuvette. The cuvette has salt windows, which allow IR radiation to pass. It is important that the salt windows are protected from corrosion by water, alcohols and acids amongst others (storage in an desiccator!). A third way to prepare a sample from a solid is to disperse it in nujol (liquid paraffin) to make a nujol mull, a drop of which is set between two clean KBr tablets.

Liquid samples. The easiest way to run a spectrum of a liquid is by placing a drop of it between KBr tablets, lightly pressed together to produce a thin film. However, volatile samples may escape from between the salt tablets, while the spectrum is run. As with solids, a liquid can be used for the

preparation of a sample solution. The spectrum is then run of the solution contained in a cuvette.

Interpreting the spectrum:

Most functional groups absorb in the region between 4000 • 1500 $cm^{•1}$. Major peaks in this region should always be interpreted first by comparing with tabulated values for known compounds. The absorption of polar groups is usually shifted towards lower frequencies and are broadened due to hydrogen bonding (e.g. hydroxy and amino groups).

Absorptions that appear between 1400 - 900 $cm^{•1}$ (so called finger print region) are usually difficult to interpreted, as numerous different vibrations caused by bending and stretching appear in this region. One should be careful with the interpretation of peaks in this region. IR absorptions that appear below 900 $cm^{•1}$ are generally only of use for the detection of substituent patterns on an aromatic ring.

Table: characteristic IR absorptions

Group	**Bond**	**Wave number $cm^{•1}$**
hydroxyl	O-H	3610•3640
amino	N-H	3300•3500
aromatic ring	C-H	3000•3100
alkene	C-H	3020•3080
alkane	C-H	2850•2960
nitrile	C≡N	2210•2260
carbonyl	C=O	1650•1750
amino	C-N	1180•1360

Abbreviations: Intensity of the spectrum (size of the peaks): s, strong; m, medium; w, weak; v, variable. Types of vibrations: ν stretching, δ symmetric bending, γ assymmetric bending.

Literature:

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E. Pretsch, T. Clerc, J. Seibl ja W. Simon, *Tabellen zur Strukturaufklärung organischer Verbindungen mit spektroskopischen Methoden*, 2. edt, Springer Verlag, Berlin 1981.

T.Hase, *Tables for Organic Spectrometry*, 6. edt, Otatieto, Espoo 1999..

R. M. Silverstein ja G. C. Bassler, *Spectrometric Identification of Organic Compounds*, 2.edt., John Wiley and Sons, New York, 1963.

K. Nakanishi, *Infrared Absorption Spectrocopy*, Holden Day, San Francisco 1962.

N. B. Colthup, L.H. Daly ja S. E.Wiberley, *Introduction to Infrared and Raman Spectroscopy*, 2. edt., Academic Press, New York 1975.

Reporting the analysis

Write a report how the analysis was carried out. Points to consider are the separation of the components (with the help of the scheme) and the purification and identification of the components

Contents of the final examination: Question sections:

1. Reaction mechanism
2. Work methods and equipment used
3. Laboratory safety
4. Extractions applied in an analysis and identification reactions
5. The interpretation of IR spectra

4 Fundamental Techniques

4.1 Crystallization

Crystallization is the most desirable method of purification for purifying organic solids. Crystallizations require less solvent, time and resources once they have been developed. In general, the compound of interest (solute) is not soluble in a solvent at low temperatures but very soluble at higher temperatures. If a saturated hot solution is allowed to cool, the solute is no longer soluble in the solvent and form crystals of pure compound, which can be separated from the dissolved impurities by filtration. Since the choice of solvent for crystallization is often not specified and is seldom obvious, testing by trial and error on a small scale is generally required.

Recrystallizations in the organic laboratory proceed in a similar manner. Just enough hot solvent is added to a small amount of an impure, solid compound in a flask to completely dissolve it. The flask then contains a hot solution, in which solute molecules – both the desired compound and impurities – move freely among the hot solvent molecules. As the solution cools, the solvent can no longer "hold" all of the solute molecules, and they begin to leave the solution and form solid crystals. During this cooling, each solute molecule in turn approaches a growing crystal and rests on the crystal surface. If the geometry of the molecule fits that of the crystal, it will be more likely to remain on the crystal than it is to go back into the solution. Therefore, each growing crystal consists of only one type of molecule, the solute. After the solution has come to room temperature, it is carefully set in an ice bath to complete the crystallization process. The chilled solution is then filtered to isolate the pure crystals and the crystals are rinsed with chilled solvent.

The table below summarizes the steps in a recrystallization. Please note that most chemists use "crystallization" and "recrystallization" interchangeably. More properly perhaps, crystallization is the process by which compounds

become solid, while recrystallization refers to a process, which a chemist will do to a solid compound in order to purify it.

Solvent	BP (°C)	Dielectric Constant	Good For	Second Solvent for Mixture	Comments
Diethyl ether	34.5	4.27	General	Acetone, hydrocarbons	May creep up side of flask and deposit precipitate
Dichloromethane	40.0	8.93	General, low-melting compounds		
Acetone	56.0	21.01	General, nitro and bromo compounds	Water, hydrocarbons, ethyl ether	Should be dried if not used with water
Chloroform	61.0	4.81	General, acid chlorides	Ethanol, hydrocarbons	Easily removed and dried
Methanol	64.6	33.00	General, esters, nitro and bromo compunds	Water, ethyl ether benzene	
Hexane	68.7	1.89	Hydrocarbons	Any solvent in the list	
Ethyl acetate	77.1	6.08	General, esters	Ethyl ether, hydrocarbons, benzene	
Ethanol	78.2	25.30	General, esters, nitro and bromo compounds	Water, hydrocarbons, ethyl acetate	
Benzene	80.0	2.28	Aromatics, hydrocarbons, molecular complexes	Ethyl ether, ethyl acetate, hydrocarbons	Cumulative poison
Cyclohexane	80.7	36.64	Hydrocarbons	Any solvent in the list	
Acetonitrile	81.6	36.64	Polar compounds	Water, ethyl ether, benzene	
Heptane	98.5	1.92	Hydrocarbons	Any solvent in the list	
Water	100.0	80.10	Salts, amides, some carboxylic acids	Acetone, alcohols, dioxane, acetonitrile	Precipitates dry slowly
1,4-Dioxane	101.5	2.22	Amides	Water, benzene, hydrocarbons	
Toluene	110.6	2.38	Aromatics, hydrocarbons	Ethyl ether, ethyl acetate, hydrocarbons	
Pyridine	115.6	12.4	High-melting, insoluble	Water, methanol,	

			compounds	hydrocarbon	
Acetic acid	117.9	6.20	Salt, amides, some carboxylic acids	Water	

Apparatus and materials: Conical bottom flasks, filtering flask, test tubes, suction glass funnel, Pasteur pipettes, activated charcoal, hot plate and heat dissipation block.

Chemicals: Acetanilide (C_6H_5-$NHCOCH_3$); acetylsalicylic acid (2-$HOOC$-C_6H_4-$OCOCH_3$); adipic acid ($HOOC$-$(CH_2)_4$-$COOH$); benzoic acid (C_6H_5-$COOH$); benzoin (C_6H_5-CO-$CH(OH)$-C_6H_5); benzyl (C_6H_5-$CO)_2$; 2-chlorobenzoic acid (2-Cl-C_6H_5-$COOH$); 4-nitroacetanilide (4-O_2N-C_6H_4-$NHCOCH_3$); phenyl benzoate (C_6H_5-$COOC_6H_5$); salicylic acid (2-HO-C_6H_4-$COOH$); acetone (CH_3COOCH_3); ethanol (CH_3CH_2OH); ethyl acetate ($CH_3COOCH_2CH_3$); hexane (C_6H_{14}); toluene (C_6H_5-CH_3).

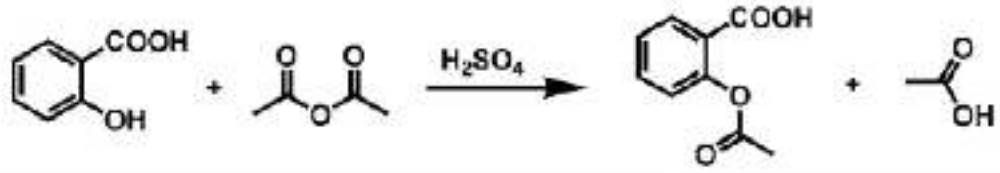
Procedure:

Solvent extraction. Place each of 10 finely crushed known samples, the size of half a grain of rice, in 6 test tubes. Add 5 drops of water, 95% ethanol, ethyl acetate, acetone, toluene and hexane to test tubes No 1-6, respectively. Swirl the content in each tube and note whether the sample in soluble in the solvent at room temperature. (NOTE: Some solvents tend to evaporate easily from the test tube so add the solvent, if necessary, to maintain the same amount of solvent for comparison). Warm the test tubes containing insoluble sample in the conical well of the heat dissipation block on hot plate. Swirl the content in each tube and note whether the sample is soluble in hot solvents. (CAUTION: Be careful not to leave the solution heating without attention). Let the solution cool and observe the crystals form. Record each solvent tested and give indication which of the six solvents is the best solvent suited for recrystallization of an unknown sample, according to the above procedures. Record the observations and the most suitable solvent for recrystallization.

Recrystallization of an unknown sample. Place 100 mg of the unknown sample for crystallization into 5 mL conical bottom flask. Add 1 mL of the suited solvent. Heat the mixture to a gentle boiling and often swirl the solution until the solid is all dissolved.

5 The Synthesis

5.1 Acetylsalisylic Acid (Aspirin)



Reagents	Safety Notes	Equipments
5.0 mL of acetic anhydride 2.8 g of salicylic acid 3•4 drops of conc. sulfuric acid	Acetic anhydride is volatile and a strong irritant	100 mL round•bottomed flask (14/29) magnetic stirrer water and ice baths suction filtration apparatus Büchner funnel rubber stopper 2 filter flasks round•bottomed flask condenser oil bath

Procedure

Place 2.8 g of salicylic acid in a dry 100 mL round•bottomed flask, then add 5.0 mL of acetic anhydride and 3•4 drops of concentrated sulfuric acid. Mix the resulting white slurry with a magnetic stirrer, and place the flask in a warm water bath (45•50°C) for 15 min. Allow the flask to cool and add 50 mL

of water and break up any lumps with a spatula.

Isolation and Purification

Allow the mixture to stand for an additional five minutes, then chill the flask in an ice bath and remove the crystals by suction. Crystallize the crude aspirin from warm 30% ethanol • water mixture not exceeding 80°C (see experimental note). Allowing the mother liquor to stand overnight may produce a second crop of crystals. Air•dry the crystals and determine the per cent yield and melting point (mp. for aspirin is 135°C). The yield is 80%.

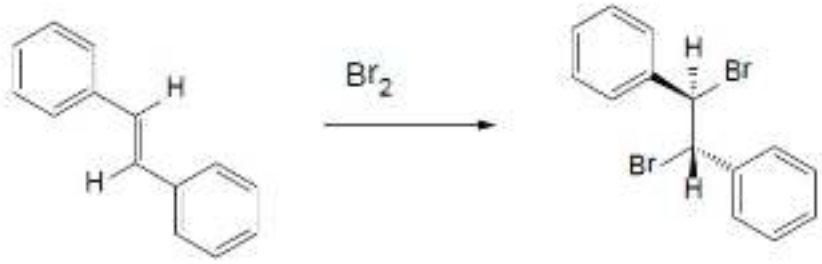
Product Characterization

Measure the melting point and run an IR spectrum of the product.

Experimental Note:

At temperatures exceeding 80°C, aspirin forms oil that dissolves organic impurities from water; in this case it may be difficult to redissolve the aspirin in water. Product characterization

5.2 Meso-1,2-dibromo-1,2-diphenylethane*



Reaction mechanism is an electrophilic addition to the double bond

Reagents	Safety Notes	Equipments
1 g of *trans*-stilbene	Bromine is extremely poisonous and	50 mL round bottomed flask (14/29)
10 ml of dichloromethane	corrosive. It has to be handled in fume	Small graduated cylinder
10 ml 10% (w/v) solution of bromine in dichloromethane	cupboard. Wear protective gloves.	Suction filtration apparatus
cyclohexene		Watch glass

Procedure

Dissolve 1.0 g of *trans*-stilbene in 10 ml of dichloromethane in a 50 ml round- bottomed flask. Stir the solution with magnetic stirrer and add 10 ml of a 10% solution of bromine in dichloromethane (0,10 g of bromine/ 1.0 ml). If the colour of bromine disappears completely, add bromine dichloromethane solution in 1 ml portions until the colour of the bromine stays permanently. The developing dibromocompound precipitates from the solution. Add to the reaction mixture cyclohexene in drops until the extra bromine is destroyed. Stop adding the cyclohexene when the colour of the

bromine disappears. Cool the solution in an ice bath to complete the precipitation.

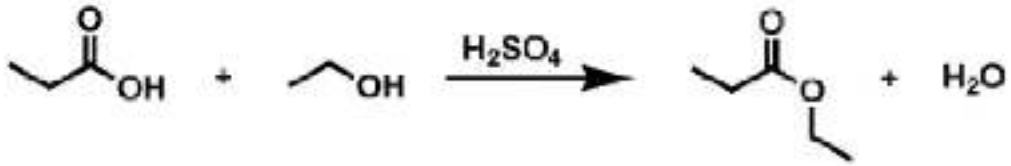
Isolation

Collect the product by suction filtration. Wash it in the funnel with 5 ml of ice cooled dichloromethane. Dry the product by sucking air though the funnel. Transfer the dry product to a watch glass and weight the dry product.

Product Characterization

Measure the melting point and run an IR-spectrum of the product. Check the purity of the product by TLC (1:2 dichloromethane:petroleum ether).

5.3 Ethyl Propanoate



The reaction in question is an acid catalyzed esterification. Propanoic acid is protonated by the acid used as a catalyst. The emerging cation is resonance stabilized. Ethanol acts as a nucleophile as it attacks the cation. This is followed by the transfer of a proton and the loss of water. The reaction is a nucleophilic substitution.

Reagents	Safety Notes	Equipments
10 mL abs. ethanol 14 mL propanoic acid Conc. H_2SO_4 40 mL ether 2M $NaHCO_3$ solution $MgSO_4$	Conc. H_2SO_4 is highly corrosive. Ether is a highly flammable and volatile liquid. Ethanol is a highly flammable and volatile liquid	Reflux apparatus Extraction/Separation equipments Apparatus for continuous feed distillation

Procedure:

In a 50 mL round-bottomed flask mix 10 mL of ethanol and 14 mL of propanoic acid. Carefully add 2-3 drops of conc. H_2SO_4 (catalyst) and mix the solution well. After addition of a few anti-bumping granules, reflux the mixture for one hour using and oil bath. Let the reaction mixture cool.

Isolation and Purification

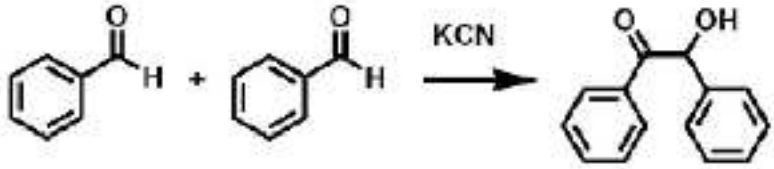
Pour the cool reaction mixture into a separatory funnel filled with 40 mL of water. Add 40 mL of ether into the funnel, stopper, and shake it gently (wear

safety glasses!!). Release any pressure inside the separation funnel by turning the stoppered funnel upside down and opening the stopcock. Let the funnel rest (upright, stopcock closed) until the layers are separated. Let the aqueous layer (the lower one) drain from the funnel into a different vessel. Wash the ether layer that remains in the funnel with 20 mL of water (shake it with the added water). Once the layer have separated, the aqueous layer is drained. Wash the ether solution with 2M $NaHCO_3$ solution to remove any unreacted acid. It is essential that you are especially careful at this stage since carbon dioxide is produced when the acid is neutralized. The gas causes pressure inside the separatory funnel. The pressure is released as described above. The acid is now present in the aqueous layer as the Na salt. Finally, wash the ether solution with 20 mL of water. Remove the aqueous layer as thoroughly as possible and then dry the ether solution with anhydrous magnesium sulfate. After 30 minutes of drying, filter the solution through a regular glass funnel fitted with a clean cotton plug into a dropping funnel. Part of ether solution is poured into a round-bottomed flash and boiling stones are added. The ether is removed by a distillation procedure used for the removal of large amounts of solvent (bp of ether = 35°C). The flask can be heated either in a water or oil bath. The product is purified by distillation. The boiling point of ethyl propanoate is 99°C (oil bath). The product yield is 60%.

Product Characterization

Measure the refractive index and run an IR spectrum of the product.

5.4 Benzoin



Aromatic aldehydes when treated with an alkali cyanide, usually in aqueous solution, undergo condensation to the α-hydroxyketone or benzoin (called benzoin condensation). The best known example is the conversion of benzaldehyde to benzoin.

Reagents	Safety Notes	Equipments
2.5 mL of benzaldehyde 35 mL of techn. Alcohol 0.25 g of Potassium Cyanide	Potassium cyanide is a poisonous and corrosive solid. For the disposal of cyanide compound please see further advice. Benzaldehyde is an irritant.	Reflux apparatus, Water bath, Erlenmeyer flask, Suction filtration apparatus.

Procedure

In a 50 mL round-bottomed flask place 5 mL alcohol, 2.5 mL purified benzaldehyde and 0.25 g potassium cyanide dissolved in 4 ml of water (CARE !! This preparation must be done in the hood! Assumed purity of KCN is 96-98%). Attach a reflux condenser and heat on a boiling water bath for 30 minutes. Cool the flask in ice-water, filter the crude benzoin and wash it with water and drain dry.

Isolation and Purification

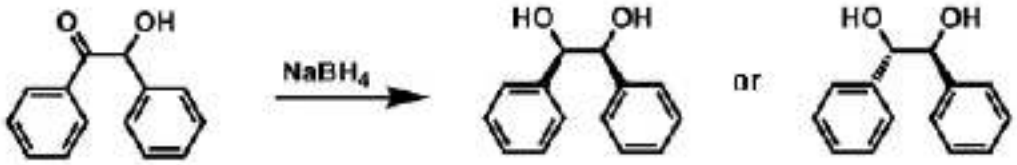
Recrystallize the crude product from ethanol; the benzoin is obtained as a

white crystalline solid, m.p. 137°C, the yield of the pure product being 2.1 g.

Product Characterisation

Measure the melting point and run an IR spectrum of the product.

5.5 Reduction of benzoin with sodium borohydride - 1,2-Diphenylethane-1,2-diol*



The stereochemical course of ketone reductions can be influenced by the presence of hydroxyl group close to the carbonyl function. This experiment illustrates the stereoselective reduction of benzoin using sodium borohydride as reducing agent. Please find the mechanism!

Reagents	Safety Notes	Equipments
2.00 g benzoin 0.40 g sodium borohydride Ethanol HCl (6M)	Irritant Corrosive, flammable Flammable, toxic Corrosive	Magnetic stirrer, suction filtration apparatus, recrystallization apparatus

Procedure

Dissolve the benzoin in 20 mL of ethanol in a 100 mL Erlenmeyer flask (CAUTION: warming may be necessary; solution need not be complete). Stir the solution magnetically and add the sodium borohydride a small portion over 5 min using a spatula (CAUTION: Care! Exothermic). If necessary, rinse in the last traces of sodium borohydride with 5 mL of ethanol. Stir the mixture at room temperature for a further 20 min and then cool it in an ice bath

whilst adding 30 mL of water followed by 1 mL of 6 M hydrochloric acid (CAUTION: foaming may occur!). Add a further 10 mL of water, and stir the mixture for a further 20 min.

Isolation and Purification

Collect the product by suction filtration, and wash it thoroughly with 100 mL water. Dry the product by suction for 30 min, and record the yield. Recrystallize from ethanol-water (1:1). Yield 90%.

Product Characterization

Check the purity by TLC (Silica plates; eluent ethyl acetate-hexane 1:1). Measure the melting point and run an IR spectrum of the product.

5.6 *p*-Benzoquinone

Reagents	Safety Notes	Equipments
2.5 g of hydroquinone	Harmful	100 mL flask (14/29) + Stopper
1.4 g of potassium bromate	Oxidative, explosive	Suction filtration apparatus
1.2 mL of sulfuric acid (2M)	NOTE: The product is highly toxic and flammable as an air-dust mixture	Sublimation apparatus

Procedure

Place potassium bromate (1.4 g), 2 M sulfuric acid (1.2 mL), water (25 mL) and hydroquinone (2.5 g) into a 100 mL round-bottomed flask. Close the flask with a stopper and stir the mixture (by swirling) for about 30 minute at room temperature. The reaction is over when the initially black mixture turns yellow (yellowish green).

Isolation and Purification

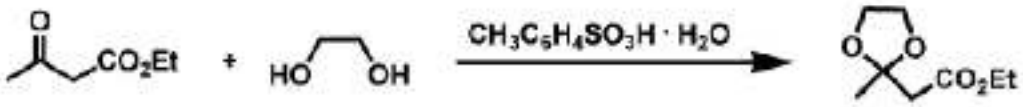
Filter by suction filtration, wash with ice cold water and spread the product on filter paper until it is dry. Purify the obtained crude product by sublimation under vacuum. *p*-Benzoquinone gives a yield of 1.3 g and has its melting point at 115°C.

NOTE: The product is water-soluble. This means that over eager washing will diminish the amount of product that is left in the funnel. The impure product in particular decomposes easily so that it is important that there isno delay in the isolation and purification of the compound.

Product Characterization

Measure the melting point and run an IR spectrum of the product

5.7 Protection of ketones as ethylene acetals (1,3-dioxolanes): ethyl acetoacetate ethylene acetal



If polyfunctional molecules it is often necessary to protect aldehyde and ketone carbonyl groups to strop undesirable side-reactions during a synthetic sequence, and then remove the protecting group at a later stage. One common protecting group for aldehydes and ketones is the ethylene acetal (1,3-dioxolane derivative) easily prepared from the carbonyl compound and ethane-1,2-diol (ethylene glycol) in the presence of an acid catalyst. The protecting group can be removed subsequently by treatment with aqueous acid.

Reagents	Safety Notes	Equipments
12.7 mL / 13.0 g ethyl acetoacetate	Irritant	Reflux with water removal (Dean and Stark apparatus) extraction/separation distillation under reduced pressure (water aspirator)
5.8 mL / 6.5 g ethane-1,2-diol	Irritant	
0.05 g toluene-4-sulfonic acid monohydrate	Corrosive, toxic	
50 mL toluene	Flammable, irritant	
sodium hydroxide solution (10%)	Corrosive	
anhydrous potassium carbonate	Corrosive, hygroscopic	

Procedure

(D. R. Paulson, A. L. Hartwig, and G. F. Moran, *J. Chem. Ed.* 1973, 30 216)

Set up a 100 mL round bottomed flask with a Dean and Stark water separator and reflux condenser. Add the ethyl acetoacetate, toluene, ethane-1,2-diol, toluene-4-sulfonic acid, and a few anti-bumping granules to the flask. Heat the flask so that the toluene refluxes vigorously (CAUTION: vapor should condense half way up condenser). Continue to heat the mixture until no more water collects in the separator (CAUTION: takes about 45 minutes).

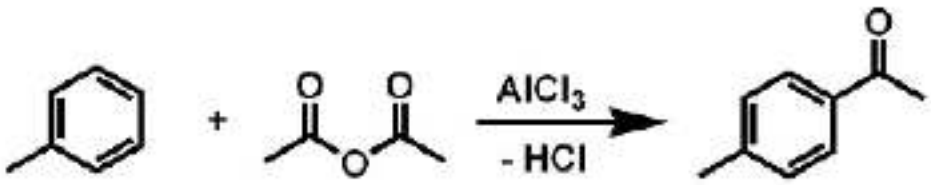
Isolation and Purification

Cool the mixture to room temperature, transfer it to a separatory funnel, and wash the solution with 15 mL sodium hydroxide solution followed by 2 x 20 mL portions of water (CAUTION: check the pH with pH-paper; the final washing should be neutral). Dry the organic layer over anhydrous potassium carbonate (NOTE: may be interrupted at this stage). Filter off the drying agent by suction, evaporate the filtrate on the rotary evaporator (NOTE: may be interrupted at this stage), transfer the residue to a 25 mL flask and distil it under reduced pressure using water aspirator. Record the exact boiling point and the yield of the product. The pure compound boils at 99.5-101°C/17 mm Hg and has the index of refraction 1.43262. Yield 64%.

Product Characterization

Measure the refractive index and run an IR spectrum of the product.

5.8 *p*-Methyl acetophenone



Reagents	Safety Notes	Equipments
15 g $AlCl_3$ 25 mL distilled toluene 4.8 mL acetic anhydride anhydrous $MgSO_4$	Aluminium chloride produces HCl when it comes into contact with dampness of the air and reacts violently with water. $AlCl_3$ dust is harmful	100 mL 3-necked round-bottomed flask dropping funnel $CaCl_2$-tube Mechanical stirrer Oil lock Glass rod Reflux condenser PVC-tube Glass funnel 250 mL beaker oil bath separation/extraction apparatus vacuum distillation apparatus

Procedure

A. I. Vogel, *A Text•Book of Practical Organic Chemistry*, 3. edt., Longman, London

1970, p. 730

In a 100 mL 3-necked flask, equipped with a separatory funnel carrying a calcium chloride tube, a mechanical stirrer, and an efficient reflux condenser attached to a gas absorption device, place 15 g of finely-powdered anhydrous aluminium chloride and 25 mL of toluene. Set the stirrer in motion and ad 5.1 g (4.8 mL) of acetic anhydride slowly through the addition funnel; the addition requires 15 minutes, during which time the temperature rises to about 90°C and much hydrogen chloride is evolved. Heat the mixture on a water bath, with stirring, for 30 minutes or until there is practically no evolution of gas. Cool the reaction mixture to room temperature and pour it into a mixture of 30 g of crushed ice and 30 mL of concentrated hydrochloric acid; stir until the aluminium salts dissolve completely.

Isolation and Purification

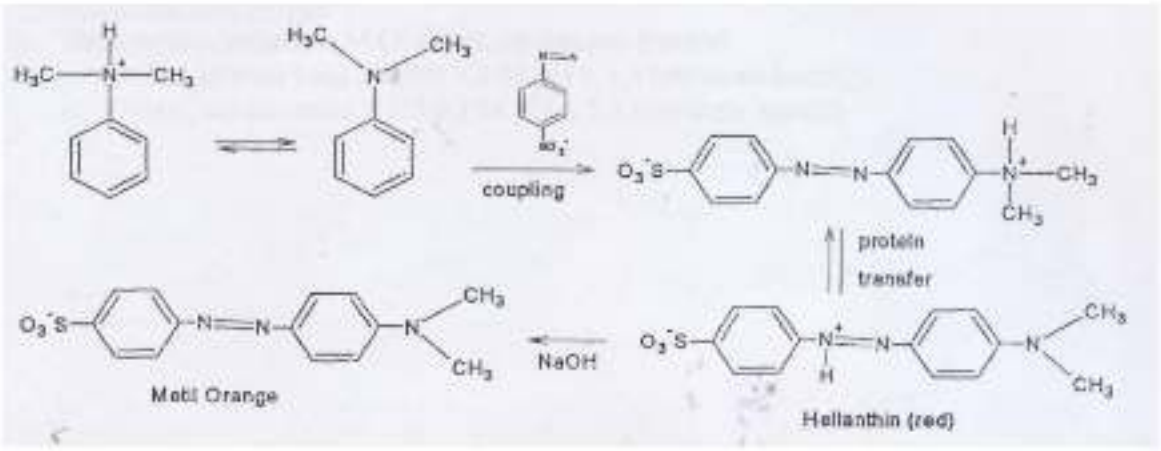
Separate the toluene layer, wash it with water, then with 10% sodium hydroxide solution until the washings remain alkaline, and finally with water; dry over anhydrous magnesium sulphate. Distill the residue using a Claisen adapter at atmospheric pressure until the temperature rises to about 125°C, then allow to cool and distil under reduced pressure. Alternatively, toluene may be removed with a rotary evaporator. Collect the *p*-methylacetophenone at 93-94°C/7 mm (the b.p. at atmospheric pressure is 225°C); the yield is 5.8 g.

Product Characterization

Measure the refractive index and run an IR spectrum of the product.

5.9 Methyl Orange

Methyl orange is a dye which can be sensitized by diazotization reaction between sulfanilic acid and N,N-dimethyl aniline. Initially helianthin is produced and then it is converted to methyl orange in the base condition. The scheme of this reaction is presented in below figure.



Reagents	Safety Notes	Equipments
2.4 g Sulfanilic acid 1 H_2O		Magnetic bar
25 ml 2.5% Na_2CO_3 solution		Beaker glass (250 mL)
0.95 g sodium nitrite		Heater and stirrer
15 g ice		Test tube
5 mL concentrated HCl		Paper filter
1.6 mL dimethyl aniline		Funnel
1.25 mL glacial acetic acid		pH indicator stick
17.5 mL 10% NaOH solution		Cylinder (25 mL)
Saturated NaCl solution		Pasteur pipette
0.1 M NaOH		Micropipette (1 mL)
0.1 M HCl		

Procedure:

Diazotization of sulfanilic acid

Dissolve 2.4 g of sulfanilic acid 1 H2O in 25 mL of 2.5% Na2CO3 solution and cool the solution in water. Add 0.95 g of sodium nitrite to this solution and then stir until dissolved completely. Pour the solution into beaker glass (250 mL) which contains ± 15 g of ice and 5 mL of concentrated HCl. In 1-2 min white precipitate of diazonium salt was formed.

Methyl orange

Dissolve 1.6 mL of dimethyl aniline and 1.25 mL of glacial acetic acid in test tube. Pour this mixture into the beaker glass containing diazonium salt, stir the solution magnetically. Wash the test tube with a small portion of water and pour it into the same beaker glass. Stirring process is continued till red color produced (in 5-10 min will be formed a hard pasta). Orange sodium salt is formed by the addition 17.5 mL of 10% NaOH solution. Stir and heat the solution till boiling so that the most salt will be dissolved. Cool the beaker glass to room temperature in water followed by the addition of ice to the cooler. During the cooling process do not shake the beaker glass. Filter the precipitate by using filter paper and use saturated NaCl solution to wash the beaker glass and the filter paper. Perform solubility (in water) and color (in different pH calues) tests from the obtained precipitate. Yellow color in pH ≥
4.4 (by the addition of 0.1 M NaOH) and red color at pH ≤ 3.2 (by the addition of 0.1 M HCl).

5.10 Endoperoxides of β-carotene

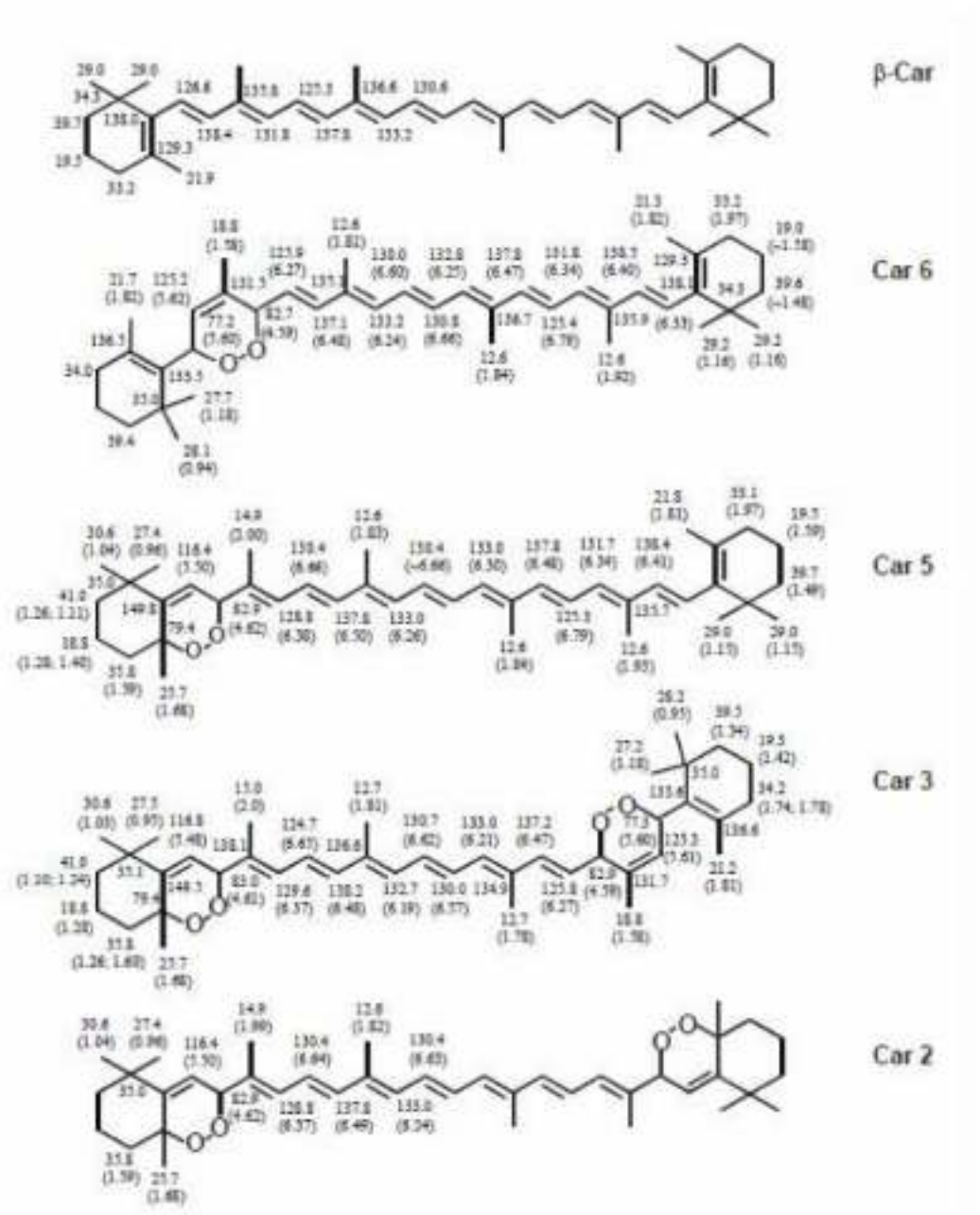


Fig. 18. NMR assignments of β-Car and its oxygenated products: Car 6 (7,10-endoperoxide), Car 5 (5,8-endoperoxide), Car 3 (7,10,5',8'-diendoperoxide), and Car 2 (5,8,5',8'-diendoperoxide) with ^{13}C and ^{1}H shifts measured in C_6D_6. Proton shifts are indicated in parenthesis (Fiedor *et al.*, 2005).

Chemicals

β-carotene was purchased from Sigma-Aldrich (≥ 95% HPLC). All solvents were spectroscopy grade or HPLC grade and were used without further purification. Hexane, methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF), acetone and glacial acetic acid were purchased from Merck.

Preparation of bacteriopheophytin a (BPheo a)

BPheo *a* was prepared from bacteriochlorophyll *a* (BChl *a*) by a short treatment with glacial acetic acid. The acid was completely removed by cycles of drying with a stream of nitrogen gas (N_2) and dissolving with acetone. BChl *a* was purified from the pigment extract, which was extracted from the cells of *Rhodobacter sphaeroides*, according to Omata and Murata (1983) and Pilch *et al* (2013).

Preparation of β-carotene oxygenation products

The singlet oxygen, generated from a BPheo-photosensitized reaction, is chemically quenched by *β*-carotene to form the photooxygenation products. The products of *β*-carotene oxygenation are endoperoxides (Fiedor et al., 2001; Fiedor et al., 2005). For instance, a solution mixture of *β*-carotene and BPheo *a* in acetone with the $OD_{1\,mm}$ of 1.5 and 0.3, respectively, was irradiated with a halogen cold-light source (Intralux 4100, Volpi) equipped with a cut off filter (RG 630, $\lambda \geq 630$ nm). The irradiation was performed at room temperature under equilibrium with air and the progress of the oxygenation of *β*-carotene was monitored from its absorption spectra. Accumulation of each oxygenation product depends on the irradiation time and it can be estimated from the shape of absorption spectra of the reaction mixture. The reaction mixture solution was dried with N_2 and was stored under vacuum at -30°C for further isolation.

Isolation and identification of β-carotene oxygenation products (Fig 18)

The oxygenation products were isolated by reverse-phase HPLC according to the modified method of Tregub *et al* (1996) and Fiedor *et al* (2001). A C18 column (Shim-pack VP-ODS, 250 mm (L) × 4.6 mm (I.D.), 4.6 µm particle size, Shimadzu) with a binary gradient elution of ACN, MeOH and THF mixture at the flow rate of 1 mL/min was used to separate the products. The following elution program was applied 100% A (ACN:MeOH:THF = 91:7:2, v/v/v) for 0-20 min, then a linear gradient to 100% B (ACN:MeOH:THF = 32:60:28 v/v/v) for 20-30 min and 100% B for 30-45 min. The analysis was performed on a LC-20AD HPLC (Shimadzu) equipped with a diode array detector SPD-M20A (Shimadzu). The purity of isolated oxygenation products was examined by HPLC.

Identification of each oxygenation product was based on the sequent of retention time on HPLC chromatogram, the shape of spectrum, and absorption maxima compared to the reference (Fiedor et al., 2001). Moreover, an electron spray ionization triple quadrupole mass spectrometer (LCMS 8030, Shimadzu) was used to confirm the identification. The detailed mass spectrometry analysis was described in Brotosudarmo et al. (2016).

Absorption spectra of β-carotene oxygenation products

The electronic absorption spectra of oxygenation products in hexane were measured on a UV 1700 spectrophotometer (Shimadzu) using 1 cm path length quartz cell.

References:

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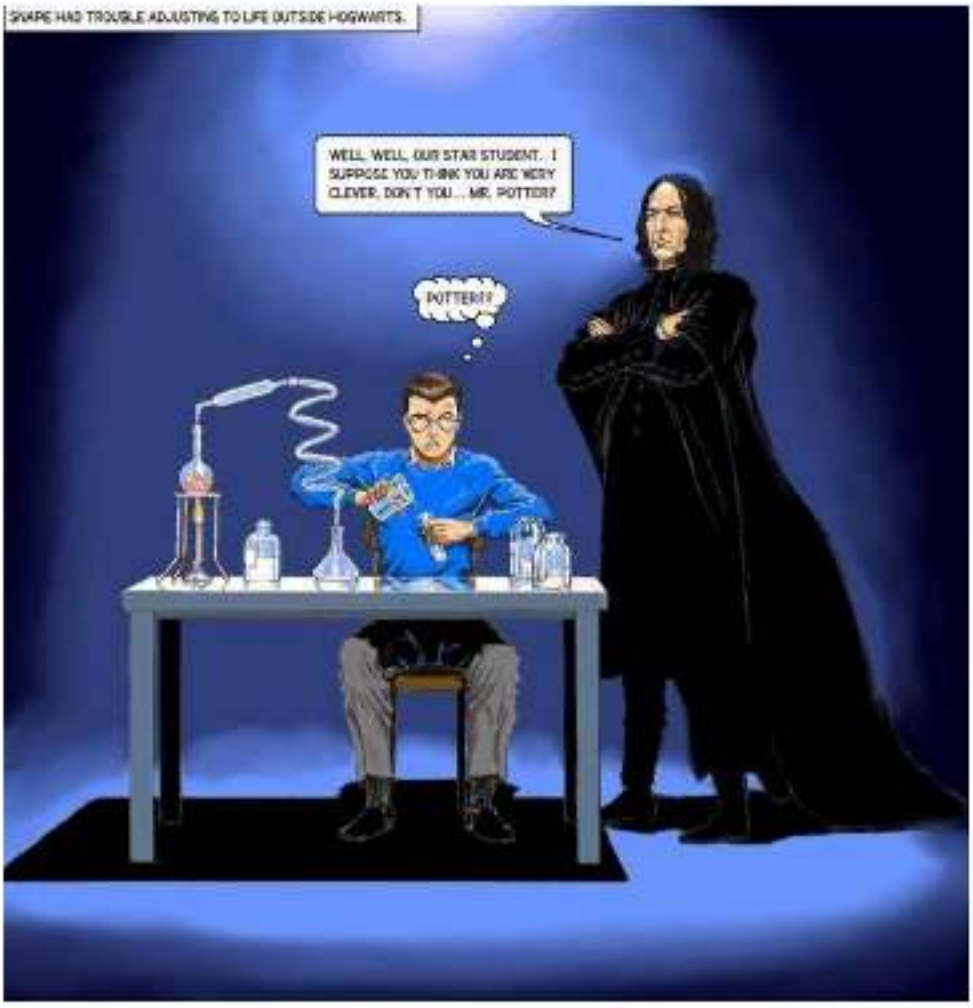
SNAPE HAD TROUBLE ADJUSTING TO LIFE OUTSIDE HOGWARTS.
WELL, WELL, OUR STAR STUDENT. I SUPPOSE YOU TI-INK YOU ARE VERY CLEVER, DON'T YOU... MR. POTTER?
POTTER!?