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DEPARTAMENTO DE QUÍMICA ORGÁNICA

COMPENDIUM OF EXPERIMENTS FOR ORGANIC CHEMISTRY IV (1606)

SEMESTRE 2027-1

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1606 – Química

Créditos y reconocimientos

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Programa de prácticas — Semestre 2027-1

Sem.	Fechas	Práctica	Nota
1	17-21 ago	Bienvenida, reglamentos, evaluación y firmas	
2	24-28 ago	Entrega de kits / Práctica 1: Ácidos carboxílicos I — Reacción del haloformo	
3	31 ago-4 sep	Práctica 2: Ácidos carboxílicos II — Reacción de Kolbe-Schmitt	
4	7-11 sep	Práctica 3: Derivados de ácidos carboxílicos I — Síntesis de benzocaína / butirato de etilo / anhídrido ftálico	
5	14-18 sep	Práctica 4: Ácidos carboxílicos III — Síntesis de ácido benzoico por reacción de Grignard	G3 sin sesión (16 sep)
6	21-25 sep	Práctica 5: Derivados de ácidos carboxílicos II — Síntesis de paracetamol / fluoresceína	
7	28 sep-2 oct	Práctica 6: Lípidos I — Aislamiento de trimiristina	
8	5-9 oct	Práctica 7: Lípidos II — Saponificación de trimiristina	
9	12-16 oct	Práctica 8: Aminoácidos y péptidos I — Síntesis del ácido hipúrico	G1 y G2 sin sesión (12 oct)
10	19-23 oct	Práctica 9: Aminoácidos y péptidos II — Reacción pseudo-Sanger e identificación de aminoácidos	
11	26-30 oct	Práctica 10: Carbohidratos I — Análisis e identificación de carbohidratos	
12	2-6 nov	Taller de RMN	G1 y G2 sin sesión (2 nov)
13	9-13 nov	Práctica 11: Carbohidratos II — Hidrólisis química del almidón (G3 y G4) / EFI (G1 y G2)	G1 y G2 realizan EFI
14	16-20 nov	EFI (G3 y G4)	G1 y G2 sin sesión (16 nov)
15	23-27 nov	Resultados, retroalimentación, cierre y devolución de kits	

ETHICS AND ACADEMIC INTEGRITY IN THE LABORATORY

Organic Chemistry Laboratory IV (1606)
Semester 2027-1

In accordance with the Code of Ethics of the Universidad Nacional Autónoma de México, the Hygiene and Safety Regulations for the laboratories of the Facultad de Química, and the current Internal Regulations of the Organic Chemistry Laboratories, the following points of mandatory observance for the course Organic Chemistry Laboratory IV (1606) are set forth below, both inside and outside the laboratory:

- Every document requested by the course instructors must be prepared by the students themselves. Plagiarism, whether partial or total, is strictly prohibited; anyone who engages in it will be subject to the corresponding academic sanctions.
- Every element (text, figure, scheme, spectrum, or data point) included in pre-lab research, assignments, homework, or laboratory reports that was not generated from the experimental work and is not of the student's own authorship must be properly referenced, or it will be considered plagiarism.
- Fabricating any data not obtained directly during an experiment is prohibited. Data that do not match expectations must be reported exactly as obtained, then analyzed and justified with chemical reasoning and critical thinking. Fabricating information in a laboratory report constitutes a serious breach of academic integrity.
- Experimental observations and any other data must be recorded at the moment they are generated, without reconstructing them afterwards. Use of the lab notebook is mandatory, as it constitutes the record of authorship of the work and the main piece of evidence in case of any doubt about a result.
- The use of generative artificial intelligence tools is permitted only as support for organizing ideas or improving writing, provided that the tool and the purpose for which it was used are explicitly declared. Its use is not permitted for generating data, discussions, or conclusions presented as the product of one's own experimental work.
- Modifying the experimental procedures of this Compendium is prohibited; any update requires written approval from the Head Office of the Department of Organic Chemistry.
- Laboratory incidents (spills, splashes, or breakage of glassware or equipment) must be reported immediately, since concealing them poses a risk to the entire group.
- Waste generated in the laboratory is disposed of according to the ecological diagram of each experiment; failure to comply constitutes an environmental infraction.
- Upon detecting any breach of academic integrity, the instructors will document the incident, hear the people involved, and apply the corresponding sanctions in accordance with the evaluation criteria handed out in the first session and with the current guidelines and regulations of the Facultad de Química. In serious cases or cases of recidivism, the matter will be referred to the Head Office of the Department of Organic Chemistry and, where applicable, to the Faculty's H. Consejo Técnico, in accordance with University Legislation.

EXPERIMENT 1

CARBOXYLIC ACIDS I

THE HALOFORM REACTION

I. Objectives.

a) To synthesize benzoic acid from acetophenone by means of an oxidative demethylation, or haloform, reaction.

b) To confirm the presence of a carboxylic acid group by means of characteristic tests.

II. Introduction.

The haloform reaction is a characteristic reaction undergone by methyl ketones in the presence of chlorine, bromine, or iodine in strongly alkaline medium (^{-}OH) to give a metal carboxylate (RCO_2Na or K) and a trihalomethane, commonly known as a haloform (CHX_3), with the particularity that the carboxylate contains one carbon fewer than the original chain. Aldehydes do not undergo this reaction, with the exception of acetaldehyde. The synthetic utility of the reaction lies in the oxidative demethylation of methyl ketones for the preparation of carboxylic acids or amides, as reported by Cao L.

The reaction proceeds through 3 successive halogenations of the 3 alpha hydrogens of the methyl group adjacent to the carbonyl; once the three halogenations have taken place, a hydroxide performs a nucleophilic attack on the carbonyl, displacing the trihalomethane as the leaving group.

III. Pre-lab Research.

1. Propose, step by step, the mechanism of trihalogenation and C–C cleavage of this reaction, and explain why it stops exactly at the third halogen.

2. The pKa of the alpha protons of a methyl ketone is ≈ 20 and that of water (the conjugate acid of hydroxide) is 15.7. Explain why the hydroxide in the medium is enough to generate the enolate that starts the reaction.

3. Calculate the equivalents of NaOCl relative to acetophenone using the amounts in this experiment. Predict which product would predominate if only 1 equivalent were used.

4. Explain, at the molecular level, what should be observed when $NaHCO_3$ is added to the purified product.

5. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents and products, and complete the table in section VI based on the balanced reaction.

IV. Reagents.

Reagents	Amount	Reagents	Amount
Acetophenone	0.5 mL	HCl solution 50% v/v	5 mL
NaOCl solution 13% v/v	18 mL	Anhydrous Na_2SO_4	2.0 g
Sodium bisulfite $NaHSO_3$	100 mg	10% KI solution	2.0 mL
Dichloromethane CH_2Cl_2	6 mL	Distilled water	15 mL
10% $NaHCO_3$ solution	0.25 mL		

V. Materials.

This experiment is carried out entirely with the kit glassware. No additional equipment needs to be requested by requisition slip (papeleta).

VI. Reaction and Stoichiometric Relationship

Reaction: Benzoic acid	Acetophenone	NaOCl 13%	Benzoic acid
Molar mass (g/mol)			
Mass (g)			
Volume (mL)			
Density (g/mL)			
Amount of substance (mmol)			
Chemical equivalents			
Physical properties			

VII. Procedure.

50% HCl: It is corrosive; handle it with care. In case of contact, wash with plenty of water for 3-5 minutes and rinse with 10% $NaHCO_3$ solution.

In the 50 mL Erlenmeyer flask, with magnetic stirring, place 0.5 mL of acetophenone and 18 mL of 13% v/v NaOCl solution (measure it with the 10 mL graduated cylinder in two successive portions). Stir vigorously at room temperature for 40 minutes.

When finished, while still stirring, add 100 mg of $NaHSO_3$ to destroy the excess hypochlorite. Confirm that the reaction is complete with the KI test: in two beakers place 1 mL of 10% KI solution in each; to one add 1 drop of NaOCl (positive control, turns brown)

and to the other 1 drop of your reaction mixture (sample). If your sample also turns brown, keep adding $NaHSO_3$ until the test is negative.

Remove the stir bar and transfer the mixture to the separatory funnel. Extract with CH_2Cl_2 (3 × 2 mL); combine the organic phases and keep them — do not discard them yet.

Rinse the Erlenmeyer flask and place the aqueous phase in it; set it in an ice bath and acidify with 50% v/v HCl to pH 2-3 — a whitish solid will begin to precipitate. Vacuum-filter with the Büchner funnel and wash the crystals with ice-cold water; set aside a small amount of the crude solid for its melting point.

Purify the benzoic acid by recrystallization using water as the solvent. Vacuum-filter, dry, and weigh the product; calculate your yield and determine the melting point of both the crude and the recrystallized material.

Place a small amount of the recrystallized product on the watch glass and add a few drops of 10% $NaHCO_3$ solution; observe and record what happens.

To the organic phase you set aside, add Na_2SO_4 (2 spatula-fuls) to remove the excess water. Run a TLC comparing acetophenone (one drop diluted in 2 mL of CH_2Cl_2 or EtOAc) against your organic phase, to check whether unreacted starting material remains (eluent hexane:EtOAc 9:1).

VIII. Post-lab Questions.

1. Calculate your experimental yield and compare it with the theoretical one. If you obtained less than 70%, point out two specific causes from your own procedure that explain the loss of product.

2. Analyze your melting points and explain the difference between the crude and the recrystallized material in terms of purity.

3. Report the result you obtained in your KI test and, based on it, state whether your first addition of bisulfite was sufficient or whether you had to repeat it. Write the equation for the reaction between bisulfite and hypochlorite.

4. Interpret your TLC plate: how many spots did you observe and with what Rf values? Do they indicate that unreacted acetophenone remained?

5. Using your yield, your melting point, and your TLC, decide which variable you would change first if you repeated the experiment, and justify your choice.

6. Look up how benzoic acid is obtained industrially and compare that route with the one you used, in terms of raw materials and conditions.

IX. References.

1. Cao, L.; Ding, J.; Gao, M.; Wang, Z.; Li, J.; Wu, L. *Org. Lett.* **2009**, 11, 3810.

2. Mayo, D. W.; Pike, R. M.; Butcher, S. S.; Trumper, P. K. Microscale Organic Laboratory **1994**, John Wiley & Sons Inc., United States of America.

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5. Williamson, K. Macro and Microscale Organic Experiments **2010**, Cengage Learning, 6th Edition, United States of America.

6. Pavia, D. L. Introduction to Organic Laboratory Techniques, a Microscale Approach **2006**, Cengage Learning, 4th Edition, United States of America.

X. Appendix: Reagent Preparation

HCl solution (50%) (1 L)

Using a graduated cylinder, measure 500 mL of concentrated hydrochloric acid (HCl) and add it slowly into a 1000 mL volumetric flask, then fill to the mark with distilled water; the volumetric flask must be kept in an ice bath. Stir gently until homogeneous and pour into the container where it will be stored. Important: do NOT reverse the order of addition; wear a lab coat, gloves, and safety glasses. The solution releases heat.

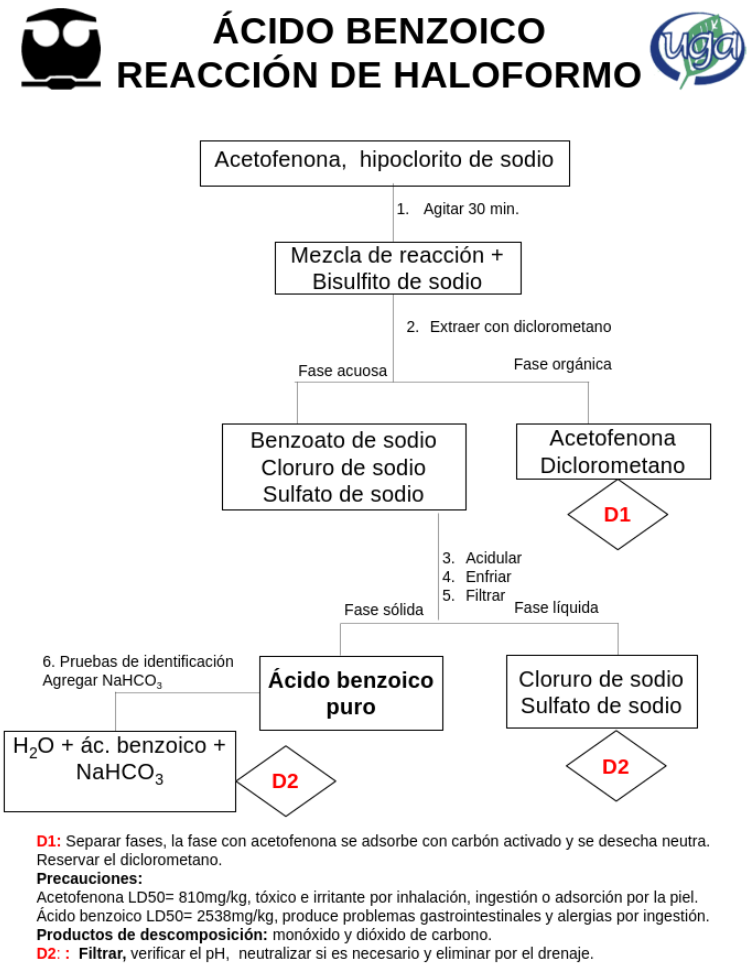
10% Sodium bicarbonate solution (1 L)

Weigh 100 g of sodium bicarbonate ($NaHCO_3$) and pour into a 1000 mL volumetric flask containing distilled water, slowly dissolve the salt until homogeneous, then fill to the mark to a final volume of 1000 mL (1 L).

10% acidic KI solution (100 mL)

Weigh 1.0 g of KI and add it into a volumetric flask, add 80 mL of distilled water, stir gently, then slowly add 5 mL of concentrated hydrochloric acid, stir gently, and finally fill to 100 mL with distilled water. Store in an amber bottle. Storage shelf life: 1 week.

XI. Waste Management.



Scheme 1. Flow diagram of the haloform reaction and its waste management.

EXPERIMENT 2

CARBOXYLIC ACIDS II

THE KOLBE-SCHMITT REACTION

I. Objectives.

a) To carry out a carboxylation reaction of phenols.

b) To apply the methodology described by Kolbe-Schmitt for the preparation of an aromatic hydroxy-carboxylic acid.

II. Introduction.

Hydroxy-aromatic carboxylic acids are very important synthetic intermediates for the preparation of a variety of value-added products, such as pharmaceuticals, cosmetics, food preservatives, antiseptic agents, fungicides, dyes, polyesters, textile auxiliaries and, currently, liquid crystal polymers. The most conventional method for preparing these acids is the Kolbe-Schmitt reaction.

The Kolbe-Schmitt reaction has been the standard procedure for the preparation of hydroxy-aromatic acids for more than 150 years. In 1860, Hermann Kolbe, at the University of Marburg, succeeded in obtaining salicylic acid from phenol, sodium, and carbon dioxide at atmospheric pressure; despite several modifications, the yield never exceeded 50%. The modification made by Rudolf Schmitt in 1884 —carrying out the reaction under pressure— gave excellent synthetic results.

In general, carboxylation occurs at the position ortho to the phenolic hydroxy group, although substitution at the para position has also been observed. Water inhibits the carboxylation of monohydroxy phenols; however, more reactive phenols (di- and tri-hydroxy) can be carboxylated in alkaline solution. With phenols such as resorcinol, monocarboxylation proceeds in excellent yields by moderate heating in the presence of mildly concentrated aqueous solutions of alkali bicarbonates, in open systems that allow access of atmospheric carbon dioxide.

III. Pre-lab Research.

1. Propose, step by step, the Kolbe-Schmitt carboxylation mechanism on resorcinol and explain why it occurs at the ortho/para positions relative to the hydroxyl and not at meta.

2. Resorcinol has pKa ≈ 9.3 and carbonic acid (the conjugate acid of bicarbonate) has pKa ≈ 6.3. Using these values, explain why sodium bicarbonate is sufficient for the carboxylation to proceed.

3. Explain, at the molecular level, what should be observed when $NaHCO_3$ is added to an aqueous solution of the purified product.

4. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents and products, and complete the table in section VI based on the balanced reaction.

IV. Reagents.

Reagents	Amount	Reagents	Amount
Resorcinol	0.5 g	Sodium bicarbonate	2.5 g
Concentrated hydrochloric acid	5 mL	10% $NaHCO_3$ solution	0.5 mL

V. Materials.

This experiment is carried out entirely with the kit glassware. No additional equipment needs to be requested by requisition slip (papeleta).

VI. Reaction and Stoichiometric Relationship

Reaction: Kolbe-Schmitt	Resorcinol	$NaHCO_3$	2,4-Dihydroxybenzoic acid
Molar mass (g/mol)			
Mass (g)			
Volume (mL)			
Density (g/mL)			
Amount of substance (mmol)			
Chemical equivalents			
Physical properties			

VII. Procedure.

Concentrated HCl: It is corrosive; handle it with care. In case of contact, wash with plenty of water for 3-5 minutes and rinse with 10% $NaHCO_3$ solution.

In the 25 mL flat-bottom boiling flask, with a magnetic stir bar, add 0.5 g of resorcinol, 4 mL of water, and 2.5 g of sodium bicarbonate. Fit the condenser in reflux position and heat the mixture slowly until it reaches reflux; keep it at reflux for 1 hour.

When finished, cool the mixture to room temperature and pour it into the 50 mL beaker containing 7.5 mL of ice-cold water. Place the beaker in an ice bath and add concentrated hydrochloric acid dropwise, with continuous stirring, until pH 1.0.

Let it cool in the ice bath until the 2,4-dihydroxybenzoic acid crystallizes. Vacuum-filter with the Büchner funnel and wash the crystals with ice-cold water; set aside a small amount of the crude solid for its melting point.

Purify the product by recrystallization using water as the solvent. Vacuum-filter, dry, and weigh the product; calculate your yield and determine the melting point of both the crude and the recrystallized material.

Dissolve a small amount of the solid obtained in a minimum volume of water and check the pH of the solution with indicator paper. Add a few drops of $NaHCO_3$ solution, observe, and record what happens.

VIII. Post-lab Questions.

1. Using your yield and your melting points (crude vs. recrystallized), assess how pure your synthesis turned out and explain the difference between both values in terms of purity.

2. Describe the result you obtained when adding $NaHCO_3$ to your dissolved product, and mention two additional ways you could confirm the identity of 2,4-dihydroxybenzoic acid, different from the one used in this experiment.

3. What other compound could you use instead of sodium bicarbonate for this carboxylation? Could you apply the same conditions to carboxylate sodium phenoxide instead of resorcinol? Justify your answer.

IX. References.

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2. Rahim, M. A.; Matsui, Y.; Kosugi, Y. *Bull. Chem. Soc. Jpn.* **2002**, 75, 619-622.

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4. Fieser, L. F.; Williamson, K. Organic Experiments **1992**, D.C. Heath & Company, United States of America.

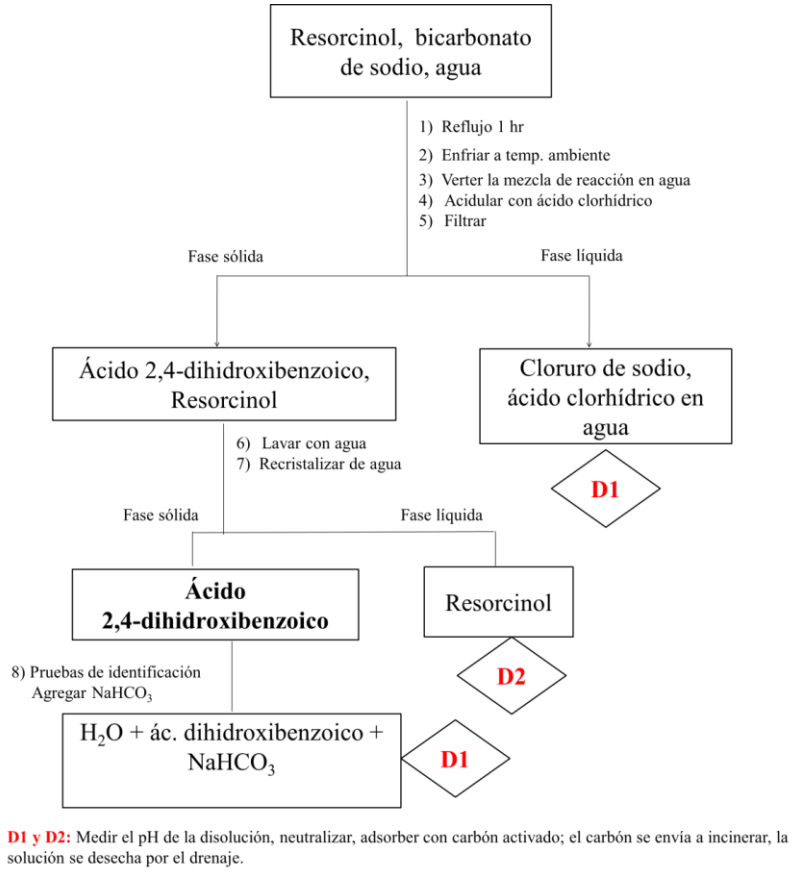
5. Ávila, G.; García, C. *et al.* Química Orgánica. Experimentos con un Enfoque Ecológico **, Dirección General de Publicaciones y Fomento Editorial, UNAM, 2nd Edition, Mexico.**, Dirección General de Publicaciones y Fomento Editorial, UNAM, 2ª Edición, México.

X. Appendix: Reagent Preparation

10% Sodium bicarbonate solution (1 L)

Weigh 100 g of sodium bicarbonate ($NaHCO_3$) and pour into a 1000 mL volumetric flask containing distilled water, slowly dissolve the salt until homogeneous, then fill to the mark to a final volume of 1000 mL (1 L).

XI. Waste Management.



Scheme 2. Flow diagram of the Kolbe-Schmitt reaction and its waste management.

EXPERIMENT 3

CARBOXYLIC ACID DERIVATIVES I
SYNTHESIS OF BENZOCAINE / ETHYL BUTYRATE / PHTHALIC ANHYDRIDE

I. Objectives.

a) To synthesize an ester by acid-catalyzed Fischer-Speier esterification: benzocaine from 4-aminobenzoic acid and ethanol, or ethyl butyrate from butyric acid and ethanol.

b) To shift the esterification equilibrium toward the product by removing the water formed within the reaction mixture.

c) To obtain a solid symmetric anhydride through the dehydrating action of an excess of a liquid anhydride on an aromatic dicarboxylic acid.

d) To assess the purity of the products obtained from the yield, the melting point, and thin-layer chromatography.

II. Introduction.

Carboxylic acids are organic compounds that bear a carbonyl and a hydroxyl on the same carbon atom, which gives this class of compounds a very particular reactivity. There are also compounds derived from them, called carboxylic acid derivatives, in which the hydroxyl has been replaced by another nucleophile such as halogen, alkoxy, thioalkoxy, acyloxy, or amino. This change of functional group substantially modifies the properties and reactivity of the molecule.

The main nucleophilic substitution reaction undergone by carboxylic acids and their derivatives is called *addition-elimination*: it starts from a trigonal planar compound with sp^2 hybridization, passes through a tetrahedral intermediate (sp^3), and ends by regenerating the hybridization and geometry of the starting material. How fast it occurs depends on how good a leaving group the substituent attached to the acyl group is, which establishes an order of reactivity among the different derivatives.

In this session, two practical consequences of that behavior are exploited. The Fischer-Speier esterification is an equilibrium: the acid and the alcohol coexist with the ester and water, so the yield depends not only on the reflux time but on how far that equilibrium can be shifted, either by using one of the reagents in excess or by removing the water as it forms. The second reaction takes advantage of the fact that a volatile liquid anhydride, used in excess, can dehydrate an aromatic dicarboxylic acid whose geometry allows closure to a five-membered cyclic anhydride; the phthalic anhydride thus obtained will be kept as starting material for Experiment 5 (Carboxylic acid derivatives II).

III. Pre-lab Research.

1. Rank the following from most to least reactive toward nucleophilic acyl substitution: acid chloride, anhydride, ester, and amide. Using that order, explain why acetic anhydride dehydrates *o*-phthalic acid and the reverse process does not occur.

2. Propose, step by step, the Fischer-Speier esterification mechanism for the synthesis your team will carry out, pointing out the tetrahedral intermediate and the step in which water is formed.

3. Calculate the equivalents of ethanol relative to the acid in your esterification. Predict, in terms of the equilibrium, what yield you would expect with equimolar amounts.

4. Explain the role of the Dean-Stark trap in the ethyl butyrate synthesis and the role of the $CaCl_2$ trap in the phthalic anhydride synthesis, and why they are not interchangeable.

5. Benzocaine is synthesized in the presence of concentrated H_2SO_4 and precipitates when the mixture is brought to pH 9-10. State in what form the amine exists during the reflux and predict what you would obtain if you stopped the NaOH addition at pH 4.

6. Look up one industrial use of benzocaine, one of ethyl butyrate, and one of phthalic anhydride, indicating which property of each compound makes it useful.

7. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents and products, and complete the tables in section VI based on the balanced reactions.

IV. Reagents.

Reagents	Amount	Reagents	Amount
Benzocaine			
4-Aminobenzoic acid	0.5 g	Absolute ethanol	5 mL
Concentrated H_2SO_4	0.8 mL	NaOH solution 10% m/v	9 mL
Absolute ethanol (recrystallization)	5 mL	Hexane:EtOAc 1:1 (eluent)	10 mL
Ethyl butyrate			
Butyric acid	5.3 mL	Absolute ethanol	10 mL
Concentrated H_2SO_4	1.5 mL	Anhydrous calcium chloride $CaCl_2$	0.5 g
Distilled water	45 mL	15% $NaHCO_3$ solution	20 mL
Brine solution	10 mL	Anhydrous Na_2SO_4	3 g
Phthalic anhydride			
o-Phthalic acid	0.5 g	Acetic anhydride	0.83 mL

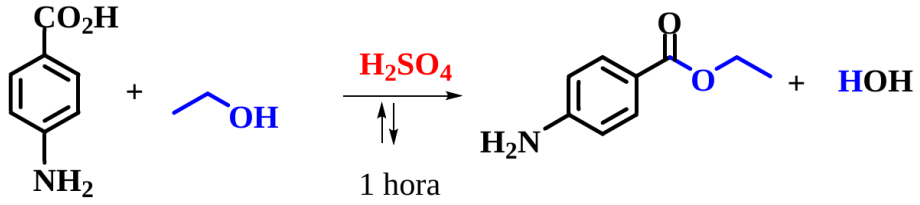
Reagents	Amount	Reagents	Amount
Anhydrous calcium chloride $CaCl_2$	0.5 g	Hexane	5 mL

V. Materials.

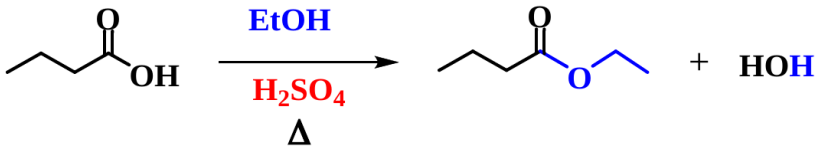
In addition to the kit, this experiment requires the following equipment, which you must request by requisition slip (papeleta). Items marked with (*) correspond only to the ethyl butyrate synthesis; if your team synthesizes benzocaine, request a single moisture trap.

Equipment	Qty.	Equipment	Qty.
10 mL flat-bottom boiling flask 14/23	2	Water-cooled condenser 14/23	1
Microscale moisture trap	2	Vial	1
Dean-Stark trap with Teflon stopcock (*)	1	50 mL separatory funnel with stopper (*)	1
Distillation T adapter 14/23 (*)	1	Short receiving adapter 14/23 (*)	1
Thermometer (*)	1	Thermometer adapter (*)	1

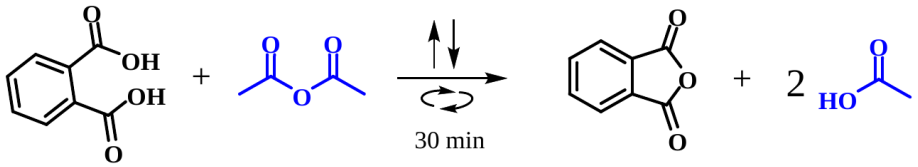
VI. Reaction and Stoichiometric Relationship



Reaction 1: Benzocaine	4-Aminobenzoic acid	EtOH	H_2SO_4	Benzocaine
Molar mass (g/mol)				
Mass (g)				
Volume (mL)				
Density (g/mL)				
Amount of substance (mmol)				
Chemical equivalents				
Physical properties				



Reaction 2: Ethyl butyrate	Butyric acid	EtOH	H_2SO_4	Ethyl butyrate
Molar mass (g/mol)				
Mass (g)				
Volume (mL)				
Density (g/mL)				
Amount of substance (mmol)				
Chemical equivalents				
Physical properties				



Reaction 3: Phthalic anhydride	*o*-Phthalic acid	Ac_2O	Phthalic anhydride
Molar mass (g/mol)			
Mass (g)			
Volume (mL)			
Density (g/mL)			
Amount of substance (mmol)			
Chemical equivalents			
Physical properties			

VII. Procedure.

Each team carries out **one** of the two esterifications —benzocaine or ethyl butyrate, as assigned by your instructor— and, in parallel, the phthalic anhydride synthesis.

Concentrated H_2SO_4: It is corrosive and causes severe burns to skin and eyes. Always add it slowly and onto the mixture, never the other way around. In case of contact, remove contaminated gloves and clothing, wash with plenty of water for 3-5 minutes, and rinse with 10% $NaHCO_3$ solution.

Benzocaine

In the 25 mL flat-bottom boiling flask, with a magnetic stir bar, place 0.5 g of 4-aminobenzoic acid and 5 mL of absolute ethanol. Slowly add, dropwise, 0.8 mL of concentrated H_2SO_4; you will observe that the initial solid dissolves. Fit the condenser in reflux position and keep the mixture at reflux with stirring for 1 hour.

Let it cool to room temperature and pour the mixture into the 50 mL beaker containing 9 mL of 10% m/v NaOH solution and approximately 10 g of ice. Stir until the benzocaine precipitates and check the pH; if necessary, add more NaOH solution until reaching pH 9-10. Vacuum-filter with the Büchner funnel and set aside a small portion of the crude solid to determine its melting point.

Purify the rest by recrystallization with the ethanol/water solvent pair: dissolve the solid in the **smallest** possible volume of hot ethanol (approx. 5 mL) and keep that volume constant throughout the process; then add the second solvent —ice-cold water or pieces of ice— until persistent cloudiness appears, and let it crystallize in an ice bath. Vacuum-filter, dry, weigh, and determine the melting point of the recrystallized material.

Run a TLC plate comparing 4-aminobenzoic acid against your benzocaine, using hexane:EtOAc 1:1 as the eluent. Measure the Rf values of both spots.

Ethyl butyrate

In the 25 mL flat-bottom boiling flask, with a magnetic stir bar, place 5.3 mL of butyric acid and 10 mL of absolute ethanol, and slowly add 1.5 mL of concentrated H_2SO_4. Fit the condenser in reflux position and place on top of it the moisture trap loaded with $CaCl_2$. Keep the mixture at reflux with stirring for 1 hour.

Stop the heating and let it cool slightly. Load the Dean-Stark trap with 10 mL of distilled water, keep the side stopcock closed, and mount it between the flask and the condenser (**Figure 1**). This pre-charge is essential: fill the trap up to the return arm, so that the condensate separates into two phases from the very first drop.

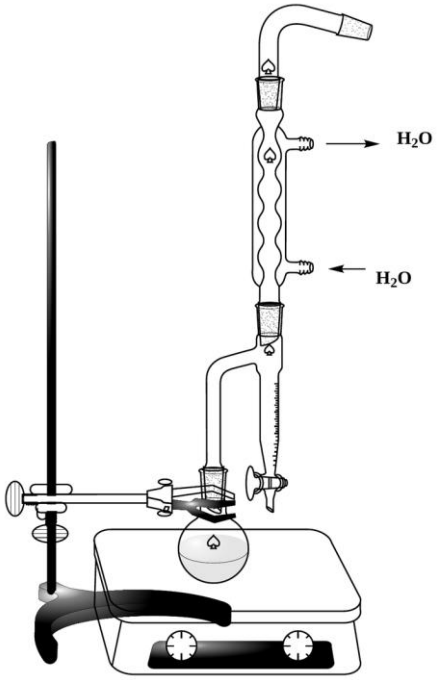


Figure 1. Dean-Stark trap setup for the synthesis of ethyl butyrate.

Resume heating at reflux for 30 more minutes. Inside the trap, the organic phase —being less dense— accumulates in the upper part and returns to the flask through the side arm, while the water and ethanol are retained in the lower aqueous phase. If the level of the aqueous phase reaches the return arm, drain it through the side stopcock. Record the volume of aqueous phase gained relative to the 10 mL pre-charged.

Turn off the heating, raise the system, and let it cool to room temperature. Pour the reaction mixture into the 50 mL separatory funnel, add 15 mL of ice-cold water, and shake; the ester stays in the organic (upper) phase, so discard the aqueous phase. Wash the organic phase with 10 mL of brine and again discard the aqueous phase.

Neutralize the organic phase with 15% $NaHCO_3$ solution (2 × 10 mL). **Caution:** CO_2 is released and the funnel becomes pressurized; shake while holding the stopper and vent frequently. Check that the aqueous phase comes out at pH 7. Finally wash with water (2 × 10 mL), recover the organic phase, and dry it with anhydrous Na_2SO_4.

Set up a simple distillation system: 10 mL flat-bottom boiling flask with a stir bar, distillation T adapter, thermometer with thermometer adapter, condenser, and short receiving adapter. Carefully decant the dried organic phase into the flask —without carrying over the drying agent— and distill. Discard as forerun everything that comes over below 110 °C and collect the fraction distilling between 115 and 125 °C into the 25 mL Erlenmeyer flask immersed in an ice bath. Weigh and measure the collected volume to calculate the yield.

Phthalic anhydride

Acetic anhydride: It is corrosive and a lachrymator. Measure and transfer it exclusively inside the fume hood,

avoiding any spills. In case of contact, wash with plenty of water for 3-5 minutes.

In the 10 mL flat-bottom boiling flask, with a magnetic stir bar, place 0.5 g of *o*-phthalic acid and, inside the fume hood, 0.83 mL of acetic anhydride. Fit the condenser in reflux position and place on top of it the moisture trap loaded with $CaCl_2$. Keep the mixture at reflux with stirring for 30 minutes.

Let it cool slightly and place the flask in an ice bath until the product solidifies. Vacuum-filter with the Büchner funnel; if necessary, use very cold hexane (approx. 5 mL) to transfer and wash the crystals. Dry, weigh, and determine the melting point.

Label the phthalic anhydride with the product name, the date, and your team number, and store it in the vial: it will be one of the starting materials for the Carboxylic acid derivatives II experiment.

VIII. Post-lab Questions.

1. Calculate the yield of the ester you synthesized and that of the phthalic anhydride. Take the lower of the two and point out two specific moments in your session in which product was lost.

2. Compare the melting point of your crude product with that of the recrystallized one. If the melting range of your final product spanned more than 2 °C, explain what that tells you about the sample you placed in the apparatus.

3. If you synthesized benzocaine: report the Rf of 4-aminobenzoic acid and that of your product, state which one eluted farther and explain it in terms of polarity; based on your plate, state whether unreacted starting material remained. If you synthesized ethyl butyrate: report the volume gained by the aqueous phase of the trap relative to the 10 mL pre-charged, compare it with the water your reaction theoretically produces, and explain the reason for the difference.

4. Pure phthalic anhydride melts at 131 °C. Compare your experimental value with that figure and, if it differs, propose a specific cause related to the way you filtered, washed, and dried your crystals.

5. Explain what you used the cold hexane for when filtering the phthalic anhydride and argue, based on what you observed, whether it could be replaced with water or with ethanol.

6. With the yield you obtained, argue whether it would make sense to replace the concentrated H_2SO_4 with dilute H_2SO_4 in your esterification. Answer based on what you observed, not just on theory.

7. Choose the variable you would change first if you repeated the experiment —reflux time, excess of ethanol, method of removing water, or recrystallization solvent— and justify your choice with your own data.

IX. References.

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X. Appendix: Reagent Preparation

10% NaOH solution

Weigh 100 g of sodium hydroxide (NaOH) and pour into a 1000 mL volumetric flask containing 500 mL of distilled water and kept in an ice bath; stir the solution gently and slowly fill the flask to a final volume of 1000 mL (1 L). The solution releases heat.

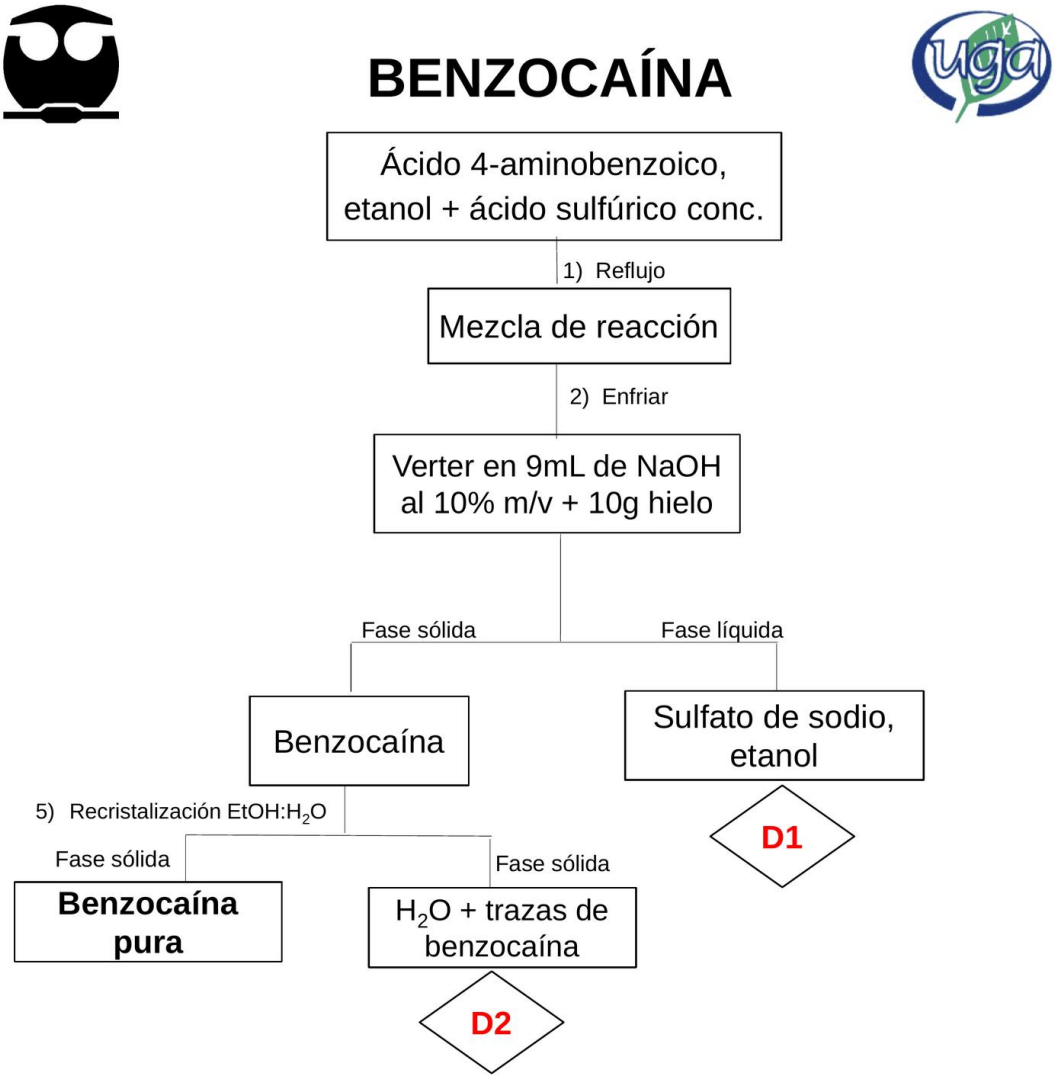
15% Sodium bicarbonate solution (1 L)

Weigh 150 g of sodium bicarbonate ($NaHCO_3$) and pour into a 1000 mL volumetric flask containing distilled water, slowly dissolve the salt until homogeneous, then fill to the mark to a final volume of 1000 mL (1 L).

Brine solution (saturated NaCl) (1 L)

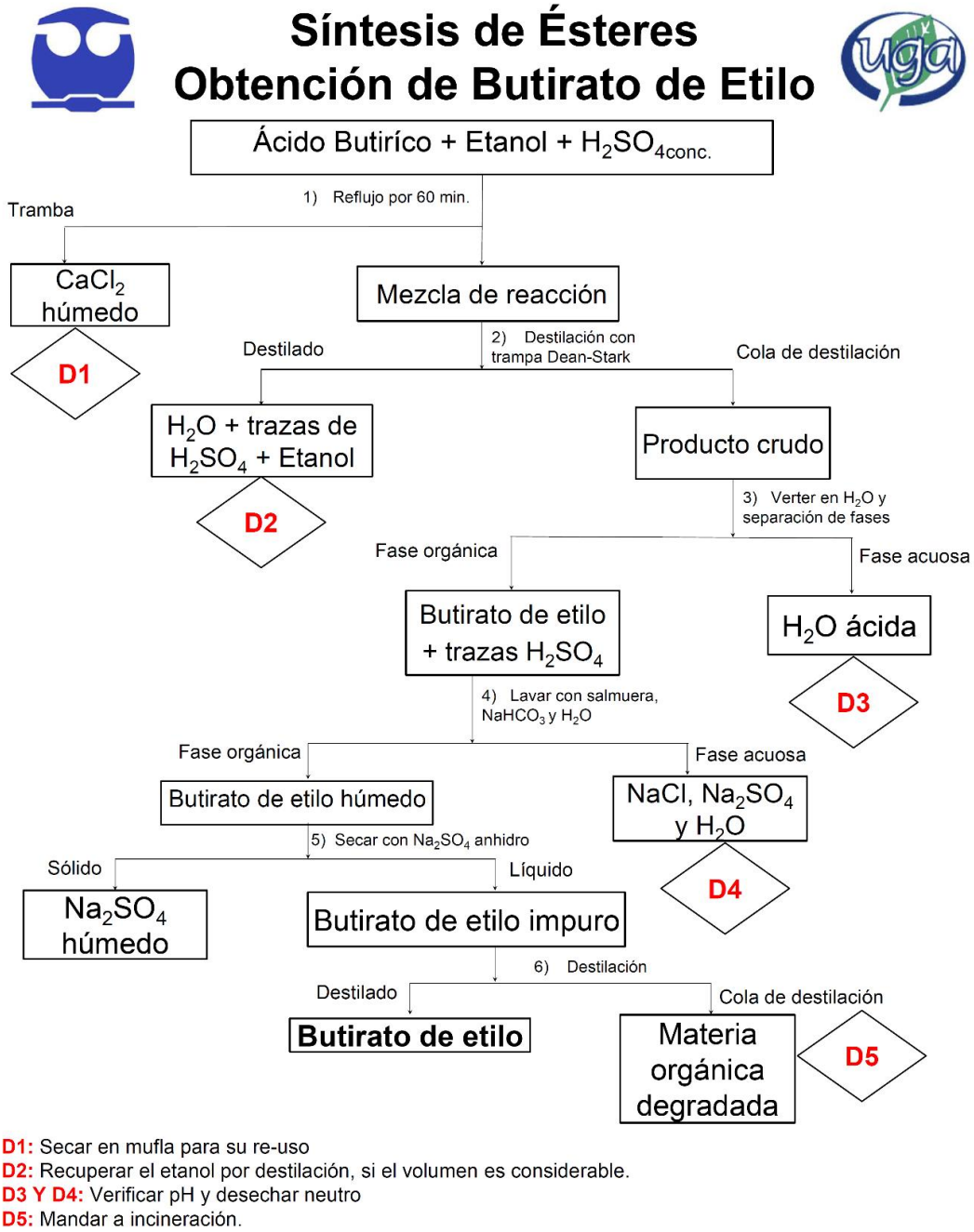
Weigh 400 g of sodium chloride (NaCl) and add them to 1 L of distilled water with constant stirring. Undissolved salt must remain at the bottom of the container: that is the sign that the solution is saturated. Decant or filter the supernatant at the time of use.

XI. Waste Management.



D1: Desechar la disolución por el drenaje si presenta un pH neutro. En caso contrario, neutralizar primero y luego desechar .
D2: Adsorber sobre carbón activado e incinerar. La fase sólida se neutraliza y desecha.

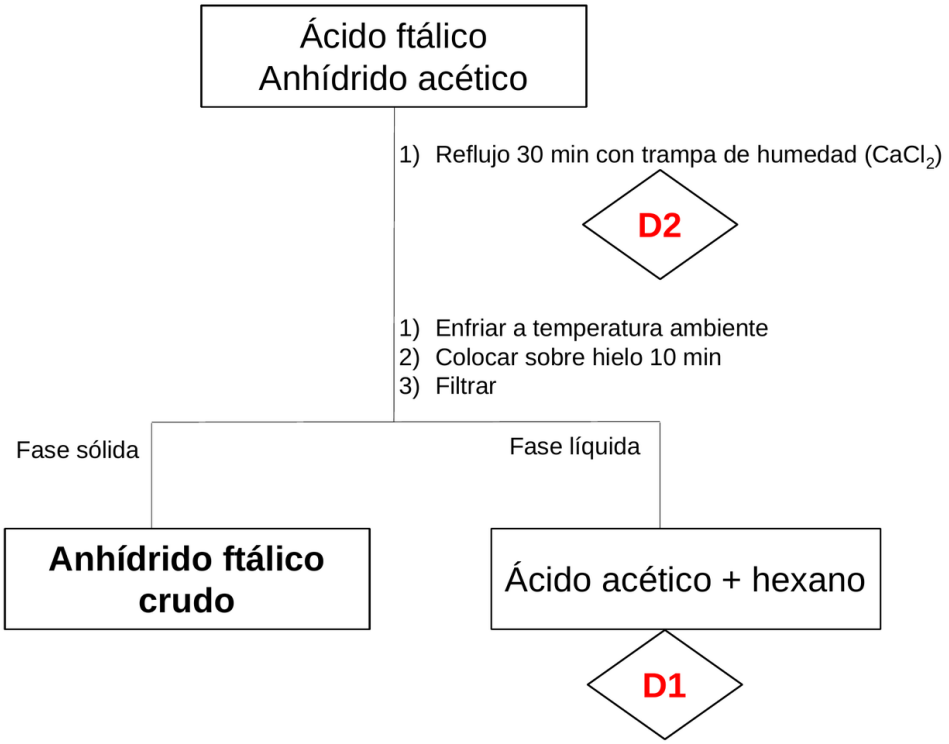
Scheme 3. Flow diagram of the benzocaine synthesis and its waste management.



D1: Secar en mufla para su re-uso
D2: Recuperar el etanol por destilación, si el volumen es considerable.
D3 Y D4: Verificar pH y desechar neutro
D5: Mandar a incineración.

Scheme 4. Flow diagram of the ethyl butyrate synthesis and its waste management.

ANHÍDRIDO FTÁLICO



D1: Recuperar el hexano por densidad y verificar el pH al residuo. Neutralizar y desechar por el drenaje.
D2: Se reserva para su reutilización o se envía a incineración.

Scheme 5. Flow diagram of the phthalic anhydride synthesis and its waste management.

EXPERIMENT 4

CARBOXYLIC ACIDS III

SYNTHESIS OF BENZOIC ACID VIA THE GRIGNARD REACTION

I. Objectives.

a) To prepare a Grignard reagent, phenylmagnesium bromide, from bromobenzene and magnesium metal under strictly anhydrous conditions.

b) To carboxylate the organomagnesium compound with solid carbon dioxide to obtain benzoic acid.

c) To apply an acid-base separation to isolate the product from the reaction mixture.

d) To recognize the experimental conditions demanded by the handling of an organometallic compound sensitive to water and oxygen.

II. Introduction.

Organometallic compounds are molecules containing at least one direct bond between a carbon atom and a metal. The difference in electronegativity between the two polarizes that bond so that the carbon carries negative electron density: it behaves as a carbanion. This inverts the usual polarity of a carbon bonded to a halogen, which in an alkyl halide is electrophilic, and that is why organometallics are one of the most powerful tools for forming carbon-carbon bonds.

Victor Grignard described in 1900 the preparation of organomagnesium compounds by insertion of magnesium metal into the carbon-halogen bond, work for which he received the Nobel Prize in Chemistry in 1912. The Grignard reagent reacts with virtually any electrophile bearing an electron-deficient carbon: aldehydes and ketones to give alcohols, esters to give tertiary alcohols, nitriles to give ketones and, as in this experiment, carbon dioxide to give carboxylic acids with one more carbon than the starting halide.

The counterpart of that reactivity is its fragility: the carbanion is a very strong base and reacts immediately with any proton source, including ambient moisture. Hence all the glassware must be dry, anhydrous solvents must be used —diethyl ether or tetrahydrofuran, which additionally stabilize the magnesium through coordination— and the system must be protected with moisture traps throughout the formation of the organometallic.

III. Pre-lab Research.

1. Propose the mechanism of formation of phenylmagnesium bromide and that of its carboxylation with CO_2, indicating the nucleophile and the electrophile at each step.

2. The Grignard reagent reacts with water. Write that reaction and predict how it would show up in your system if the glassware were not dry.

3. Explain the role of the iodine crystal in initiating the reaction and why the bromobenzene is first added in a small volume and without stirring.

4. Justify the use of dry ice instead of gaseous CO_2, considering moisture, stoichiometry, and temperature control.

5. The workup extracts the ether phase with 5% NaOH and then acidifies the aqueous phase. Using the structures involved and their pKa values, explain the phase change of the product at each step and predict which impurities remain in the ether.

6. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents and products —paying attention to the flammability of diethyl ether and THF— and complete the table in section VI based on the balanced reaction.

IV. Reagents.

Reagents	Amount	Reagents	Amount
Magnesium turnings	0.15 g	Iodine (crystal)	0.1 g
Bromobenzene	0.65 mL	Anhydrous tetrahydrofuran	4 mL
Solid carbon dioxide	10 g	Diethyl ether	20 mL
5% H_2SO_4 solution	5 mL	5% NaOH solution	15 mL
Concentrated hydrochloric acid	3 mL	Silica gel with indicator	15 g

The silica gel with indicator is collected at the end of the session: if it is pink it contains moisture and must be dried in the oven at 100 °C for one hour; if it is blue it is anhydrous and ready for use.

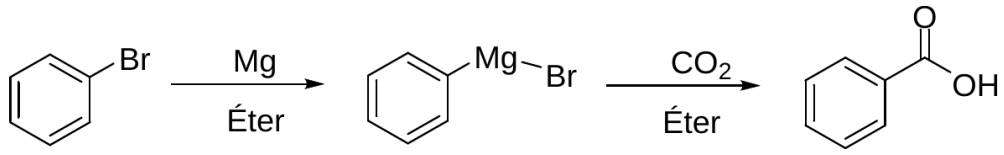
V. Materials.

In addition to the kit, request the following equipment by requisition slip (papeleta). Items marked with (†) are delivered oven-dried and must be kept under anhydrous conditions: do not rinse them or leave them uncovered.

Equipment	Qty.	Equipment	Qty.
Two-neck flat-bottom boiling flask, 25 mL (†)	1	Addition funnel with stopper (†)	1
Moisture trap (†)	2	Water-cooled condenser 14/23 (†)	1
125 mL Erlenmeyer flask	1	50 mL separatory funnel with stopper	1

Equipment	Qty.	Equipment	Qty.
Powder funnel	1	5 mL graduated pipette (†)	1
Vial (†)	1		

VI. Reaction and Stoichiometric Relationship



Reaction: Benzoic acid	Bromobenzene	Mg	CO_2	Benzoic acid
Molar mass (g/mol)				
Mass (g)				
Volume (mL)				
Density (g/mL)				
Amount of substance (mmol)				
Chemical equivalents				
Physical properties				

VII. Procedure.

Diethyl ether and THF: They are extremely flammable. Work with the fume hood on, with no ignition source nearby, and keep the solvents cold to reduce their volatility.

Concentrated H_2SO_4 and HCl: They are corrosive. In case of contact, wash with plenty of water for 3-5 minutes and rinse with 10% $NaHCO_3$ solution. Dry ice causes cold burns: handle it with tongs or thermal gloves, never with bare hands.

A) Formation of phenylmagnesium bromide

Prepare the two moisture traps: place a small plug of cotton, then silica gel with indicator —it must be blue— and another plug of cotton. Use the spatula and the powder funnel to help you.

In the 25 mL two-neck boiling flask, with a magnetic stir bar, place 0.15 g of magnesium turnings, one iodine crystal, and 0.5 mL of anhydrous THF. On the angled neck fit the addition funnel with its stopcock closed and, on top of it, a moisture trap; on the straight neck fit the condenser in reflux position and, at its top, the second trap (**Figure 2**).

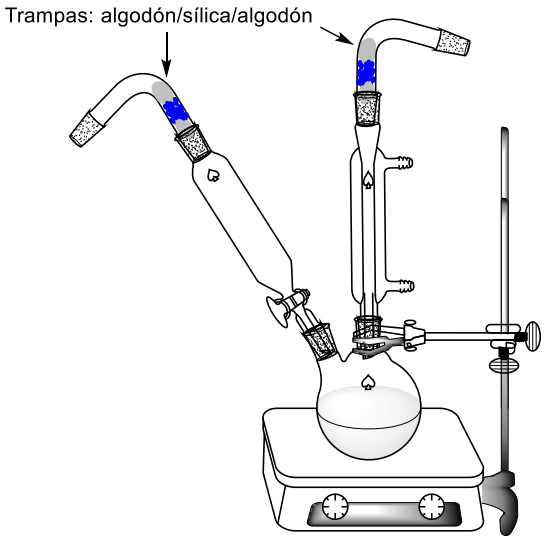


Figure 2. Setup for the formation of the Grignard reagent under anhydrous conditions.

Place 0.65 mL of bromobenzene in the addition funnel and immediately cap it with the trap. Let about 0.1 mL drip into the flask, **without stirring**, to achieve a high local concentration that initiates the reaction. You will know it has started because the iodine color disappears and a sustained boiling appears.

If the reaction does not start, you can induce it by adding another iodine crystal, heating gently with continuous stirring, rubbing the iodine crystal against the magnesium on the flask wall with a circular motion, or adding a few drops from an already-initiated reaction.

Once initiated, add 3.5 mL of anhydrous THF to the addition funnel to dilute the remaining bromobenzene and add that solution dropwise, maintaining a gentle, continuous boil without letting it stop. If the boiling decreases noticeably, heat gently. The reaction is over when all the magnesium has disintegrated and the solution takes on a brown turbidity; if necessary, heat at reflux for 10 more minutes.

B) Carboxylation and isolation of benzoic acid

Carefully pour the Grignard reagent solution into the 125 mL Erlenmeyer flask containing 10 g of dry ice. At this stage the corresponding magnesium carboxylate is formed.

Once the carbon dioxide has been completely consumed, add 10 mL of diethyl ether and place the flask in a water bath. Slowly add 5% H_2SO_4 solution until the medium is acidic, controlling the addition because the process is exothermic.

Transfer the mixture to the 50 mL separatory funnel. Rinse the Erlenmeyer flask with another 10 mL of diethyl ether and add it to the funnel to ensure complete transfer of the

product. Shake carefully, venting frequently, and let the phases separate: discard the aqueous phase and keep the ether phase.

Extract the ether phase with 5% NaOH solution (3 × 5 mL) and check that the aqueous phases come out basic. Combine the aqueous fractions in a single container and place them in an ice-water bath.

Add hydrochloric acid until pH 1: a white precipitate of benzoic acid will form. Recover the solid by vacuum filtration with the Büchner funnel, wash it with ice-cold water, and let it dry to constant mass.

Weigh the product, calculate the yield, and determine its melting point. Assess the purity by comparing your experimental value with the one reported in the literature (121-123 °C).

VIII. Post-lab Questions.

1. Report your yield and your melting point. If the yield was below 50%, identify in which specific stage of your session the most product was lost and explain what you base that claim on.

2. Describe what your mixture looked like at the moment you considered the reaction initiated and how long it took to start. If you had to induce it, state which method you used and whether it worked.

3. When the addition was finished, was there unreacted magnesium left in your flask? Based on that, calculate what fraction of the bromobenzene may have actually been converted into the Grignard reagent.

4. Report the pH measured for your basic aqueous phases and the final pH at which your product precipitated. Explain what would have happened if you had stopped the acidification at pH 5.

5. Compare your experimental melting point with the reported range (121-123 °C) and decide whether your product requires an additional recrystallization. Justify with your own data.

6. In Experiment 1 you obtained benzoic acid by the haloform reaction. Compare both methods using the yields you obtained in each session and point out what practical advantage each route has.

IX. References.

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X. Appendix: Reagent Preparation

Preparation of glassware under anhydrous conditions

Place the glassware marked on the requisition slip inside the oven at 120 °C overnight or for a minimum of 4 hours.

Treatment of magnesium turnings

The magnesium turnings must be free of oxide (not black). If oxidized, they must be treated: 1) wash the turnings with petroleum ether or hexane; 2) wash with 2% hydrochloric acid until the magnesium is shiny; 3) wash with water until neutral, placing the magnesium in a Büchner funnel and flooding it with cold water, stirring without tearing the filter paper and opening the vacuum valve, or alternatively transferring the magnesium to a beaker with cold water, stirring and vacuum-filtering; 4) wash with cold acetone following the same procedure and place it in a container that can be sealed tightly; 5) dry under vacuum; 6) seal the container and keep it in a tightly closed desiccator until the moment of use. Oven drying is best avoided to prevent oxidation.

Silica with indicator and anhydrous calcium chloride

The anhydrous calcium chloride $CaCl_2$ or the silica with indicator must also be placed in the oven in a beaker, in an amount sufficient to fill 2 traps per student.

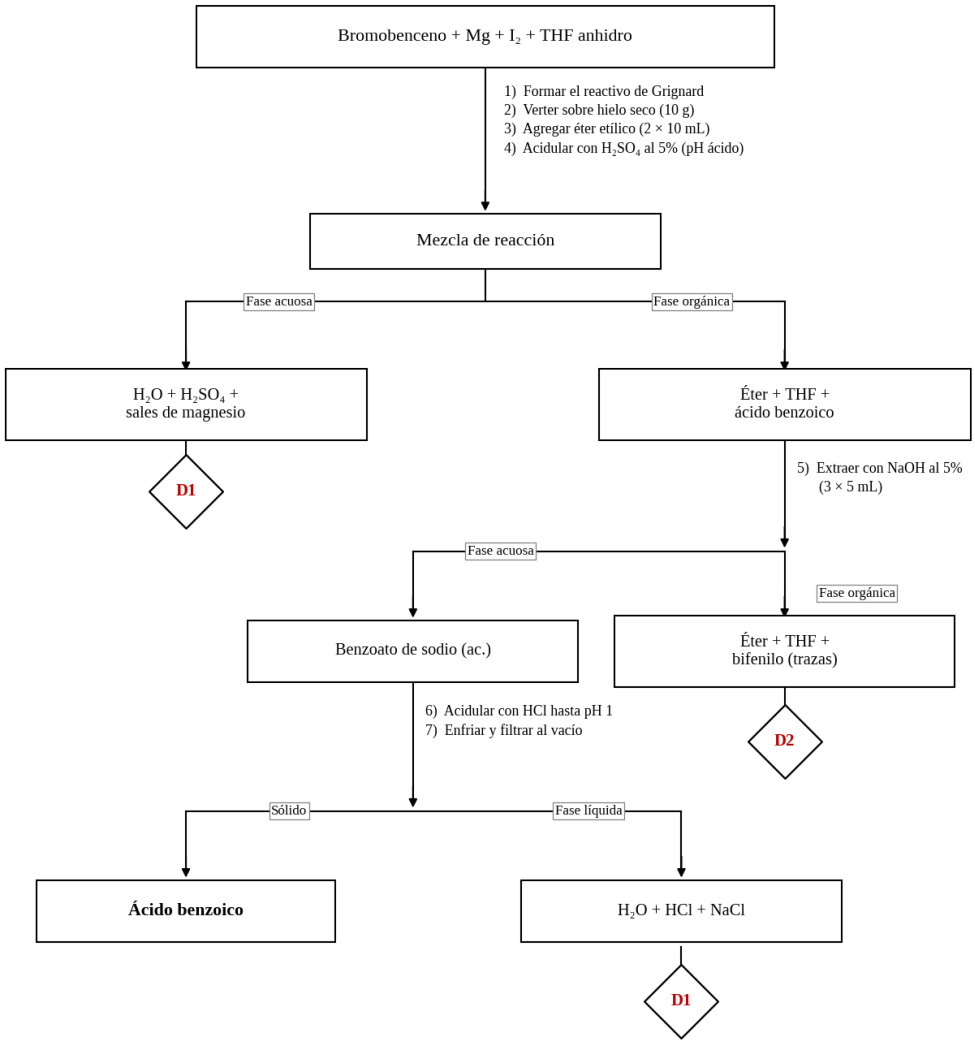
Preparation of 5% sulfuric acid

To 400 mL of distilled water, slowly add 19.5 mL of concentrated sulfuric acid down the walls of the container. **CAUTION!** The reaction is exothermic and spatters, so it should preferably be done over an ice bath. Finish by filling to 500 mL. Note: $\delta = 1.8$ g/mL for H_2SO_4.

10% ammonium chloride solution

To 300 mL of distilled water, add 50 g of ammonium chloride in portions and finish by filling to 500 mL.

XI. Waste Management.



Scheme 6. Flow diagram of the synthesis of benzoic acid via the Grignard reaction and its waste management.

EXPERIMENT 5

CARBOXYLIC ACID DERIVATIVES II
SYNTHESIS OF PARACETAMOL / FLUORESCEIN

I. Objectives.

a) To obtain an amide through the regioselective acetylation of a primary aromatic amine: paracetamol from 4-aminophenol and acetic anhydride.

b) To use an acid anhydride as the acylating agent in a double Friedel-Crafts acylation to synthesize fluorescein from phthalic anhydride and resorcinol.

c) To relate the delocalized π structure of fluorescein to its fluorescent properties and their pH dependence.

II. Introduction.

Acetylation is a substitution reaction in which an acetyl group (CH_3CO) is introduced by displacing the active hydrogen of a hydroxyl or an amino group. When the hydrogen of a hydroxyl is displaced, an ester is obtained; when that of an amine is displaced, an amide is obtained. The acetyl group also serves as a protecting group for hydroxyls and is widely used in the synthesis of non-selective COX-1 inhibitor drugs, such as aspirin and paracetamol, where it facilitates the drug's access to the cell membrane.

Friedel-Crafts acylation is one of the most important reactions in organic synthesis: it produces aromatic ketones from aromatic rings and acid halides or anhydrides, generally catalyzed by a Lewis acid, although it sometimes proceeds perfectly well in the presence of Brønsted acids, as is the case in this experiment. Anhydrides are the second most reactive carboxylic acid derivative, preceded only by acid halides.

Luminescence, a term introduced by Wiedemann in 1888, describes light-emission phenomena that are not conditioned on an increase in temperature; more precisely, it is the spontaneous emission of radiation from an electronically excited species. In fluorescence, the central role is played by *fluorophores*, the part of the molecule that absorbs energy of a specific wavelength and emits radiation of a longer wavelength: the electrons promoted to excited levels relax down to the LUMO and release the previously absorbed energy in the form of light.

Fluorescein is one of the best-known fluorescent pigments. It was first synthesized by Adolf von Baeyer in 1871, absorbs at $\lambda max = 494$ nm and emits at $\lambda max = 523$ nm, shows a strong pH dependence, and has applications ranging from medicine to the tracing of pollutants in rivers.

III. Pre-lab Research.

1. Propose the mechanism of acetylation of 4-aminophenol with acetic anhydride and explain, using nucleophilicity arguments, why the amino group is acetylated and not the hydroxyl.

2. Compare acetic anhydride, acetyl chloride, and acetic acid as acylating agents, and justify why this synthesis uses the anhydride.

3. Propose the mechanism of the double Friedel-Crafts acylation between phthalic anhydride and resorcinol, and explain why concentrated H_2SO_4 is sufficient as the catalyst without a Lewis acid.

4. Explain what structural requirements a molecule must meet to be fluorescent and which of them fluorescein exhibits but its starting materials do not.

5. Draw the fluorescein species that predominate in acidic and in alkaline medium, and predict in which one you will observe greater emission under UV light.

6. Look up what aggregation-caused quenching of emission is and predict what you will observe when comparing the concentrated sodium fluorescein solution with its 1:10 dilution.

7. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents and products, and complete the tables in section VI based on the balanced reactions.

IV. Reagents.

Reagents	Amount	Reagents	Amount
Paracetamol			
4-Aminophenol	0.4 g	Acetic anhydride	0.35 mL
Distilled water	15 mL	EtOAc:Hexane 9:1 (eluent)	10 mL
Fluorescein			
Phthalic anhydride	0.05 g	Resorcinol	0.07 g
Concentrated H_2SO_4	0.15 mL	NaOH solution 1 M	10 mL

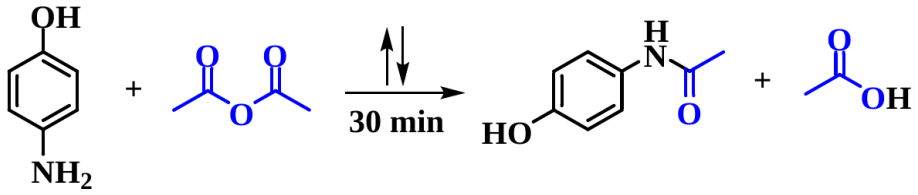
The phthalic anhydride may be the one your team synthesized and stored in Experiment 3.

V. Materials.

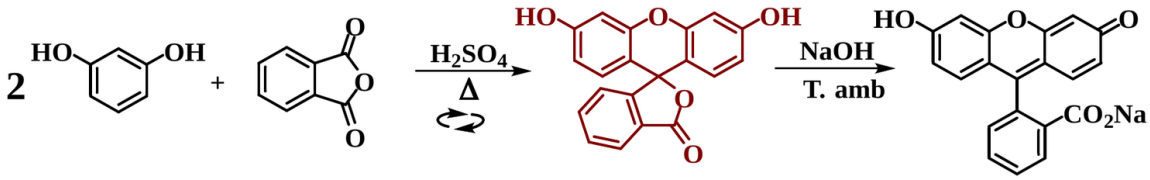
In addition to the kit, request the following equipment by requisition slip (papeleta).

Equipment	Qty.	Equipment	Qty.
10 mL flat-bottom boiling flask 14/23	2	1 mL graduated pipette	2
Test tube	2	Vial	1

VI. Reaction and Stoichiometric Relationship



Reaction 1: Paracetamol	4-Aminophenol	Ac_2O	Paracetamol
Molar mass (g/mol)			
Mass (g)			
Volume (mL)			
Density (g/mL)			
Amount of substance (mmol)			
Chemical equivalents			
Physical properties			



Reaction 2: Fluorescein	Resorcinol	Phthalic anhydride	Fluorescein
Molar mass (g/mol)			
Mass (g)			
Volume (mL)			
Density (g/mL)			
Amount of substance (mmol)			
Chemical equivalents			
Physical properties			

VII. Procedure.

A) Paracetamol

Acetic anhydride: It is corrosive and a lachrymator. Measure and transfer it exclusively inside the fume hood. In case of contact, wash with plenty of water for 3-5 minutes.

In the 10 mL flat-bottom boiling flask, with a magnetic stir bar, add 0.35 mL of acetic anhydride, 0.4 g of 4-aminophenol, and 0.5 mL of water. Fit the condenser in reflux position and keep the mixture at reflux with stirring for 30 minutes.

Let it cool slightly and place the flask in an ice bath for about 10 minutes, until the product precipitates. Vacuum-filter with the Büchner funnel and wash with cold water; set aside a portion of unrecrystallized paracetamol for its melting point.

Purify the rest by recrystallization from water. Crude paracetamol usually shows a slight violet color; if you consider it necessary, use activated carbon during the recrystallization. Vacuum-filter, dry, weigh, and calculate the yield; determine and compare the melting point of the crude against the recrystallized material.

Run a TLC plate comparing 4-aminophenol against your paracetamol, using EtOAc:hexane 9:1 as the eluent. Measure the Rf values of both spots.

B) Fluorescein

Concentrated H_2SO_4: It is corrosive and causes severe burns to skin and eyes. In case of contact, remove contaminated gloves and clothing, wash with plenty of water for 3-5 minutes, and rinse with 10% $NaHCO_3$ solution.

1 M NaOH: It is corrosive; handle it with care. In case of contact, wash with plenty of water for 3-5 minutes.

In the second 10 mL flat-bottom boiling flask, with a magnetic stir bar, add 0.05 g of phthalic anhydride and 0.07 g of resorcinol. Place the flask in an ice bath, take it to the fume hood, and add 3 drops of concentrated H_2SO_4 with a clean Beral pipette; let it cool for a couple of minutes.

Before heating, carefully place the reagent mixture inside the UV light chamber and observe whether the starting materials emit fluorescence on their own. Record what you see.

Heat on the hot plate with magnetic stirring until the solids melt and react with each other; you will obtain a reddish solid. Let it cool to room temperature, place the mixture back inside the UV light chamber, and observe whether it now emits fluorescence.

Dilute the cold mixture with 3 mL of water and 5 mL of 1 M NaOH solution until pH 8, and stir on the hot plate until completely homogeneous. Observe the sodium fluorescein solution again under UV light.

In the 30 mL beaker, prepare 10 mL of a **1:10** solution of sodium fluorescein in water. Transfer about 3 mL to a test tube and observe it inside the UV light chamber. Write down all your observations and compare them with those of the concentrated solution.

Note: rinse all the glassware with a small amount of 1 M NaOH solution before washing it, and place the rinse solution in the corresponding waste container.

VIII. Post-lab Questions.

1. Calculate your paracetamol yield and compare the melting points of the crude and the recrystallized material. If you used activated carbon, explain what changed in the appearance of your product and why.

2. Interpret your TLC plate: report the Rf of 4-aminophenol and that of your paracetamol, state which one eluted farther, and explain it in terms of polarity. Based on your plate, did unreacted starting material remain?

3. Describe, in the order in which you made them, the four observations under UV light: starting materials, molten mixture, alkaline solution, and 1:10 dilution. Explain each change based on the structure of the species present at that moment.

4. Based on the time it took for your mixture of phthalic anhydride and resorcinol to melt and react, argue how reactive acid anhydrides are compared with the esters you worked with in Experiment 3.

5. If you used the phthalic anhydride you synthesized yourself, comment on whether its melting point and appearance influenced the outcome of the fluorescein. If you used the commercial reagent, explain what difference you would expect.

6. Explain why fluorescein is a pH-dependent compound and point out, structurally and optically, two differences between fluorescein and rhodamine.

IX. References.

1. Carey, F. A.; Giuliano, R. M. Química Orgánica **2014**, McGraw Hill, 9th Edition, Mexico.

2. Williamson, K. Macro and Microscale Organic Experiments **2010**, Cengage Learning, 6th Edition, United States of America.

3. Ávila, G.; García, C. *et al.* Química Orgánica. Experimentos con un Enfoque Ecológico **2009**, Dirección General de Publicaciones y Fomento Editorial, UNAM, 2nd Edition, Mexico.

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5. Sauer, M.; Hopkens, J.; Enderlein, J. Handbook of Fluorescence Spectroscopy and Imaging **2011**, Wiley-VCH, 1st Edition, Weinheim, Germany.

6. Pay, A. L.; Kovash, C.; Loge, B. A. *J. Chem. Educ.* **2017**, 94(10), 1580-1583.

7. Burgess, K.; Ueno, Y.; Jiao, G. S. *Synthesis* **2004**, (15), 2591-2593.

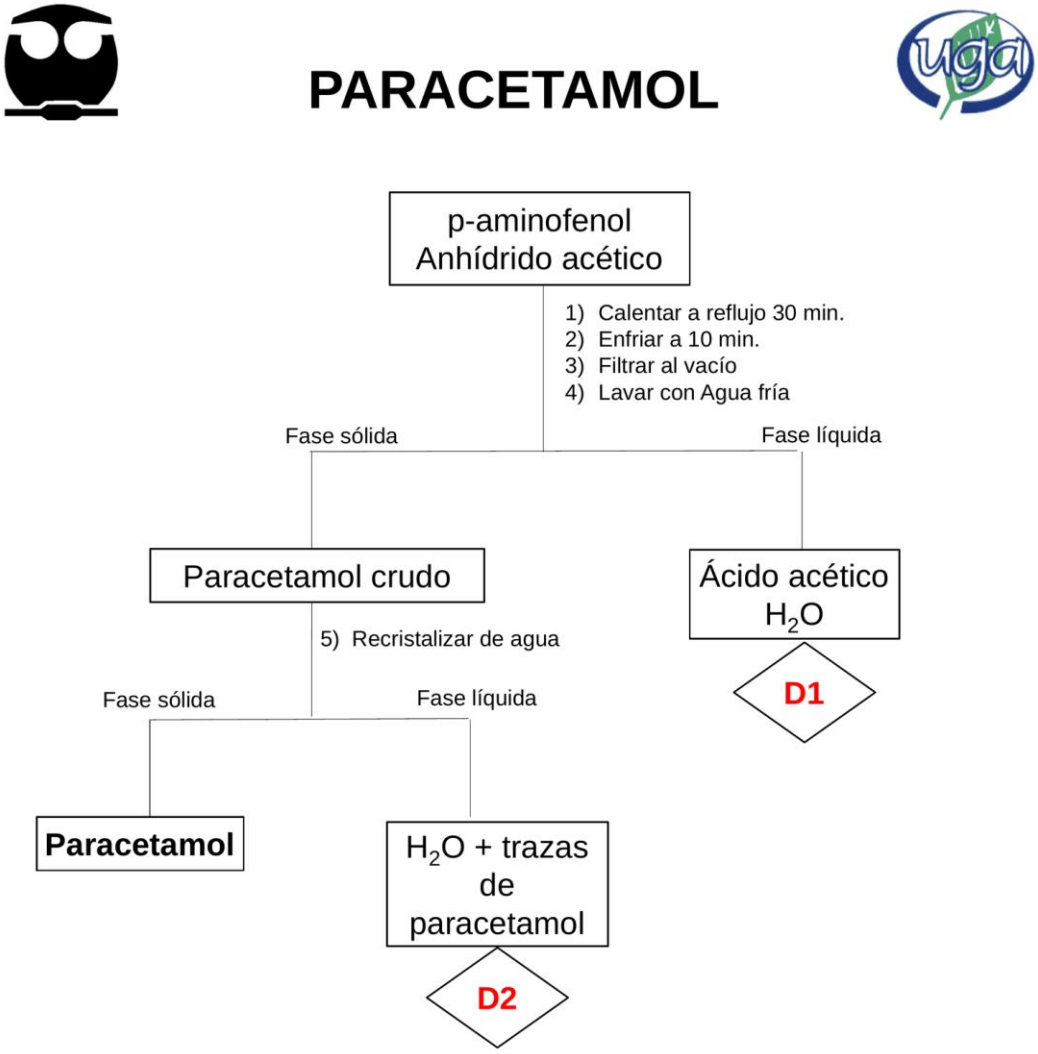
8. Mayo, D. W.; Pike, R. M.; Trumper, P. K. Microscale Organic Laboratory **1994**, 3rd Edition, John Wiley & Sons Inc., United States of America.

X. Appendix: Reagent Preparation

1 M NaOH solution (1 L)

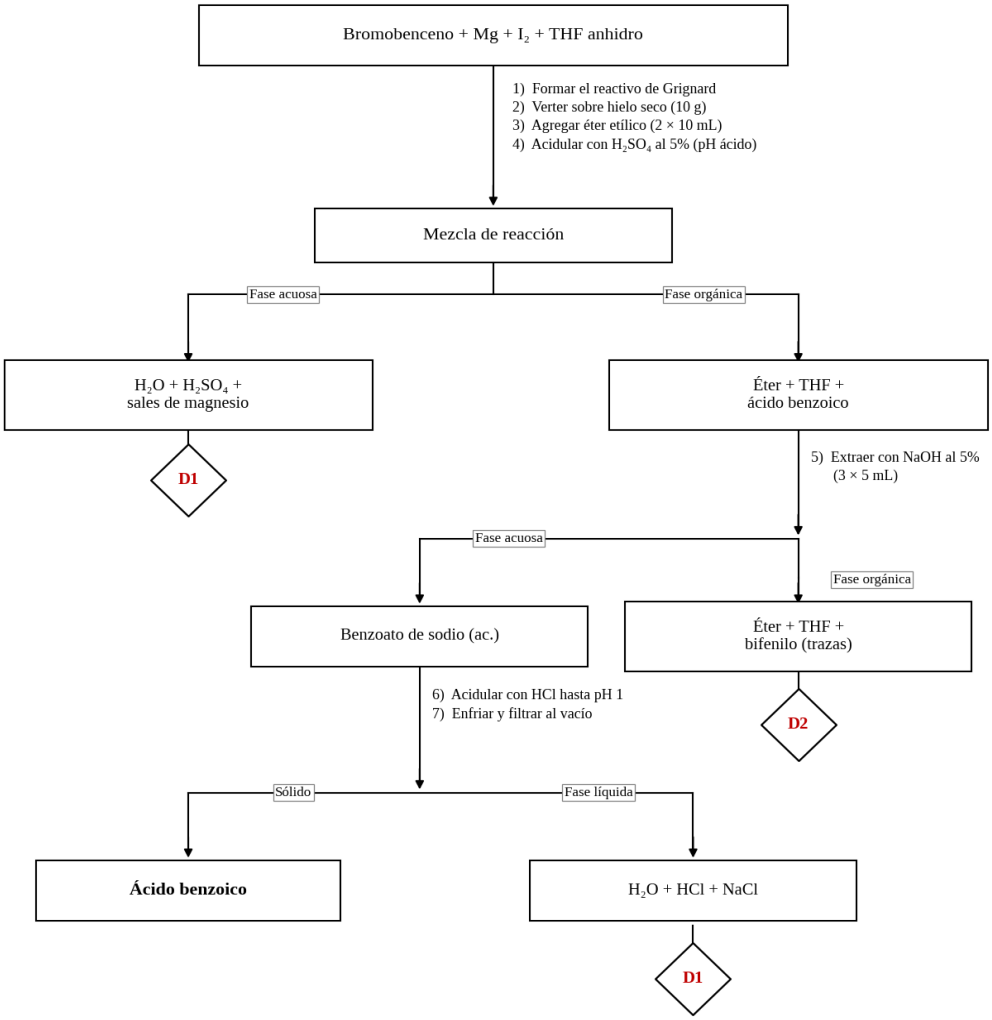
Weigh 40 grams of NaOH and place them inside a 1000 mL volumetric flask, set it in an ice bath, and fill to 1 L with distilled water. Stir gently once the solution is at room temperature and store.

XI. Waste Management.



D1: Neutralizar y desechar.
D2: Adsorber sobre carbón activado y enviar a incineración. La fase líquida se neutraliza y desecha.

Scheme 7. Flow diagram of the paracetamol synthesis and its waste management.



Scheme 8. Flow diagram of the sodium fluorescein synthesis and its waste management.

EXPERIMENT 6

LIPIDS I

ISOLATION OF TRIMYRISTIN

I. Objectives.

a) To isolate a triglyceride from a natural source by means of a continuous solid-liquid extraction in a Soxhlet system.

b) To purify the trimyristin obtained by precipitation with a solvent pair and assess its purity by melting point and thin-layer chromatography.

II. Introduction.

Lipids are a very diverse group of chemical compounds whose only common denominator is their solubility in nonpolar solvents, due to the presence of large hydrocarbon regions and the absence or scarcity of polar functional groups.

The vast majority of organisms use lipids as an energy reserve, since they are easily metabolized and generate a large amount of energy per mole. Mammals accumulate them as fats; fish and some insects, as waxes; plants, as oils, some even with very pleasant aroma and flavor. Phospholipids and sterols make up about half of biological membranes, and other lipids serve functions as enzyme cofactors, electron carriers, light-absorbing pigments, emulsifying agents, vitamins, or hormones.

Precisely because of their high solubility in nonpolar organic solvents and their almost complete insolubility in water, lipids can be easily separated from other biomolecules through extraction processes. The Soxhlet system takes that idea to the extreme: it recirculates the solvent continuously, so that the sample comes into contact again and again with clean, hot solvent, achieving an exhaustive extraction while using a much smaller volume of solvent than a batch extraction.

III. Pre-lab Research.

1. Draw the structure of trimyristin and identify the fatty acid it is composed of. Write down its molar mass and its reported melting point.

2. Explain the cycle of a Soxhlet system and why it extracts with “always clean” solvent. Predict what would happen to the yield if you stopped the extraction at the second cycle instead of the sixth.

3. Compare the polarity of dichloromethane and methanol, and explain why trimyristin dissolves in the former and precipitates when the latter is added. Propose another viable extraction solvent and justify your choice.

4. Nutmeg also contains aromatic essential oils. Predict whether they will be extracted along with the triglyceride and how their presence would show up in your product.

5. Review the physical, chemical, and toxicological properties (CRETIB) of the solvents used in this experiment.

IV. Reagents.

Reagents	Amount	Reagents	Amount
Ground nutmeg *	5-6 g	Dichloromethane	75 mL
Methanol	80 mL		

* The nutmeg must be provided by the student.

V. Materials.

In addition to the kit, request the following equipment by requisition slip (papeleta).

Equipment	Qty.	Equipment	Qty.
Complete Soxhlet apparatus	1	125 mL flat-bottom boiling flask	1
250 mL Erlenmeyer flask	1	100 mL Erlenmeyer flask	1
25 mL graduated cylinder	1	Distillation T adapter 14/23	1
Short receiving adapter 14/23	1	Ground-glass stopper 14/23	1
100 mL beaker	1	Clips	2

VI. Experimental Data.

Record the data of your extraction in the following table; you will need them to answer the post-lab questions.

Data	Experimental value
Mass of nutmeg used (g)	
Cycles completed in the Soxhlet	
Mass of trimyristin obtained (g)	
Extraction yield (% w/w)	
Experimental melting point (°C)	
Reported melting point (°C)	
Rf of trimyristin	

VII. Procedure.

Dichloromethane and methanol: They are volatile and harmful by inhalation. Work with the fume hood on and do not evaporate them on the benchtop. Methanol is additionally flammable and toxic by ingestion.

Grease all the joints of the Soxhlet system well. Place 75 mL of CH_2Cl_2 and a magnetic stir bar in the 125 mL flat-bottom boiling flask and assemble the extraction system.

Make a filter-paper thimble and fill it with approximately 5-6 g of nutmeg; weigh the nutmeg accurately and write down the value. Place the thimble inside the Soxhlet chamber, start the magnetic stirring, and bring the system to dichloromethane reflux. Allow at least 6 siphoning cycles to complete.

Carefully remove the thimble and discard it. If traces of solids remain in the flask, perform a gravity filtration before continuing.

Set up a simple distillation system and distill approximately 50 mL of dichloromethane, until the trimyristin solution is reduced to about 25 mL. Collect the distillate in the 100 mL Erlenmeyer flask immersed in an ice bath; that solvent is recovered for reuse.

Transfer the concentrated solution to the 250 mL Erlenmeyer flask, rinse the distillation flask with 5 mL of dichloromethane, and combine both fractions.

Measure 70 mL of cold methanol in the graduated cylinder and add it slowly, with gentle stirring, onto the concentrate. Let the flask stand in a container with ice until the solid forms. Vacuum-filter and wash the solid with 2 portions of 10 mL of dichloromethane:methanol 1:1.

Dry the solid and calculate the extraction yield relative to the initial mass of nutmeg. Determine the experimental melting point and run a TLC plate to verify its purity.

Label the product with the name, the date, and your team number, and store it: it will be your starting material for the next session.

VIII. Post-lab Questions.

1. Report your extraction yield in % w/w relative to the nutmeg used. Compare it with the trimyristin content reported for nutmeg and explain the difference.
2. Using your experimental melting point and your TLC plate, assess how pure your trimyristin turned out. If you observed more than one spot, propose what the additional spots are.
3. State how many cycles your system completed and at what point you noticed that the returning solvent was coming out practically colorless. Based on that, argue whether extending the extraction was worthwhile.

4. Explain how the dichloromethane and methanol used in this experiment could be separated and recovered for reuse.

5. Based on what you observed, propose a specific modification to the procedure that would increase your extraction percentage and justify why it would work.

IX. References.

1. Carey, F. A.; Giuliano, R. M. Química Orgánica **2014**, McGraw Hill, 9th Edition, Mexico.

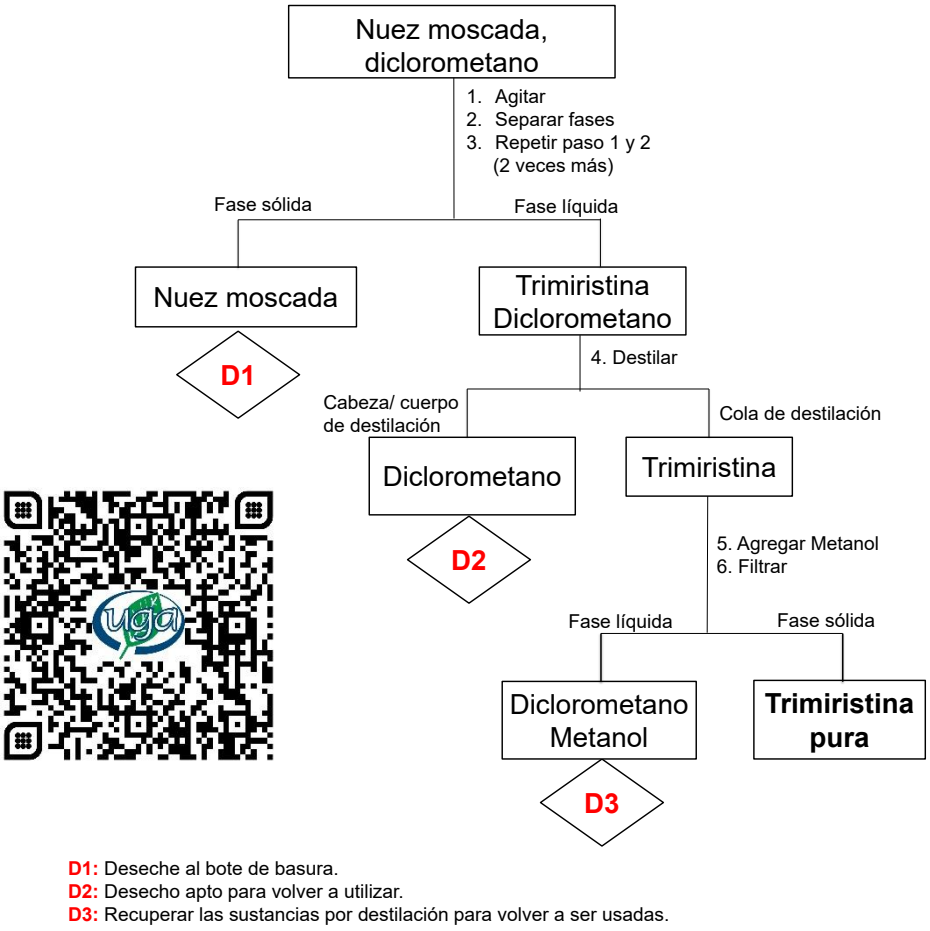
2. Doyle, P. M.; Mungall, W. Experimental Organic Chemistry **1980**, John Wiley & Sons, United States of America.

3. Ávila, G.; García, C. *et al.* Química Orgánica. Experimentos con un Enfoque Ecológico **2009**, Dirección General de Publicaciones y Fomento Editorial, UNAM, 2nd Edition, Mexico.

X. Appendix: Reagent Preparation

This experiment does not require prepared solutions: the solvents are used as received. The dichloromethane recovered in the distillation is stored, labeled, for reuse.

XI. Waste Management.



Scheme 9. Flow diagram of the trimyristin extraction and its waste management.

EXPERIMENT 7

LIPIDS II

SAPONIFICATION OF TRIMYRISTIN

I. Objectives.

a) To carry out an alkaline hydrolysis (saponification) reaction on a triglyceride.

b) To obtain myristic acid from the trimyristin isolated in the previous experiment and assess its purity.

II. Introduction.

The alkaline hydrolysis of triglycerides or of certain vegetable oils is commonly known as fat saponification, since the sodium or potassium salt of the corresponding fatty acid is formed, better known as soap; hence the name of the reaction, from the Latin *sapo* = soap.

The dissociation of fats in basic medium is an exothermic process; nevertheless, heat must be supplied to the system for the reaction to be completed in a reasonable time. Unlike acid hydrolysis, which is an equilibrium, saponification is irreversible: the carboxylate formed is no longer electrophilic and cannot be re-esterified under those conditions, which shifts the process completely toward the products.

The direct product of the reaction is the sodium carboxylate, soluble in the aqueous-alcoholic medium. To isolate the free fatty acid it is necessary to acidify: upon protonating the carboxylate, a long-chain acid is obtained that is practically insoluble in water, precipitates, and can be recovered by filtration.

III. Pre-lab Research.

1. Write the balanced saponification reaction of trimyristin and calculate the equivalents of NaOH relative to the triglyceride. Explain why more than one equivalent is required.

2. Propose the mechanism of the alkaline hydrolysis of an ester and explain why, unlike the acidic version, saponification is irreversible.

3. The mixture is acidified to pH 2-3. State which species predominates before and after acidifying, and predict what you would obtain if you stopped the addition at pH 8.

4. The recrystallization uses the methanol-water pair. Based on the structure of myristic acid, explain why methanol is a better choice than ethanol.

5. Identify the second organic product released by the reaction, state in which phase it remains after acidification, and explain how you would verify its presence.

6. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents and products, and complete the table in section VI based on the balanced reaction.

IV. Reagents.

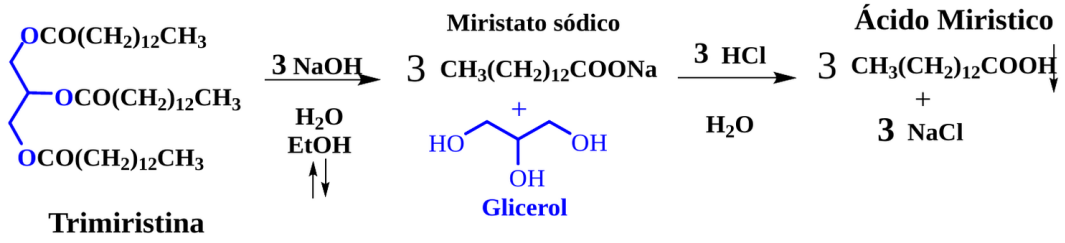
Reagents	Amount	Reagents	Amount
Trimyristin	0.5 g	Hexane	5 mL
Ethanol	10 mL	Methanol	10 mL
Diethyl ether	5 mL	HCl solution 20% v/v	10 mL
20% NaOH solution	2.5 mL	Distilled water	10 mL

The trimyristin is the one your team isolated and stored in Experiment 6.

V. Materials.

This experiment is carried out entirely with the kit glassware. No additional equipment needs to be requested by requisition slip (papeleta).

VI. Reaction and Stoichiometric Relationship



Reaction: Saponification	Trimyristin	NaOH	Myristic acid
Molar mass (g/mol)			
Mass (g)			
Volume (mL)			
Density (g/mL)			
Amount of substance (mmol)			
Chemical equivalents			
Physical properties			

VII. Procedure.

20% NaOH and 20% HCl: Both are corrosive. In case of contact, wash with plenty of water for 3-5 minutes; for the acid, rinse afterwards with 10% $NaHCO_3$ solution.

In the 25 mL flat-bottom boiling flask, with a magnetic stir bar, add 0.5 g of trimyristin, 10 mL of ethanol, and 2.5 mL of 20% m/v sodium hydroxide solution. Fit the condenser in reflux position and heat the mixture at reflux for 1 hour, to favor the complete conversion of the triglyceride into the soluble sodium salt.

When finished, pour the reaction mixture into the 50 mL beaker containing approximately 10 g of ice and 10 mL of 20% v/v hydrochloric acid solution. Stir vigorously for 10 minutes, let it stand, and check that the pH is 2-3.

Dilute with 10 mL of distilled water, vacuum-filter the solid formed with the Büchner funnel, and wash it with ice-cold water (3 × 2 mL). Set aside a portion of the crude for its melting point.

Purify the myristic acid by recrystallization with the methanol-water solvent pair. Vacuum-filter, dry, and weigh the product; calculate the yield of the reaction.

Determine the experimental melting point and compare it with that of the starting trimyristin.

Run a TLC plate to verify the purity of the product, comparing it with the starting material, using hexane:Et_2O 1:1 as the eluent; you may also use hexane:EtOAc 9:1. If necessary, develop the plate with I_2 vapors.

VIII. Post-lab Questions.

1. Report your yield and explain what specific experimental evidence from your session indicates that the solid you isolated is myristic acid and not unreacted trimyristin.

2. Compare the melting point of your product with that of the trimyristin you isolated in the previous experiment. Explain the difference between both values based on the structures.

3. Interpret your TLC plate: report the Rf values of trimyristin and myristic acid and state which one ran farther. Explain the result in terms of polarity.

4. If the hydrochloric acid solution had been 1% instead of 20%, predict what would have happened to your isolation and justify your answer with the pH you measured.

5. Describe two advantages of saponification over acid hydrolysis for this substrate, drawing on what you observed during the session.

IX. References.

1. Ávila, G.; García, C. *et al.* Química Orgánica. Experimentos con un Enfoque Ecológico **2009**, Dirección General de Publicaciones y Fomento Editorial, UNAM, 2nd Edition, Mexico.

2. Carey, F. A.; Giuliano, R. M. Química Orgánica **2014**, McGraw Hill, 9th Edition, Mexico.

3. Doyle, P. M.; Mungall, W. Experimental Organic Chemistry **1980**, John Wiley & Sons Inc., United States of America.

X. Appendix: Reagent Preparation

20% HCl solution (1 L)

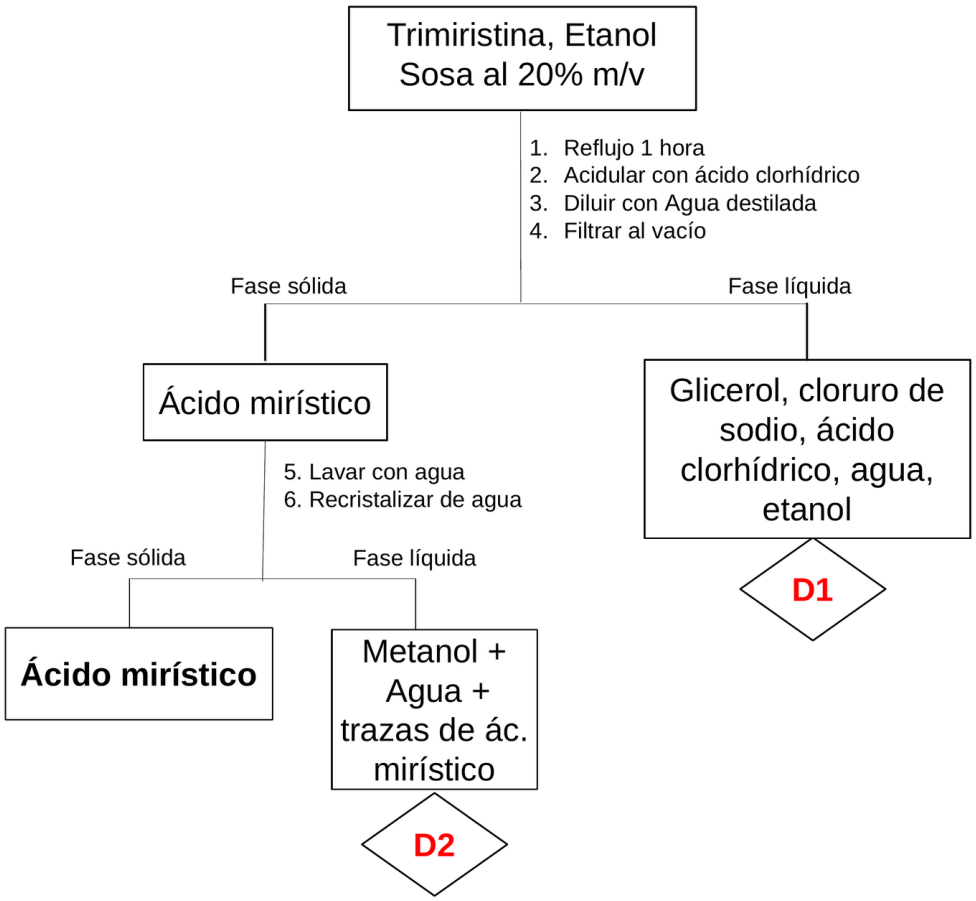
Using a graduated cylinder, measure 200 mL of concentrated hydrochloric acid (HCl) and add it slowly into a 1000 mL volumetric flask, then fill to the mark with distilled water; the volumetric flask must be kept in an ice bath. Stir gently until homogeneous and pour into the container where it will be stored. Important: do NOT reverse the order of addition; wear a lab coat, gloves, and safety glasses. The solution releases heat.

20% NaOH solution (1 L)

Weigh 200 g of sodium hydroxide (NaOH) and pour into a 1000 mL volumetric flask containing 500 mL of distilled water and kept in an ice bath; stir the solution gently and slowly fill the flask to a final volume of 1000 mL (1 L). The solution releases heat.

XI. Waste Management.

ÁCIDO MIRÍSTICO



D1: Si es necesario neutralice el ácido y emulsificar el glicerol.
D2: Neutralizar y desechar, debido a que el porcentaje de metanol es menor al 10%

Scheme 10. Flow diagram of the preparation of myristic acid and its waste management.

EXPERIMENT 8

AMINO ACIDS AND PEPTIDES I
SYNTHESIS OF HIPPURIC ACID

I. Objectives.

a) To exemplify the formation of an amide bond analogous to the peptide bond.

b) To apply the conditions of a Schotten-Baumann reaction to acylate the amino group of an amino acid.

c) To synthesize hippuric acid from glycine and benzoyl chloride, and assess its purity by melting point.

II. Introduction.

Amino acids are chemical compounds that bear a carboxylic acid group and an amino group within the same molecule; when the amino group sits on the α carbon they are called *α*-amino acids.

Each *α*-amino acid bears, on the carbon atom adjacent to the carboxyl, in addition to the amino group, a side chain *R* that is unique to each one. Having four different substituents on the C*α*, amino acids are chiral molecules with optical activity; the only exception is glycine, whose *R* is a second hydrogen atom. According to Fischer projections, all proteinogenic amino acids are *L*-amino acids.

Amino acids are the building blocks of proteins and intermediates of metabolism. For the human species there are 21 amino acids, of which the body can synthesize only 11; the rest must be ingested in the daily diet. The human body does not store amino acids for later use.

The Schotten-Baumann reaction allows amines to be acylated with acid chlorides in aqueous alkaline medium. The base fulfills two simultaneous functions: it keeps the amino group deprotonated so that it retains its nucleophilicity, and it neutralizes the HCl released in the acylation. The result is an amide bond formed under mild conditions, the same type of bond that links amino acids in a peptide.

III. Pre-lab Research.

1. Propose the mechanism of the reaction between glycine and benzoyl chloride, and explain why the amino group reacts and not the carboxylate.

2. Draw the glycine species that predominate at pH 1, 6, and 13, and explain why the reaction requires an alkaline medium to proceed.

3. Benzoyl chloride is also hydrolyzed by water. Write that competing reaction, explain why the synthesis works even in aqueous medium, and predict what by-product the excess benzoyl chloride generates.

4. The workup acidifies to pH 2 and then washes the solid with dichloromethane. Explain what is removed in each of those steps.

5. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents and products, and complete the table in section VI based on the balanced reaction.

IV. Reagents.

Reagents	Amount	Reagents	Amount
Glycine	0.3 g	NaOH pellets	1.0 g
Benzoyl chloride	0.5 mL	HCl solution 50% v/v	2 mL
40% NaOH solution	0.8 mL	Dichloromethane CH_2Cl_2	5 mL
Distilled water	12 mL		

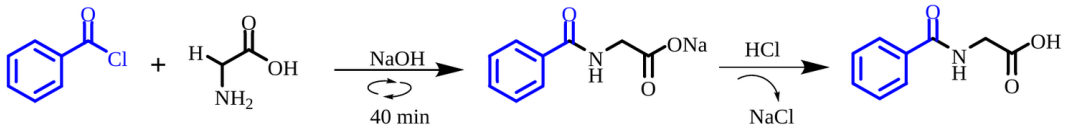
On the day of the experiment, the student must bring a 3 mL syringe with a needle.

V. Materials.

In addition to the kit, request the following equipment by requisition slip (papeleta).

Equipment	Qty.	Equipment	Qty.
10 mL flat-bottom boiling flask 14/23	1	Claisen adapter with rubber stopper	1
Microscale moisture trap 14/23	1		

VI. Reaction and Stoichiometric Relationship



Reaction: Hippuric acid	Benzoyl chloride	Glycine	NaOH 40%	Hippuric acid
Molar mass (g/mol)				
Mass (g)				
Volume (mL)				
Density (g/mL)				
Amount of substance (mmol)				
Chemical equivalents				
Physical properties				

VII. Procedure.

Benzoyl chloride: It is a lachrymator and corrosive. Handle it inside the fume hood. In case of contact, remove contaminated gloves and clothing, wash with plenty of water for 3-5 minutes, and take the person to fresh air for 3-5 minutes.

40% NaOH and 50% HCl: They are corrosive; handle them with care. In case of contact, wash with plenty of water for 3-5 minutes; for the acid, rinse afterwards with 10% $NaHCO_3$ solution.

Set up the system shown in **Figure 3**. In the 10 mL flat-bottom boiling flask, with a magnetic stir bar, add 0.3 g of glycine and 2 mL of water; stir until the glycine is completely dissolved and then add 0.5 mL of benzoyl chloride.

Fit the Claisen adapter: on one inlet place a moisture trap containing 4 NaOH pellets inside, and on the other a rubber stopper pierced by the syringe loaded with 1 mL of 40% NaOH solution.

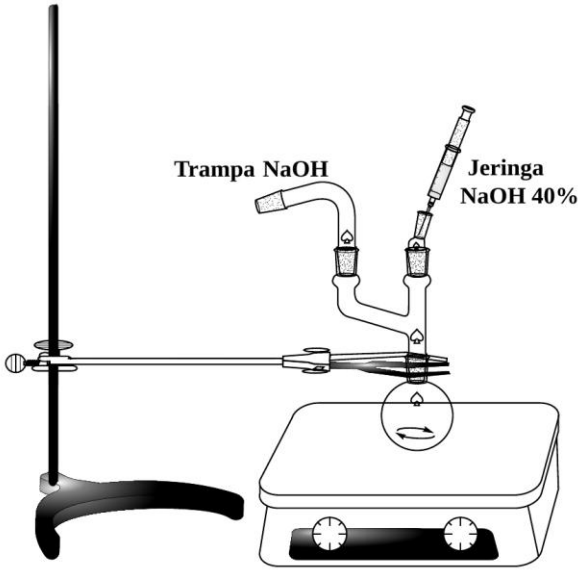


Figure 3. Setup for the synthesis of hippuric acid.

Add the 40% NaOH solution dropwise, with magnetic stirring and at room temperature. When the addition is finished you will observe two phases; keep stirring for 40 more minutes at room temperature. As the reaction progresses, the two phases will disappear to give a single one.

Pour the contents of the flask into the 30 mL beaker, place it in an ice bath, and add 2 mL of 50% HCl solution dropwise until pH 2.

Vacuum-filter the solid formed with the Büchner funnel, wash it with 10 mL of ice-cold distilled water to remove traces of HCl and then with 5 mL of dichloromethane. Set aside a portion of the crude product to determine its melting point.

Purify the product by simple recrystallization from water. Vacuum-filter, dry, and weigh the product; determine the melting point of the crude against the recrystallized material and calculate the experimental yield.

VIII. Post-lab Questions.

1. Report your yield and your two melting points (crude and recrystallized). Explain the difference between them in terms of purity.

2. Describe at what moment of your session the two phases disappeared and how long it took. Explain at the molecular level what changed in the system for that to happen.

3. Report the pH at which your product precipitated. Predict what you would have obtained if you had stopped the acidification at pH 6 and justify with the structure of hippuric acid.

4. The dichloromethane wash removes a by-product. State which one it is, explain why it dissolves in that solvent, and describe whether you noticed any change in the appearance of the solid after the wash.

5. Explain why the benzoyl chloride is added before the base and what would have happened if the additions had been reversed.

6. Look up from what natural source hippuric acid is obtained and what role it plays in the organism.

IX. References.

1. Ávila, G.; García, C. *et al.* Química Orgánica. Experimentos con un Enfoque Ecológico **2009**, Dirección General de Publicaciones y Fomento Editorial, UNAM, 2nd Edition, Mexico.

2. Carey, F. A.; Giuliano, R. M. Química Orgánica **2014**, McGraw Hill, 9th Edition, Mexico.

3. Williamson, K. Macro and Microscale Organic Experiments **2010**, Cengage Learning, 6th Edition, United States of America.

X. Appendix: Reagent Preparation

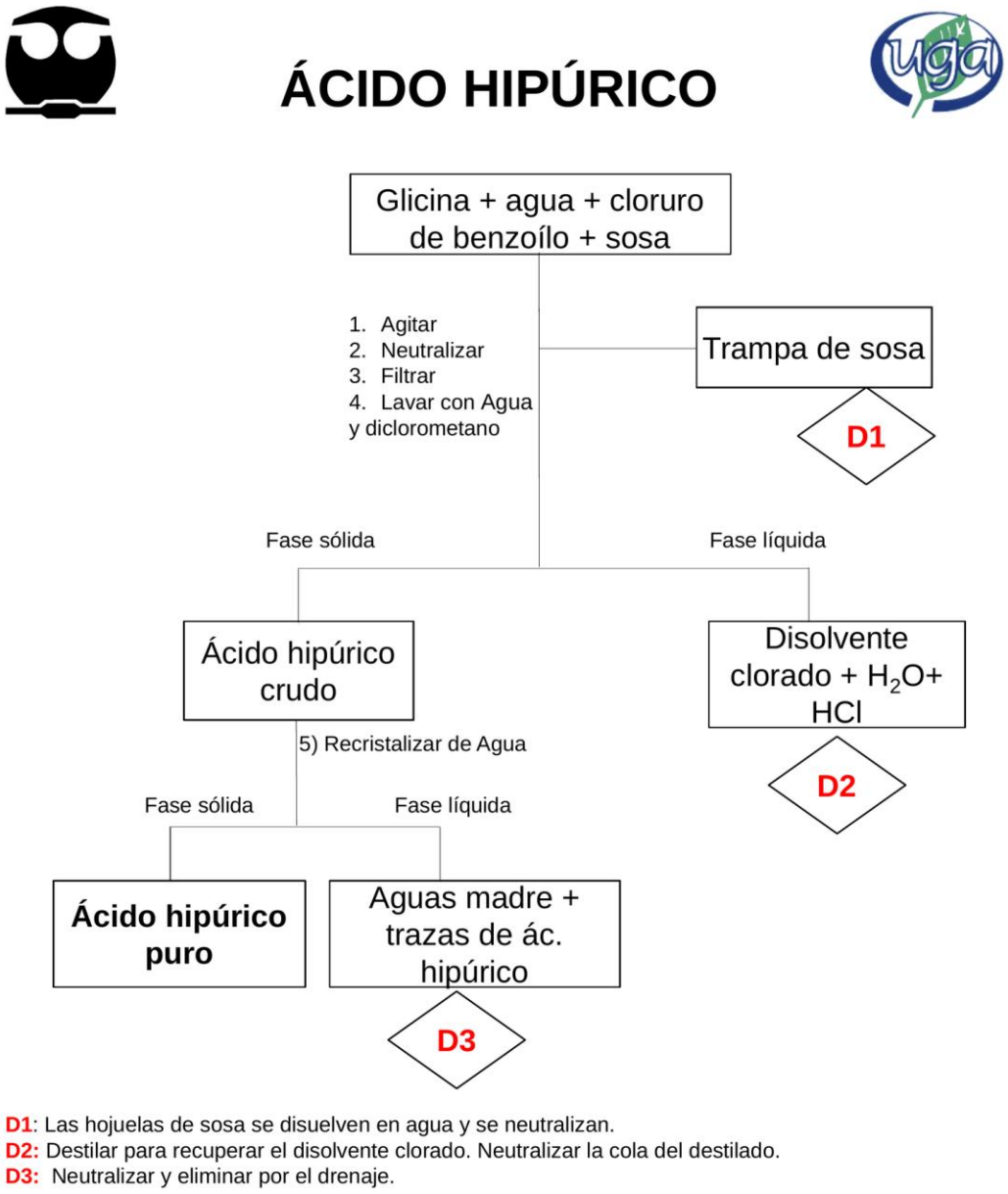
50% HCl solution (1 L)

Using a graduated cylinder, measure 500 mL of concentrated hydrochloric acid (HCl) and add it slowly into a 1000 mL volumetric flask, then fill to the mark with distilled water; the volumetric flask must be kept in an ice bath. Stir gently until homogeneous and pour into the container where it will be stored. ***Important***: *do NOT** reverse the order of addition; wear a lab coat, gloves, and safety glasses. The solution releases heat.

40% NaOH solution (1 L)

Weigh 400 g of sodium hydroxide (NaOH) and pour into a 1000 mL volumetric flask containing 500 mL of distilled water and kept in an ice bath; stir the solution gently and slowly fill the flask to a final volume of 1000 mL (1 L). ***Important***: The solution releases heat. Wear a lab coat, gloves, and safety glasses.

XI. Waste Management.



D1: Las hojuelas de sosa se disuelven en agua y se neutralizan.
D2: Destilar para recuperar el disolvente clorado. Neutralizar la cola del destilado.
D3: Neutralizar y eliminar por el drenaje.

Scheme 11. Flow diagram of the hippuric acid synthesis and its waste management.

EXPERIMENT 9

AMINO ACIDS AND PEPTIDES II

PSEUDO-SANGER REACTION AND IDENTIFICATION OF AMINO ACIDS

I. Objectives.

a) To exemplify the Sanger-type reaction for the identification of the amino terminus of a peptide chain.

b) To synthesize a 2,4-dinitrophenyl-amino acid derivative through an S_nAr reaction.

c) To qualitatively identify various amino acids through the reaction with ninhydrin.

d) To observe the presence of amino acids in fingerprint traces.

II. Introduction.

The amino acid sequence constitutes the primary structure of proteins, and knowing it is of great importance. Today there are automated and highly efficient methods for determining it, but this was not always the case: in 1945 Frederick Sanger proposed a reaction of great simplicity and incalculable relevance for its time, a sober nucleophilic aromatic substitution using 2,4-dinitrofluorobenzene on a peptide, followed by a mild acid hydrolysis. In this way, in 1953 he was able to determine the sequence of the 51 amino acids that make up insulin, work for which he received the Nobel Prize in Chemistry in 1958.

The amino acid identification in this experiment is based on the formation of a highly colored complex called Ruhemann's purple, which absorbs at 570 nm. It is a simple imine-forming reaction between an aromatic triketone, ninhydrin, which functions as the chromophore group, and the free amino group of amino acids. In the field of criminalistics this reaction is used for the search and detection of fingerprints, since the sweat of palms and fingers contains moderate amounts of certain amino acids.

III. Pre-lab Research.

1. Propose the mechanism of the nucleophilic aromatic substitution between 2,4-dinitrochlorobenzene and the amino acid. Explain the role of the nitro groups and why the reaction does not proceed with chlorobenzene.

2. The reaction uses 4 equivalents of K_2CO_3 and not NaOH. Justify the choice of base and the number of equivalents.

3. Propose the mechanism of the reaction between an amino acid and ninhydrin up to Ruhemann's purple, and indicate which fragment of the amino acid ends up in the chromophore.

4. Proline is a secondary amino acid. Based on the previous mechanism, predict whether it will give the same color as the other amino acids and explain why.

5. After the extraction with CH_2Cl_2 the product remains in the aqueous phase. Explain why and what is removed in the organic phase.

6. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents and products, and complete the table in section VI based on the balanced reaction.

IV. Reagents.

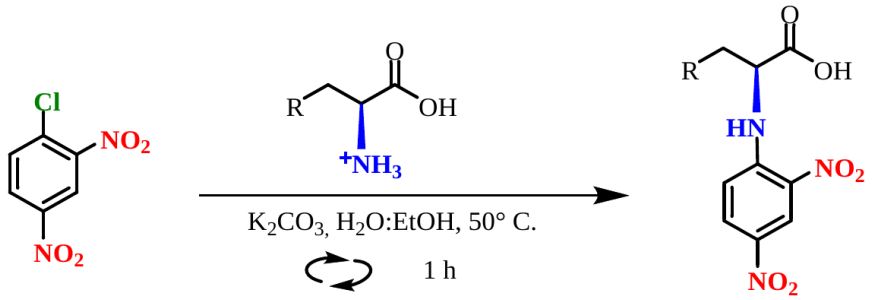
Reagents	Amount	Reagents	Amount
2,4-Dinitrochlorobenzene	0.4 g	Glycine or *L*-Phenylalanine	0.15 / 0.33 g
Potassium carbonate K_2CO_3	1.1 g	Dichloromethane CH_2Cl_2	30 mL
Ethanol	10 mL	Concentrated HCl	1.5 mL
0.2% ninhydrin solution	8 mL	Distilled water	10 mL
0.5% solutions of *L*-glutamic acid, *L*-phenylalanine, glycine, *L*-arginine, *L*-leucine, methionine, and *L*-proline	1 mL each		

V. Materials.

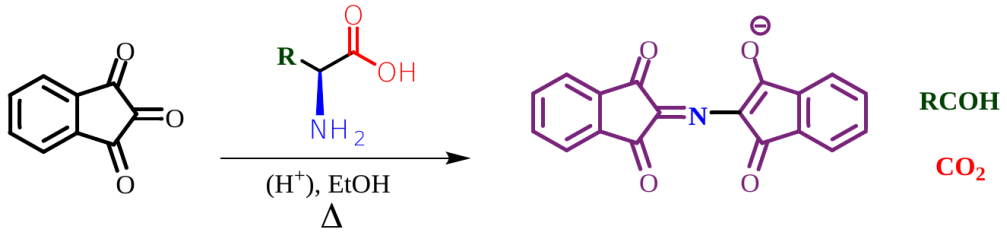
In addition to the kit, request the following equipment by requisition slip (papeleta).

Equipment	Qty.	Equipment	Qty.
Test tube rack	1	16 × 150 mm test tubes	10
Test tube holder	1	Air condenser 14/23	1
Thermometer	1	50 mL separatory funnel with stopper	1
150 mL beaker	1		

VI. Reaction and Stoichiometric Relationship



Reaction 1: Pseudo-Sanger	2,4-Dinitrochloro-benzene	Phe / Gly	K_2CO_3	DNB-AA product
Molar mass (g/mol)				
Mass (g)				
Volume (mL)				
Density (g/mL)				
Amount of substance (mmol)				
Chemical equivalents				
Physical properties				



Reaction 2. Formation of Ruhemann's purple between an amino acid and ninhydrin.

VII. Procedure.

A) Pseudo-Sanger reaction

2,4-Dinitrochlorobenzene: It is harmful by ingestion and by contact with skin and eyes. Wear gloves at all times. In case of skin contact, wash with plenty of water and soap; in case of eye contact, wash with plenty of water.

Concentrated HCl: It is corrosive; handle it with care. In case of contact, wash with plenty of water for 3-5 minutes and rinse with 10% $NaHCO_3$ solution.

Prepare a 50 °C water bath and place in it the 25 mL flat-bottom boiling flask with a magnetic stir bar (**Figure 4**). Add 0.33 g of *L*-phenylalanine or 0.15 g of glycine, as assigned to you, and 1.1 g of K_2CO_3 dissolved in 5 mL of distilled water. Stir for 3 minutes.

To that solution add 0.4 g of 2,4-dinitrochlorobenzene and 5 mL of ethanol, and attach the air condenser to the flask. Stir vigorously at 50 °C for 1 hour.

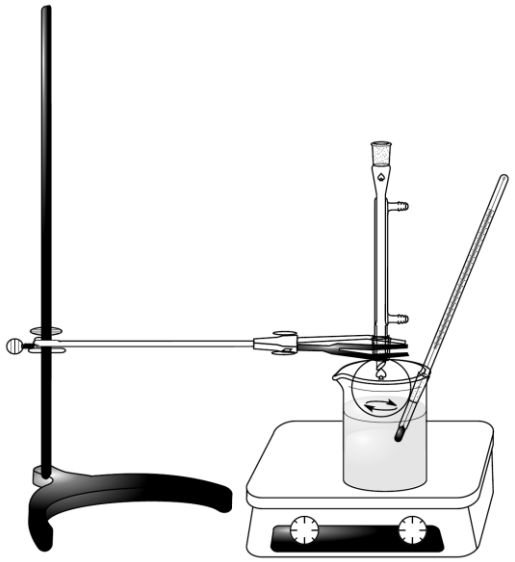


Figure 4. Setup for the pseudo-Sanger reaction.

Let it cool to room temperature, dilute the mixture with 5 mL of water, pour it into the 50 mL separatory funnel, and extract with CH_2Cl_2 (3 × 10 mL). The desired product remains in the aqueous phase.

Transfer the aqueous phase to the 50 mL Erlenmeyer flask, place it in an ice bath, and acidify with concentrated HCl to pH 1, until an amorphous yellow solid forms.

Vacuum-filter, remove the mother liquor from the Kitasato flask, and place it in the appropriate waste container. Dry the solid, weigh it, and determine its melting point (DNB-Phe: 185-186 °C; DNB-Gly: decomposes at 195-202 °C).

B) Identification of amino acids with ninhydrin

First prepare a blank: add 1 mL of water and 1 mL of ninhydrin solution to a test tube, and heat it for 1 minute in the water bath.

In each of the 7 remaining tubes place 1 mL of one of the available amino acid solutions (*L*-glutamic acid, *L*-phenylalanine, glycine, *L*-arginine, *L*-leucine, methionine, and *L*-proline)

and add 1 mL of ninhydrin solution. Shake by hand to homogenize and wait 5 minutes at room temperature; observe whether there is any change.

Then heat the tubes for 1-2 minutes in the water bath. Write down your observations for each amino acid, including the color and the time at which it appeared, and take photographs.

C) Development of fingerprints

On a piece of cardboard or paper draw two boxes. In box A) place a pair of fingerprints by removing your glove and pressing your fingerprint directly. In box B) rub a gloveless finger on the surface of your forehead or nose and press a pair of fingerprints again.

Put your gloves back on, spray the ninhydrin solution over both boxes, and let it dry for a couple of minutes. Gently heat the paper over the hot surface of the hot plate. Observe, take notes, and photograph both boxes.

VIII. Post-lab Questions.

1. Report your yield and the melting point of your DNB-amino acid derivative. Compare it with the value indicated in the procedure and decide whether your product is pure.
2. Organize in a table the results of the ninhydrin test for the 7 amino acids: color observed at room temperature, color after heating, and time of appearance. Point out which one gave a different color and explain it based on its structure.
3. Compare the two boxes of fingerprints you developed. Explain why fingerprints from oily areas develop with greater intensity and what that tells you about the composition of the fingerprint residue.
4. Based on the result you obtained, argue whether the reaction would proceed the same way in a slightly acidic medium and justify your answer with the mechanism.
5. The reaction is carried out in water with ethanol as a cosolvent. Explain what function each one fulfills and propose an alternative solvent, justifying your choice.

IX. References.

1. Sanger, F. *Biochemistry J.* **1945**, 39(5), 507-515.
2. Levy, A. L.; Chung, D. *J. Am. Chem. Soc.* **1955**, 2899-2900.
3. Friesen, J. B. *J. Chem. Educ.* **2015**, 92, 487-504.
4. Girod, A.; Ramotoswki, R.; Weyermann, C. *Forensic Sci. Int.* **2012**, 223(1-3), 10-24.
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X. Appendix: Reagent Preparation

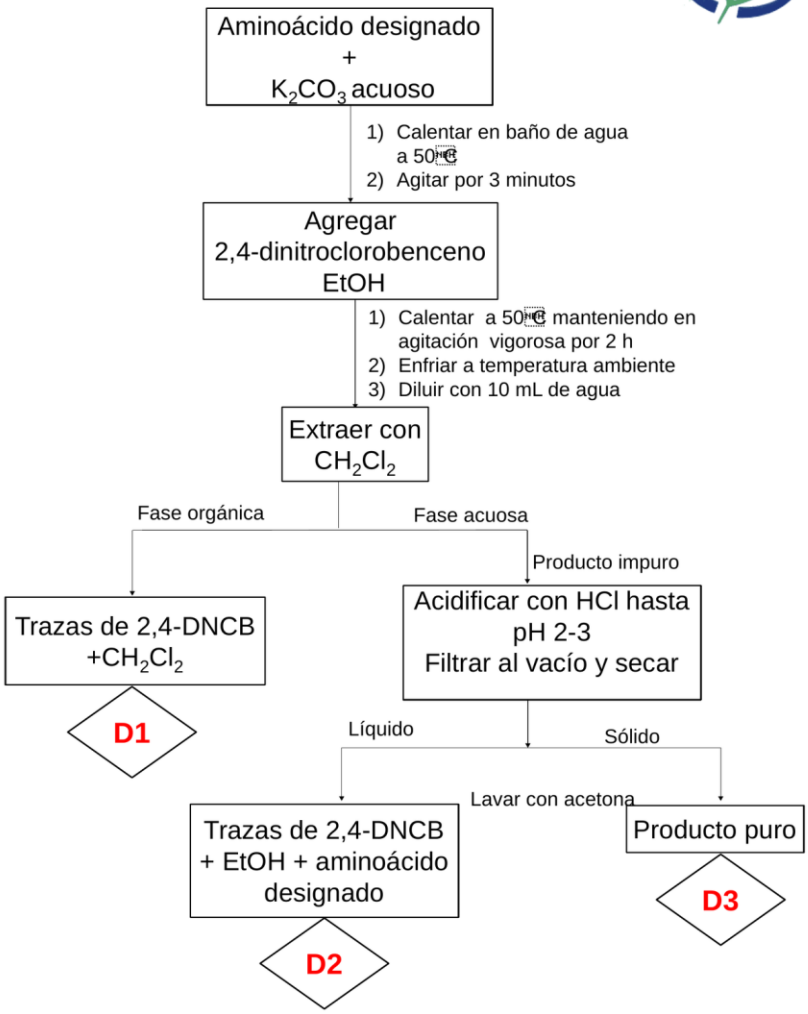
0.5% amino acid solutions (100 mL)

Weigh and place 0.5 g of the corresponding amino acid inside a 100 mL volumetric flask, add 50 mL of distilled water, stir gently to complete its dissolution, then fill to 100 mL with distilled water. Prepare these solutions no more than 1 week in advance since they are nutrient broths for microorganisms.

0.2% Ninhydrin solution (100 mL)

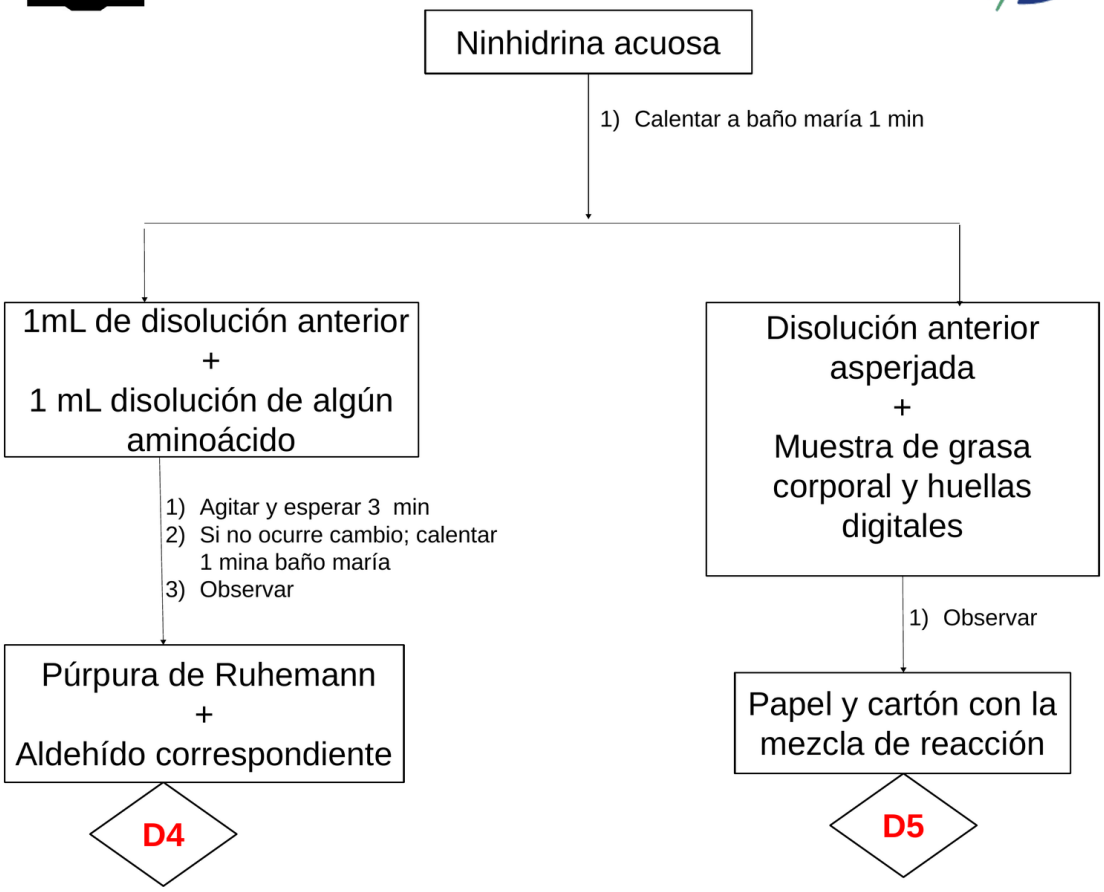
Weigh and place 0.2 grams of ninhydrin inside a 100 mL volumetric flask and add 60 mL of ethanol, then add 0.5 mL of acetic acid and 4.5 mL of distilled water, and fill to 100 mL. Shake and store in an amber container. *Note*: wear a lab coat and gloves when preparing this solution; ninhydrin stains the skin on contact.

XI. Waste Management.



D1, D3 y D4: Enviar a incineración.
D2: Si el contenido de EtOH es menor a 10% adsorber sobre carbón activado, enviar el sólido a incineración y desechar neutro. Si el contenido es mayor a 10% destilar el EtOH para recuperarlo y efectuar lo antes descrito.

Scheme 12. Flow diagram of the synthesis of the 2,4-dinitrophenyl-amino acid derivative and its waste management.



D5: Adsorber con carbón activado y enviar a incineración, la disolución se neutraliza y se desecha.
D6: Desechar en la charola de papel filtro correspondiente para su posterior incineración.

Scheme 13. Flow diagram of the identification of amino acids with ninhydrin, the development of fingerprints, and their waste management.

EXPERIMENT 10

CARBOHYDRATES I

ANALYSIS AND IDENTIFICATION OF CARBOHYDRATES

I. Objectives.

a) To identify different types of carbohydrates through simple chemical tests.

b) To differentiate a pentose from a hexose, an aldose from a ketose, and a disaccharide from a monosaccharide, using characteristic reactions.

c) To analyze the different reactivities of mono- and disaccharides toward the typical identification reactions, including the recognition of false positives.

II. Introduction.

Carbohydrates are organic molecules made up of carbon, hydrogen, and oxygen, generally in a hydrogen:oxygen ratio of 2:1, the same as in water; in other words, they have an empirical formula $Cx(H_2O)y$. Structurally they are considered polyhydroxyaldehydes and polyhydroxyketones.

Another more widely used term is saccharides, which are divided into four main groups: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. The word saccharide comes from the Greek *sacaron* (sweet). The names of mono- and disaccharides generally end with the suffix ***-ose***: grape sugar is the most widely distributed monosaccharide in nature, glucose; cane sugar is the disaccharide sucrose; and the milk sugar of mammals is the disaccharide lactose.

Carbohydrates play countless roles in living organisms: polysaccharides serve as energy stores (starch and glycogen) and as structural components (cellulose in plants and chitin in arthropods). Saccharides and their derivatives also participate in the immune system, fertilization, prevention of pathogenesis, human development, and blood group factors.

The four tests in this experiment exploit fine differences in reactivity. Barfoed and Benedict are based on the reduction of Cu^{2+} to Cu^{+}, but they differ in the pH of the medium and, with it, in their selectivity. Seliwanoff and Bial are based on acid-catalyzed dehydrations that generate furfurals, which condense with a phenol to give a colored product. In all cases, time and temperature are critical variables: prolonged heating produces false positives.

III. Pre-lab Research.

1. Define what a reducing sugar is and identify the anomeric carbon. With that definition, predict which carbohydrates in this experiment will give a positive Benedict's test.

2. Barfoed and Benedict are based on the same reduction of Cu^{2+} to Cu^{+}, but only one is selective for monosaccharides. Compare the composition and pH of both reagents and explain that difference in selectivity.

3. Propose the mechanism of the Lobry de Bruyn-van Ekenstein rearrangement in alkaline medium and explain how it allows a ketose to give a positive result in a test designed for aldoses.

4. Explain the basis of the Seliwanoff and Bial tests —which intermediate is formed and with which phenol it condenses—, predict the color of each positive result, and explain why prolonged heating generates false positives.

5. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents used, paying attention to those containing concentrated HCl.

IV. Reagents.

Reagents	Amount	Reagents	Amount
5% arabinose solution	2 mL	5% ribose solution	2 mL
5% fructose solution	2 mL	5% sucrose solution	2 mL
5% galactose solution	2 mL	Barfoed's solution	15 mL
5% glucose solution	2 mL	Benedict's solution	10 mL
5% lactose solution	2 mL	Bial's solution	15 mL
5% maltose solution	2 mL	Seliwanoff's solution	15 mL
5% mannose solution	2 mL		

V. Materials.

In addition to the kit, request the following equipment by requisition slip (papeleta).

Equipment	Qty.	Equipment	Qty.
Test tube rack	1	Test tubes	30
Test tube holder	2	400 mL beaker	1

VI. Recording of Results.

Compile the results of the four tests in this table. For each cell, write down the result (positive, negative, or false positive) and the time at which the change appeared.

Carbohydrate	Barfoed	Benedict	Seliwanoff	Bial
Arabinose				
Ribose				
Fructose				
Glucose				
Galactose				
Mannose				
Lactose				
Maltose				
Sucrose				

VII. Procedure.

Bial's and Seliwanoff's reagents: They contain concentrated hydrochloric acid and are corrosive. Handle them with gloves and safety glasses. In case of contact, wash with plenty of water for 3-5 minutes and rinse with 10% $NaHCO_3$ solution.

a) Barfoed's test — identification of monosaccharides

$$RCHO + 2\,Cu^{+2} + 2\,H_2O \longrightarrow RCOOH + Cu_2O\downarrow + 4\,H^+$$

In a test tube add 0.5 mL of the corresponding sugar solution and 1.5 mL of Barfoed's reagent, and heat in a boiling water bath until the reaction occurs.

Observe and time how long it takes for a reddish precipitate or a color change to appear in each tube. Monosaccharides react in less than 15 minutes; disaccharides require more than 15 minutes. Record the reaction time of each carbohydrate in the table in section VI.

b) Benedict's test — reducing sugars

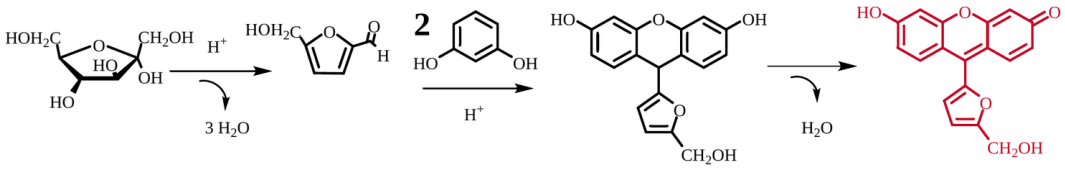
$$2\,RCHO + 2\,Cu^{+2} + 6\,OH \longrightarrow 2\,RCOOH + Cu_2O\downarrow + 6\,H_2O$$

Place 1 mL of Benedict's reagent and 3 drops of the solution of the carbohydrate to be analyzed in a test tube. Heat to boiling and let it cool to room temperature. A positive result shows up as the appearance of a reddish-orange precipitate.

Perform this test with only five carbohydrates: fructose, glucose, galactose, lactose, and sucrose. Time how long each one takes to react.

The chemical basis is the same as Barfoed's —the reduction of Cu^{2+} to Cu^{+}— but this test **is not selective** for monosaccharides. Write down your results and be ready to justify that lack of selectivity in the post-lab questions.

c) Seliwanoff's test — identification of ketoses

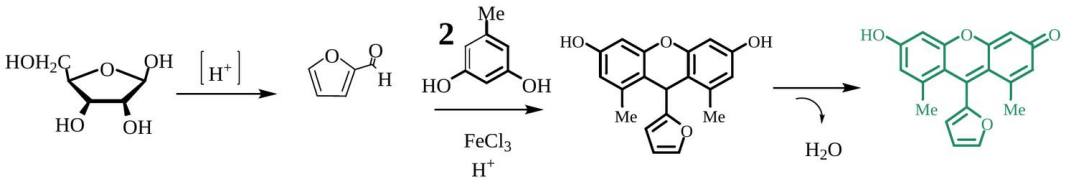


In a test tube add 0.5 mL of the corresponding sugar solution and 1.5 mL of Seliwanoff's reagent, and heat in a boiling water bath for a maximum of 1-3 minutes. Observe.

Ketoses react quickly, dehydrating to give 5-hydroxymethylfurfural, which condenses with resorcinol to give a highly conjugated aromatic compound of cherry-red color. Prolonged heating favors hydrolysis and rearrangements, and makes other carbohydrates give false positives.

Structure your data by indicating in one column the positive results and in another the false positives and negatives, with the corresponding reaction times.

d) Bial's test — identification of pentoses



In a test tube place 0.1 mL (5 drops with a Pasteur pipette) of the corresponding sugar solution, add 1.5 mL of Bial's reagent, and place the tube in a boiling water bath for 2-3 minutes. Observe.

Pentoses give a blue-green or olive-green coloration. If overheated, the rest of the carbohydrates in the presence of Bial's reagent form colored compounds that constitute false positives.

VIII. Post-lab Questions.

1. With your table of results, classify the nine carbohydrates into monosaccharides and disaccharides using only the times you measured in Barfoed's test. Point out whether any result did not match what was expected and propose why.

2. Report which carbohydrates gave a positive Benedict's test in your session and explain, based on your own results, why this test **is not** selective for monosaccharides.

3. Sucrose gave negative results in the Barfoed and Benedict tests. Explain this from its structure, pointing out which carbon is involved.

4. State which carbohydrates gave you false positives in Seliwanoff or Bial and at what time they appeared. With those data, propose an optimal heating time for each test.

5. Look up what the Molisch test consists of and for which carbohydrates it is used. Compare it with the four tests you performed in terms of selectivity.

IX. References.

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X. Appendix: Reagent Preparation

5% carbohydrate solutions (1 L)

Weigh 50 g of the carbohydrate in question and dissolve in 800 mL of distilled water inside a volumetric flask, then fill to 1000 mL with distilled water. Stir gently to complete its dissolution. These solutions are prepared no more than 1 week before use and kept refrigerated to prevent microbial growth.

Barfoed's solution (1 L)

Weigh 65 g of cupric acetate ($Cu(OAc)_2 \cdot H_2O$), add it to 500 mL of distilled water inside a 1 L volumetric flask, stir gently, then measure 9 mL of glacial acetic acid and pour it into the previously prepared solution, finally filling the solution to 1 L with distilled water. It can be stored in an amber container without problem for up to 12 months.

Bial's solution (1 L)

Measure 450 mL of distilled water and place it in a 1 L beaker, which must be inside an ice bath. Once the water is cold, weigh 3.0 grams of orcinol and dissolve it in the water, also adding 3.0 mL of 10% $FeCl_3$ solution; then, WITH EXTREME CARE, pour in 500 mL of concentrated hydrochloric acid (HCl) with gentle stirring. ALL OF THIS INSIDE AN ICE BATH. The solution releases a great deal of heat. Once the addition of the concentrated HCl is finished and the solution has cooled, pour it into a 1 L volumetric flask and fill to 1000 mL with distilled water. Store in an amber container for no more than 2 months. Wear a lab coat, gloves, and safety glasses.

Seliwanoff's solution (1 L)

Measure 600 mL of distilled water and place it in a 1 L beaker, which must be inside an ice bath. Once the water is cold, weigh 1.0 gram of resorcinol and add it to the cold water; then, WITH EXTREME CARE, pour in 330 mL of concentrated hydrochloric acid (HCl) with gentle stirring. ALL OF THIS INSIDE AN ICE BATH. The solution releases a great deal of heat. Once the addition of the concentrated HCl is finished and the solution has cooled, pour it into a 1 L volumetric flask and fill to 1000 mL with distilled water. Store in an amber container for no more than 2 months. Wear a lab coat, gloves, and safety glasses.

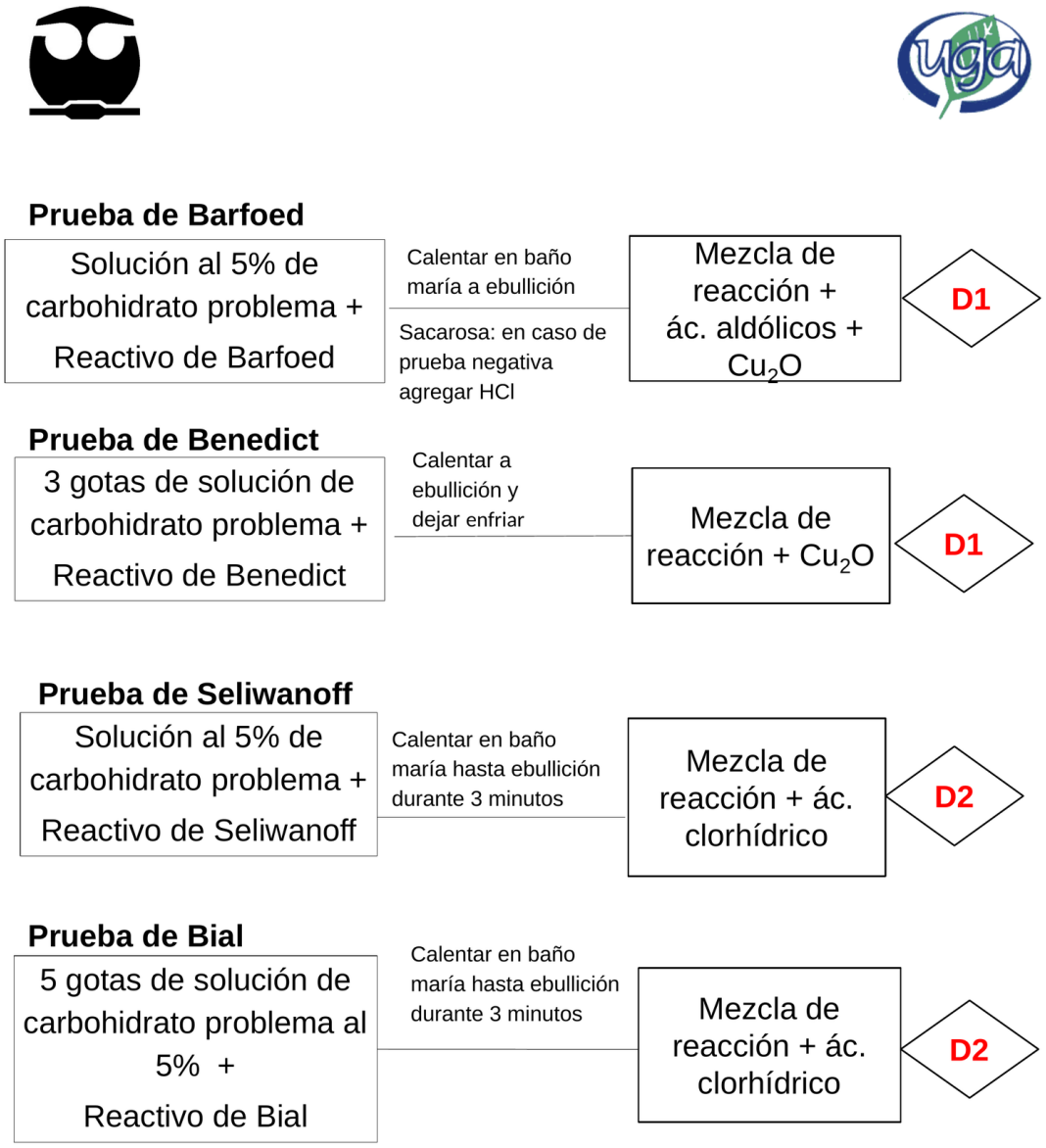
10% $FeCl_3$ solution (100 mL)

Weigh 10 g of $FeCl_3$ and dissolve in 80 mL of distilled water inside a 100 mL volumetric flask; once the $FeCl_3$ has dissolved, fill to 100 mL with distilled water and stir gently until completely dissolved. Store in an amber bottle; no shelf-life issues.

Benedict's solution (1 L)

Weigh 173 g of sodium citrate and 100 g of anhydrous sodium carbonate. Dissolve both solids in 700 mL of distilled water and stir gently until maximum dissolution is obtained; if necessary, heat the solution slightly to help the solids dissolve. If undissolved particles remain, filter the solution. Separately, weigh 17.3 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) and dissolve it in 100 mL of distilled water. Mix both solutions with constant stirring and add distilled water until reaching a final volume of 1000 mL.

XI. Waste Management.



Scheme 14. Flow diagram of the carbohydrate identification reactions and their waste management.

EXPERIMENT 11

CARBOHYDRATES II

CHEMICAL HYDROLYSIS OF STARCH

I. Objectives.

a) To recognize the chemical structure of starch as a carbohydrate biopolymer.

b) To carry out the chemical hydrolysis of starch and follow its progress over time using identification tests.

c) To identify the factors that favor hydrolysis and verify whether the product obtained is a reducing sugar.

II. Introduction.

Starch is a glucose polymer produced by plants as their main reserve polysaccharide. Unlike cellulose, another polysaccharide present in dietary fiber, starch is easily digested by humans and represents one of the main energy sources of our species: bread, potatoes, rice, and pasta are examples of this.

Starch is in fact a mixture of two different polysaccharides: amylose and amylopectin. Both are homopolymers of D-glucopyranose, but amylose is a linear polymer joined through α-(1,4′) glycosidic bonds, while amylopectin is a branched polymer that also includes α-(1,6′) bonds. As a consequence of its linearity, amylose disperses easily in cold water and dissolves in hot water. The amylose:amylopectin ratio varies according to the botanical source, but typical starches contain around 25% amylose.

Polysaccharides are by far the most abundant biopolymers on Earth, and starch dominates among them. It is one of the materials with the greatest potential in polymer technology: it can easily be converted into high value-added products such as ethanol, acetone, and organic acids.

The acid hydrolysis of starch to glucose using inorganic acids must be finished with a neutralization. High temperatures and low pH accelerate the rate of hydrolysis and, at the same time, provide sterile conditions that prevent the growth of fermenting microorganisms. The progress of this reaction is monitored with two complementary tests: Lugol's, which detects the presence of long amylose chains, and Benedict's, which detects the appearance of reducing ends.

III. Pre-lab Research.

1. Draw the α-(1,4′) and α-(1,6′) bonds, indicate which one distinguishes amylose from amylopectin, and predict which one is hydrolyzed more easily in acidic medium.

2. Explain the basis of Lugol's test: what interaction produces the color, why it disappears as the hydrolysis progresses, and why the solution must be cold. Predict the color at t_0 and at t_{50}.

3. Explain why intact starch gives a negative Benedict's test and why the aliquots for Benedict must be neutralized before adding the reagent, while those for Lugol need not be.

4. Look up what dextrins are, how they are named according to their chain length, and at what point of the hydrolysis they appear.

5. Review the physical, chemical, and toxicological properties (CRETIB) of the reagents used in this experiment.

IV. Reagents.

Reagents	Amount	Reagents	Amount
Soluble starch	0.6 g	Concentrated hydrochloric acid	1 mL
Benedict's solution	15 mL	Iodine-KI solution (Lugol's)	2 mL
5% NaOH solution	10 mL	Phenolphthalein solution	1 mL
Distilled water	80 mL		

V. Materials.

In addition to the kit, request the following equipment by requisition slip (papeleta).

Equipment	Qty.	Equipment	Qty.
125 mL Erlenmeyer flask	1	Test tube rack	1
Test tubes	16	Test tube holder	1
1 mL graduated pipette	2	25 mL graduated cylinder	1
250 mL beaker	1		

VI. Recording of Results.

Record in this table the color observed in each tube. Take photographs of the complete series to attach them to your report.

Time	Lugol	Benedict
t_0		
t_{10}		
t_{20}		
t_{30}		
t_{40}		
t_{50}		

VII. Procedure.

Concentrated HCl: It is corrosive; handle it with care. In case of contact, wash with plenty of water for 3-5 minutes and rinse with 10% $NaHCO_3$ solution.

Place 0.6 g of soluble starch in the 125 mL Erlenmeyer flask, with a magnetic stir bar, and add 60 mL of water at room temperature. Heat the solution to boiling with stirring: the starch must be completely dissolved, forming an opalescent solution.

Label 12 test tubes for the two tests, marked t_0, t_{10}, t_{20}, t_{30}, t_{40}, and t_{50} minutes.

Once the starch has dissolved, take two 1 mL samples with the graduated pipette and place them in the tubes labeled t_0, one for each test. These tubes correspond to time zero of the hydrolysis.

Remove the rest of the solution from the heat, let it cool for 1 minute, and slowly and carefully add 1 mL of concentrated HCl. Return the flask to boiling for 50 minutes and observe what happens almost immediately. Take 1.0 mL samples every 10 minutes for each of the tests and place them in the corresponding t_{10} to t_{50} tubes.

Take a third 1 mL sample at time t_{10}, which will serve as a **neutralization blank**: add 1 drop of phenolphthalein solution and count how many drops of 5% NaOH are required to neutralize 1 mL of the solution. That number of drops is what you must add to each Benedict tube from t_{10} onward.

Neutralize with 5% NaOH all the Benedict test tubes from t_{10} to t_{50} before adding the reagent. Place water in the 250 mL beaker and bring it to a boil. Once neutralized, add 1 mL of Benedict's solution to each tube (t_0 to t_{50}), place them in the boiling water bath for 3-5 minutes, and observe.

Place all the Lugol test tubes (t_0 to t_{50}) in an ice bath. When they are all cold, add 2 drops of Lugol's solution to each one and observe.

Note 1: Lugol's test requires the starch solution to be cold, since the starch-iodine complex is soluble when hot. Also prepare a positive control: in a test tube add 2 mL of distilled water and 2 drops of Lugol's.

When you have all the tubes ready, add 1 mL of extra distilled water to each one to dilute them; that way the shades will be easier to observe. Analyze, photograph, and report your observations in the table in section VI.

VIII. Post-lab Questions.

1. Describe how the color of your Lugol tubes evolved from t_0 to t_{50} and state at what time you stopped observing the characteristic color. Explain what happened to the amylose chain at that point.

2. State from what time onward your Benedict's test was positive. Compare that time with the one you recorded in the previous question and explain why both tests change at different moments.

3. Report how many drops of 5% NaOH your neutralization blank required. Explain what would have happened to Benedict's test if you had not neutralized the tubes.

4. Explain why the Lugol and Benedict tests are performed before starting the hydrolysis and what information that time zero provides.

5. With your results, estimate whether 50 minutes were enough to completely hydrolyze the starch and justify your answer. Propose a specific modification to the procedure if you consider they were not. Also explain why it was necessary to keep the mixture boiling throughout the hydrolysis.

6. Look up the correct chemical name of the enzyme that carries out the enzymatic hydrolysis of starch and how its action differs from the acid hydrolysis you performed.

IX. References.

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X. Appendix: Reagent Preparation

Phenolphthalein/Indicator solution (100 mL)

Weigh 0.1 grams of phenolphthalein and dissolve it in a mixture of 50 mL of ethanol and 50 mL of distilled water, shaking vigorously to complete its dissolution. Store inside an amber container.

Iodine-Iodide (Lugol's) solution (1 L)

Weigh 200 g of potassium iodide (KI) and dissolve in 500 mL of distilled water; to this solution add 100 g of iodine (I_2) and stir gently, then add distilled water to complete a final volume of 1 L. Note: if it does not dissolve completely, heat the solution gently until fully dissolved. Once the solution is prepared, it must be **diluted 1:10**. Store inside an amber container.

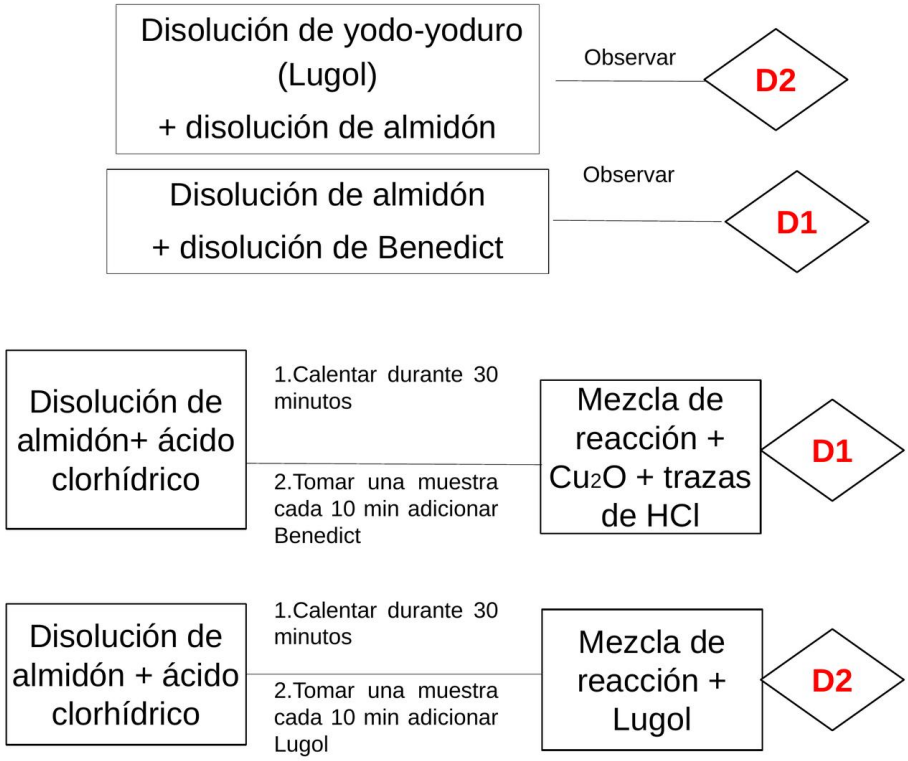
5% Sodium hydroxide solution (1 L)

Weigh 50 g of sodium hydroxide (NaOH) and pour into a beaker containing 500 mL of distilled water and kept in an ice bath; stir the solution gently and bring the solution to a final volume of 1000 mL (1 L). The solution releases heat.

Benedict's solution (1 L)

Weigh 173 g of sodium citrate and 100 g of anhydrous sodium carbonate. Dissolve both solids in 700 mL of distilled water and stir gently until maximum dissolution is obtained; if necessary, heat the solution slightly to help the solids dissolve. If undissolved particles remain, filter the solution. Separately, weigh 17.3 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$) and dissolve it in 100 mL of distilled water. Mix both solutions with constant stirring and add distilled water until reaching a final volume of 1000 mL.

XI. Waste Management.



D1: Llevar la disolución a pH= 10 y filtrar el complejo de cobre para enviar a confinamiento. La disolución restante desechar neutra.

D2: Reducir con disolución de bisulfito de sodio ($NaHSO_3$) hasta la desaparición del color. Confirmar el pH neutro y desechar al drenaje.

Scheme 15. Flow diagram of the chemical hydrolysis of starch and its waste management.