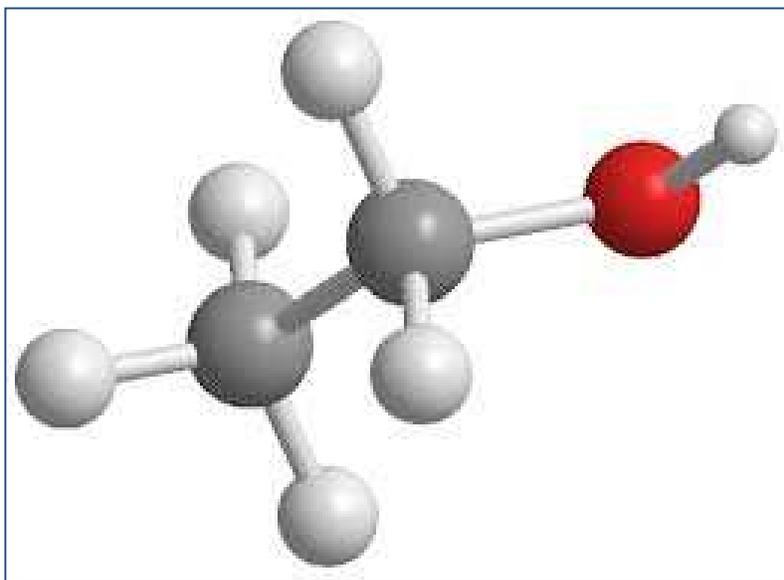




Procedure Of

Qualitative Organic Chemistry



By:

Dr. Fattahi & Khazai

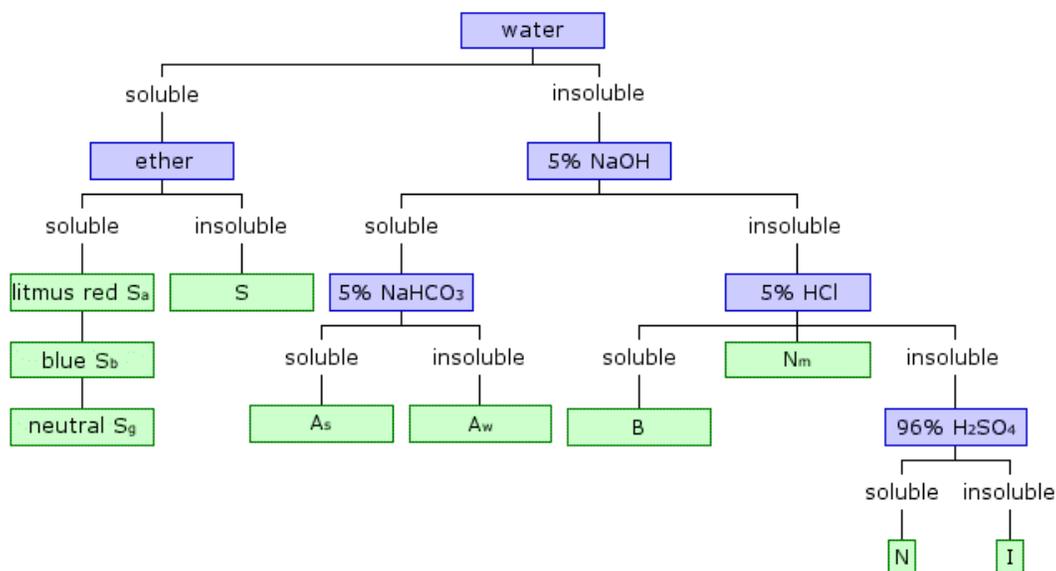
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In the Name of Merciful GOD

Qualitative Organic Chemistry

Solubility



Classes of Organic Compounds

Class	Functional Group Possibilities
S_a	monofunctional carboxylic acids (<5C), arylsulfonic acids
S_b	monofunctional amines (<6C)
S_g	monofunctional alcohols, aldehydes, ketones, esters, nitriles, and amides (all <5C)
S	salts of organic acids, amine hydrochlorides, amino acids, polyfunctional compounds with hydrophilic functional groups
A_s	strong organic acids: carboxylic acids (>6C), phenols with electron-withdrawing groups in the ortho and/or para position(s), β -diketones
A_w	weak organic acids: phenols, enols, oximes, imides, sulfonamides, thiophenols (all >5C), β -diketones, nitro compounds with α -hydrogens
B	aliphatic amines (>8C), anilines (only one phenyl group attached to N), some ethers
N_m	miscellaneous neutral compounds containing N or S (>5C)

N	alcohols, aldehydes, ketones, monofunctional esters (>5C but <9C), ethers, epoxides, alkenes, alkynes, some aromatic compounds (with activating groups)
I	saturated hydrocarbons, haloalkanes, aryl halides, other deactivated aromatic compounds, diaryl ethers

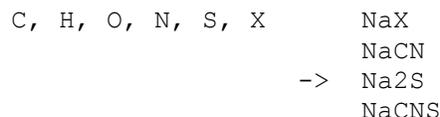
Notes:

- Acyl halides and acid anhydrides have not been classified due to their high reactivity.

Procedure for Determining Solubility of Organic Compounds

1. **Water Solubility.** Place 0.05 mL or 25 mg of compound in a small test tube, and add 0.75 mL of water in small portions. Shake test tube vigorously after the addition of each portion of solvent. If water soluble, go on to step 2; otherwise proceed to step 3.
2. **Ether Solubility.** Place 0.05 mL or 25 mg of compound in a small test tube, and add 0.75 mL of diethyl ether in small portions. Shake test tube vigorously after the addition of each portion of solvent. If the compound is both water and ether soluble, the acid-base properties of the compound should be determined with litmus.
 - litmus turns red - water soluble acidic compound (class S_a)
 - litmus turns blue - water soluble basic compound (class S_b)
 - litmus neutral - water soluble general compound (class S_g)
 If the compound is not ether soluble it is a salt, amino acid, or contains many hydrophilic functionalities (class S)
3. **5% NaOH Solubility.** Place 0.05 mL or 25 mg of compound in a small test tube, and add 0.75 mL of NaOH solution in small portions. Shake test tube vigorously after the addition of each portion of solvent. If NaOH soluble, go on to step 4; otherwise proceed to step 5.
4. **5% NaHCO₃ Solubility.** Place 0.05 mL or 25 mg of compound in a small test tube, and add 0.75 mL of NaHCO₃ solution in small portions. Shake test tube vigorously after the addition of each portion of solvent. If NaHCO₃ soluble, then it is a strong organic acid (class A_s). If not NaHCO₃ soluble, then it is a weak organic acid (class A_w).
5. **5% HCl Solubility.** Place 0.05 mL or 25 mg of compound in a small test tube, and add 0.75 mL of HCl solution in small portions. Shake test tube vigorously after the addition of each portion of solvent. If HCl soluble, then it is an organic base (class B). If not HCl soluble and (from elemental analysis) is found to contain nitrogen or sulfur, then it is a miscellaneous neutral compound (class N_m). If not HCl soluble, then go on to step 6.
6. **96% H₂SO₄ Solubility.** Place 0.6 mL of H₂SO₄ in a small test tube, and add 0.05 mL or 25 mg of compound. Shake test tube vigorously. If H₂SO₄ soluble, then it is a neutral compound (class N). If not H₂SO₄ soluble, then it is an inert compound (class I).

Lassaigne's Sodium Fusion Test



PROCEDURE

Place a piece of clean sodium metal, about the size of a pea into a fusion tube. Add a little of the compound (50 mg or 2 - 3 drops). * Heat the tube gently at first, allowing any distillate formed to drop back onto the molten sodium. When charring begins, heat the bottom of the tube to dull redness for about three minutes and finally plunge the tube, while still hot, into a clean dish containing cold distilled water (6 mL) and cover immediately with a clean wire gauze. **

*For liquids it is better to first melt the sodium add the liquid drop by drop.

**CAUTION: The tube shatters, and any residual sodium metal reacts with water. Stir the mixture, boil for 1 - 2 minutes, on a tripod and filter hot through a fluted paper.

The 'fusion' filtrate which should be clear and colourless, is used for the SPECIFIC TESTS DESCRIBED BELOW:

1. Nitrogen: to a portion (2 mL) of the 'fusion' filtrate add 0.2 g of powdered ferrous sulphate crystals. Boil the mixture for a half a minute, cool and acidify by adding dilute sulphuric acid dropwise. Formation of a bluish-green precipitate (Prussian blue) or a blue solution indicates that the original substance contains nitrogen. If no precipitate appears, allow to stand for 15 minutes, filter and inspect filter paper.

2. SULPHUR (SULPHIDE)

To the cold 'fusion' filtrate (1 mL) add a few drops of cold, freshly prepared, dilute solution of sodium nitroprusside. The latter may be prepared by adding a small crystal of the solid to 2 mL of water. Production of a rich purple colour indicates that the original substance contains sulphur. This test is very sensitive. Only strong positive results are significant.

3. HALGENS (HALIDES)

Acidify a portion (1 mL) of the 'fusion' filtrate with 2N nitric acid, and if nitrogen and/or sulphur are present, boil for 1 - 2 minutes.* Cool and add aqueous silver nitrate (1 mL), compare with a blank. Formation of a heavy, white or yellow precipitate of silver halide indicates halogen. If a positive result is obtained: acidify the remaining portion of the 'fusion' filtrate with dilute sulphuric acid, boil

and cool. Add carbon tetrachloride (1 mL) and a few drops of freshly prepared chlorine water. Shake the mixture.

(a) If the carbon tetrachloride layer remains colourless - indicates chlorine.

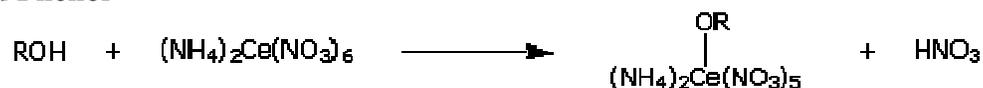
(b) If the carbon tetrachloride layer is brown - indicates bromine.

(c) If the carbon tetrachloride layer is violet - indicates iodine.

*If nitrogen and/or sulphur are also present, the addition of silver nitrate to the acidified 'fusion' solution will precipitate silver cyanide and/or silver sulphide in addition to the silver halides. The removal of hydrogen cyanide and/or hydrogen sulphide is effected by boiling the 'fusion' solution.

Ceric Ammonium Nitrate

Alcohol and Phenol



Procedure

For Water Soluble Compounds: To 0.5 mL of the ceric ammonium reagent add 2 drops of a liquid unknown or 0.1g of a solid. Mix thoroughly and note if the yellow color of the reagent changes to red. Alcohols react with the reagent to form a red alkoxy cerium(IV) compound. If a red color develops, watch the solution carefully and note the time for the mixture to become colorless. If no change is noted in 15 min, the test tube may be stoppered and allowed to stand several hours or overnight. Also note if bubbles of carbon dioxide are liberated.

For Water Insoluble Compounds: Add 1 mL of dioxane to 0.5 mL of the ceric ammonium nitrate reagent. If a red color develops or if the solution becomes colorless, the dioxane must be purified. If the mixture remains yellow or is only a light orange-yellow, it may be used to test water insoluble compounds. Divide the 6 mL of the solution in half, reserving 3 mL for observation as a control. To the other 3 mL of the dioxane containing reagent, add 2 drops of a liquid unknown or 0.05 g of a solid. Mix thoroughly and make the same observations as above.

Ceric Ammonium Nitrate Reagent: Add 1.3 mL of concentrated nitric acid to 40 mL of distilled water and then dissolve 10.96 g of yellow ceric ammonium nitrate in the dilute nitric acid solution. After the solid has dissolved, dilute to 50 mL.

Positive Test

Formation of a red alkoxy cerium(IV) compound is a positive test.

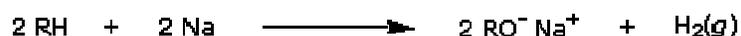
Phenols give a brown color or precipitate as a positive test.

Complications

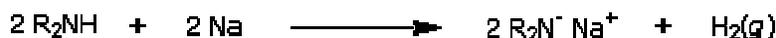
Hot solutions of Ce(IV) oxidize many organic compounds.

Sodium Detection of Active Hydrogen

Alcohol



Amine



Alkyne



Procedure

To 0.25 mL or 0.25 g of the sample, add small thin slices of freshly cut sodium until no more will dissolve. Evolution of hydrogen gas indicates the presence of an acidic hydrogen, such as a hydroxyl group in an alcohol, a hydrogen attached to the nitrogen in a primary or secondary amine, or a hydrogen in a terminal alkyne. Cool the solution, and observe. Add an equal volume of ether. Another positive test is the formation of the solid salt. Liquid samples should be dried with calcium sulfate, prior to testing. This test may be applied to solid compounds or very viscous liquids by dissolving them in an inert solvent such as anhydrous ligroin or toluene.

Positive Test

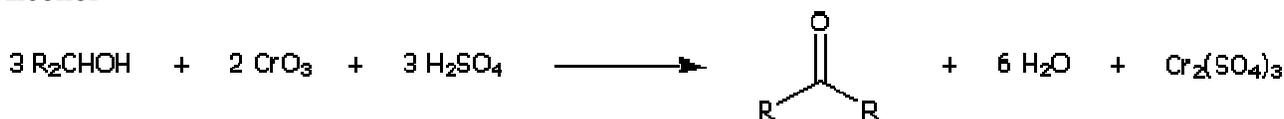
Formation of hydrogen gas is a positive test.

Complications

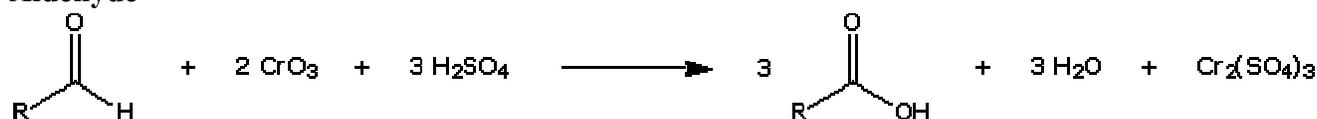
Dealing with sodium metal can be exciting. Make sure that all samples are dry before proceeding with test.

Chromic Anhydride (Jones Oxidation)

Alcohol



Aldehyde



Procedure

To 1 mL of acetone in a small test tube, add 1 drop of the liquid or about 10 mg of a solid compound. Then add 1 drop of the Jones reagent and note the result within 2 sec. Run a control test on the acetone and compare the result.

Jones Reagent: A suspension of 25 g of chromic anhydride (CrO_3) in 25 mL of concentrated sulfuric acid is poured slowly with stirring into 75 mL of water. The deep orange-red solution is cooled to room temperature before use. A good grade of acetone should be used. Some samples of acetone may become cloudy in appearance in 20 sec, BUT this does not interfere, providing the test solution becomes yellow. If the acetone gives a positive test, it should be purified by adding a small amount of potassium permanganate and distilling.

Positive Test

A positive test for primary or secondary alcohols consists in the production of an opaque suspension with a green to blue color. Tertiary alcohols give no visible reaction within 2 sec, the solution remaining orange in color. Disregard any changes after 2 sec.

Complications

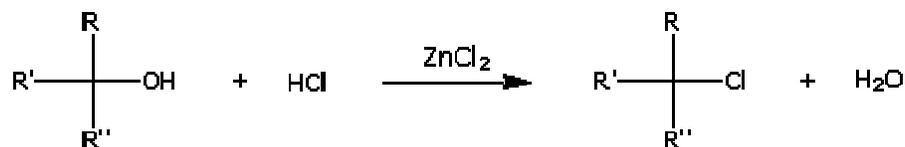
Aldehydes are better characterized in other ways. The color usually develops in 5 - 15 seconds.

Enols may give a positive test.

Phenols give a dark colored solution which is not blue-green like a positive test.

Lucas Test

Alcohol



Procedure

(a) To 0.1 mL or 0.1 g of the sample in a test tube add 1 mL of the Lucas reagent at 26-27°C. Stopper the tube and shake; then allow the mixture to stand. Note the time required for the formation of the alkyl chloride, which appears as an insoluble layer or emulsion. Note by means of a watch the time required for the reaction to take place.

Lucas Reagent: Dissolve 13.6 g (0.1 mole) of anhydrous zinc chloride in 10.5 g (0.1 mole) of concentrated hydrochloric acid, with cooling.

Positive Test

Appearance of a cloudy second layer or emulsion.

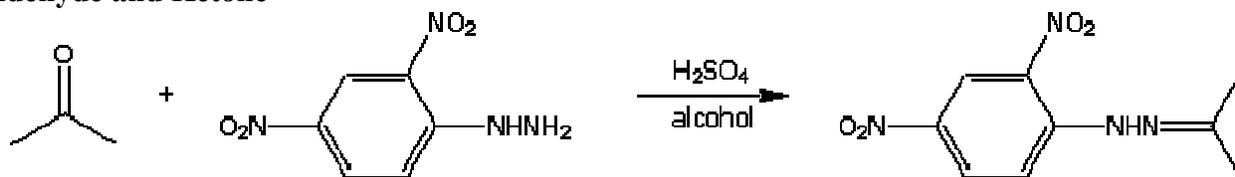
- 3° alcohols - immediate to 2 - 3 minutes
- 2° alcohols - 5 - 10 minutes
- 1° alcohols - no reaction

Complications

The test applies only to those alcohols soluble in the reagent (monofunctional alcohols lower than hexyl and some polyfunctional alcohols).

2,4-Dinitrophenylhydrazine

Aldehyde and Ketone



Procedure

Add a solution of 1 or 2 drops or 30 mg of unknown in 2 mL of 95% ethanol to 3 mL of 2,4-dinitrophenylhydrazine reagent. Shake vigorously, and, if no precipitate forms immediately, allow the solution to stand for 15 minutes.

2,4-Dinitrophenylhydrazine reagent: Dissolve 3 g of 2,4-Dinitrophenylhydrazine in 15 mL of conc. sulfuric acid. This solution is then added with stirring to 20 mL of water and 70 mL of 95% ethanol. This solution is mixed thoroughly and filtered.

Positive Test

Formation of a precipitate is a positive test.

Complications

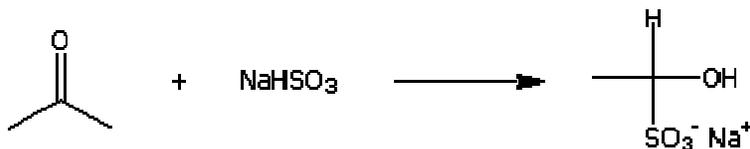
Some ketones give oils which will not solidify.

Some allylic alcohols are oxidized by the reagent to aldehydes and give a positive test.

Some alcohols, if not purified, may contain aldehyde or ketone impurities.

Sodium Bisulfite Addition Complex

Aldehyde and Ketone



Procedure

Place 0.25 mL of sodium bisulfite reagent in a test tube and add 0.07 mL or 75 mg of unknown. Stopper the test tube and shake vigorously.

Sodium bisulfite reagent: Add 1 mL of ethanol to 4 mL of a 40% aqueous solution of sodium bisulfite. Decant or filter the solution prior to use.

Positive Test

Formation of a precipitate is a positive test.

Complications

Aryl methyl ketones form the precipitate slowly or not at all.
Addition complex stable only in neutral solution.

Tollens Test

Aldehyde and Carbohydrate



Procedure

Add one drop or a few crystals of unknown to the freshly prepared Tollens reagent. Gentle heating can be employed if no reaction is immediately observed.

Tollens reagent: Into a test tube which has been cleaned with 10% sodium hydroxide, place 2 mL of a 5% silver nitrate solution, and add a drop of 10% sodium hydroxide. Add 2% ammonia solution, drop by drop, with constant shaking, until the precipitate of silver oxide just dissolves.

Positive Test

Formation of silver mirror or black precipitate is a positive test.

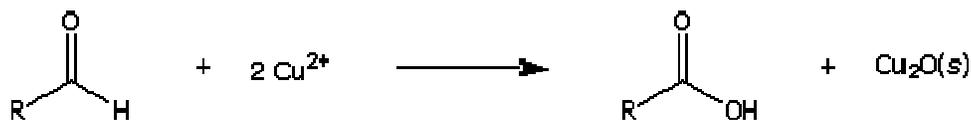
Complications

The test tube must be clean and free of oil if a silver mirror is to be observed.

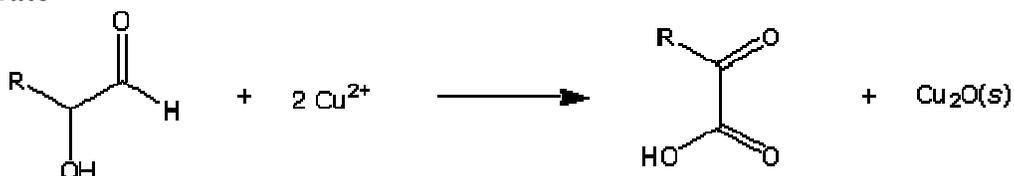
Easily oxidized compounds give a positive test. For example: aromatic amine, some phenols, α -alkoxy and α -dialkylaminoketones.

Benedict's Solution

Aldehyde



Carbohydrate



Procedure

To a solution or suspension of 0.05 g of unknown in 1.2 mL of water, add 1.2 mL of Benedict's solution. If no precipitate is formed, heat the mixture to boiling and cool.

Benedict's solution: A solution of 17.3 g of sodium citrate and 10.0 g of anhydrous sodium carbonate in 80.0 mL of water is heated until the salts are dissolved. Additional water is added to bring the volume up to 85.0 mL. A solution of 1.73 g of hydrated copper sulfate in 10.0 mL of water is poured slowly with stirring into the solution of the citrate and the carbonate. Add water to make a final volume of 100 mL.

Positive Test

Precipitation of copper(I) oxide as a red, yellow, or yellowish-green solid is a positive test.

Complications

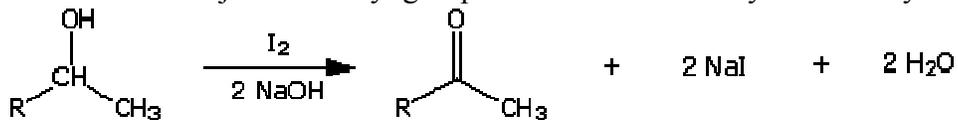
Not general for simple aldehydes and ketones.

Hydrazine derivatives give a positive test.

Iodoform Test

Alcohol

Secondary alcohols with an adjacent methyl group are oxidized to methyl ketones by iodine bleach.



Ketone



Procedure

Add four drops or 0.05 g of unknown to a test tube. Add 3 mL of dioxane, and shake until unknown dissolves. Add 0.5 mL of 10% NaOH solution, and then slowly add the iodine-potassium iodide solution with shaking, until a slight excess yields a definite dark color of iodine. Heat the mixture to 60°C. The addition of iodine is continued until the dark color is not discharged by 2 minutes of heating at 60°C. Add a few drops of 10% NaOH solution to discharge iodine color. Now fill the test tube with water and let stand for 15 minutes. Filter the precipitate and check the melting point; iodoform melts at 119-121°C.

Iodine-potassium iodide solution: Add 20.0 g of potassium iodide and 10.0 g of iodine to 80.0 mL of water and stir until the reaction is complete.

Positive Test

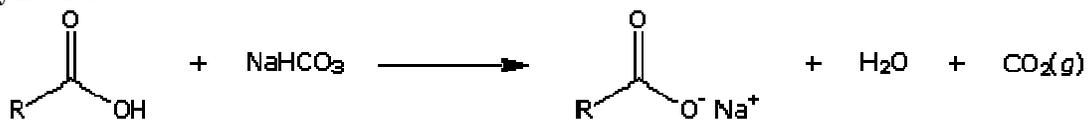
Formation of solid iodoform (yellow) is a positive test.

Complications

Test will not be positive if the R group is a di-ortho substituted aryl group.

Sodium Bicarbonate Test

Carboxylic Acid



Procedure

A few drops or a few crystals of the unknown sample are dissolved in 1 mL of methanol and slowly added to 1 mL of a saturated solution of sodium bicarbonate.

Positive Test

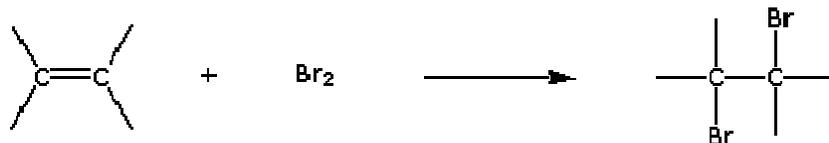
Evolution of carbon dioxide gas is a positive test for the presence of the carboxylic acid.

Complications

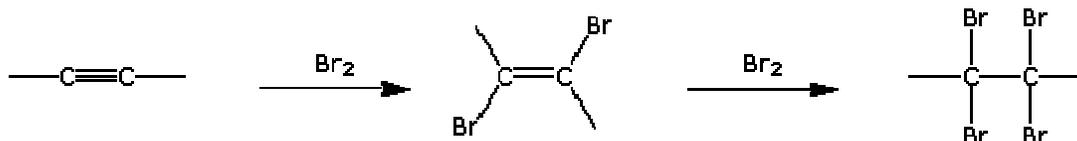
None.

Bromine Test

Alkene



Alkyne



Procedure

In a hood, 0.05 g or 0.1 mL of the unknown is added to 1 mL of carbon tetrachloride, and a 5% solution of bromine in carbon tetrachloride is added drop by drop, with shaking, until the bromine color persists.

Positive Test

Discharging of the bromine color without the evolution of hydrogen bromide gas is a positive test.

Complications

Should be employed in conjunction with Baeyer test (dilute KMnO_4).

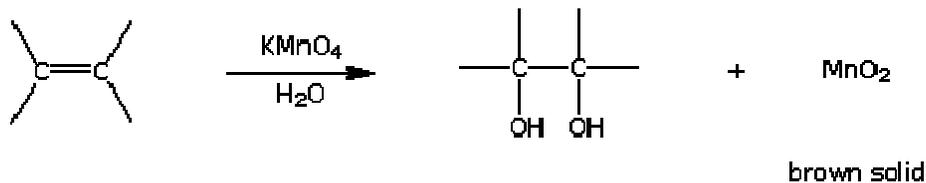
Electron-withdrawing groups in the vinylic position can slow down bromine addition to the point that a negative test is erroneously produced.

Tertiary amines (like pyridine) form perbromides upon treatment with bromine and lead to false positive tests.

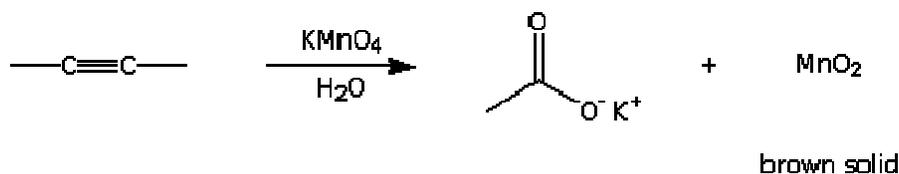
Aliphatic and aromatic amines discharge the bromine color without the evolution of HBr gas.

Baeyer Test

Alkene



Alkyne



Procedure

To 2 mL of water or ethanol add 0.1 g or 0.2 mL of the unknown. Then add a 2% aqueous potassium permanganate solution drop by drop with shaking until the purple color of the permanganate persists.

Positive Test

The disappearance of the purple color and the appearance of a brown suspension is a positive test.

Complications

Water insoluble compounds should be dissolved in ethanol, methanol, or acetone.

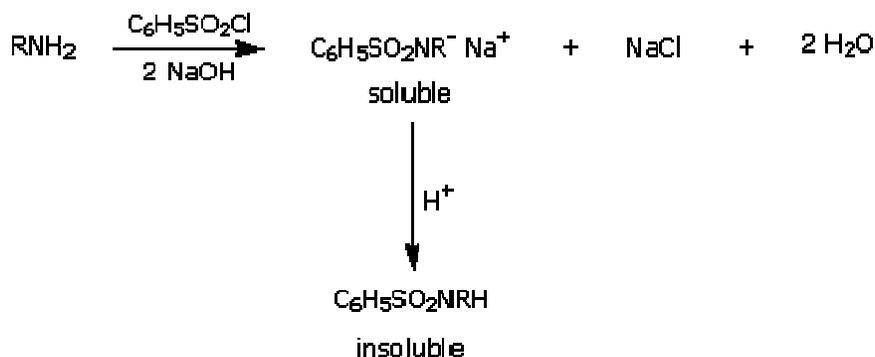
Often, the brown precipitate fails to form and the solution turns reddish-brown.

Easily oxidized compounds give a positive test:

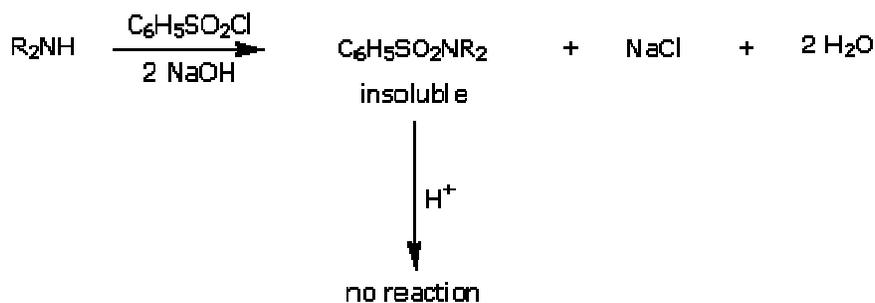
- most aldehydes give a positive test
 - formic acid and its esters give a positive test
 - alcohols with trace impurities give a positive test
 - phenols and aryl amines give a positive test
 - mercaptans and thioethers give a positive test
 - carbonyl compounds which decolorize bromine/carbon tetrachloride usually give a negative test
-

Hinsberg Test

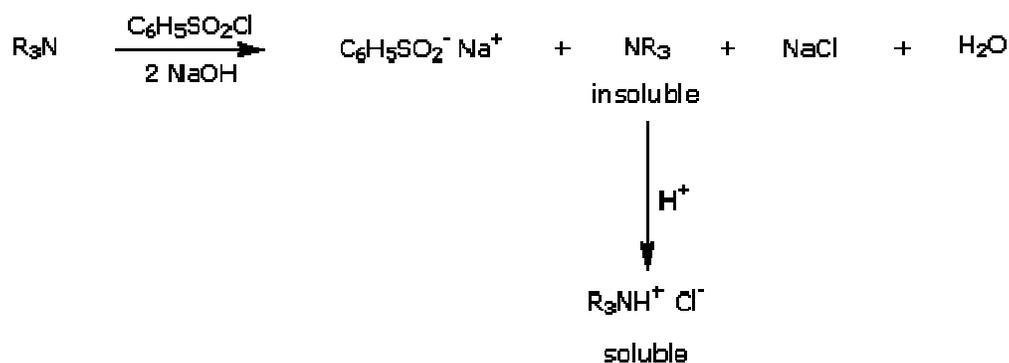
1° Amine



2° Amine



3° Amine



Procedure

To 0.15 mL or 150 mg of unknown in a test tube, add 2.5 mL of 10% NaOH solution and 0.2 mL of benzenesulfonyl chloride. Stopper the test tube, and shake the mixture vigorously. Test the solution to make sure that it is still alkaline. After all of the benzenesulfonyl chloride has reacted, cool the solution and separate the residue, if present, from the solution. Test the residue for solubility in 10% HCl solution. If no residue remains, then treat the solution with 10% HCl solution and observe whether a precipitate forms.

Positive Test

1° amines - dissolves in base and precipitates from acid is a positive test.

2° amines - precipitates from base and no change from acid is a positive test.

3° amines - precipitates from base and dissolves in acid is a positive test.

Complications

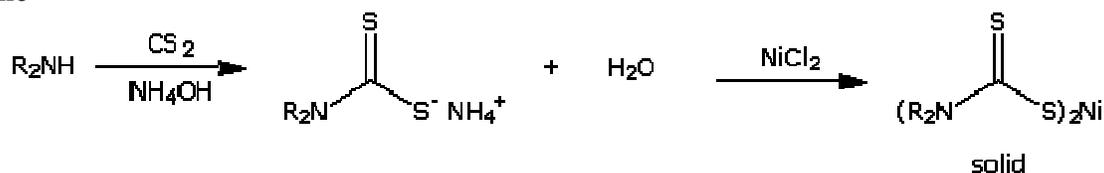
Amphoteric compounds give erroneous results.

Some sodium salts of benzenesulfonamides of primary amines are insoluble in the Hinsberg solution and may appear to be secondary amines.

Some tertiary amine hydrochloride salts are insoluble in dilute HCl and water and may also appear to be secondary amines.

Nickel Chloride, Carbon Disulfide, Ammonium Hydroxide Test

2° Amine



Procedure

Add 1 or 2 drops or 50 mg of unknown to 5 mL of water. If necessary, 1 or 2 drops of conc. HCl may be added to dissolve the amine. To 1 mL of nickel chloride in carbon disulfide reagent in a test tube, add 0.5 - 1 mL of conc. ammonium hydroxide, followed by 0.5 - 1 mL of amine solution.

Positive Test

2° amines- precipitate is a positive test.

Complications

Primary and tertiary amines with secondary amine impurities will yield a positive test.

Carbohydrates

COLORIMETRIC IDENTIFICATION OF UNKNOWN SUGARS

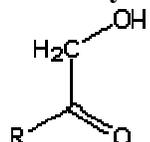
Purpose:

Identify which samples contain real maple syrup, and which contain fake maple syrup, and determine which carbohydrates the imposters contain.

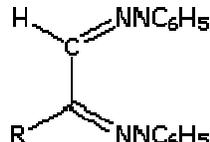
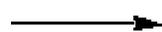
Qualitative Carbohydrate Assays (It is useful to run both negative and positive controls at the same time as the sample.)

Osazone

Carbohydrate



+ 3 H₂NNHC₆H₅



+ C₆H₅NH₂ + NH₃ + 2 H₂O

Procedure

Place 0.05 g of the unknown sample in a test tube and add 0.1 g of phenylhydrazine hydrochloride, 0.15 g of crystallized sodium acetate, and 1 mL of distilled water. Place the test tube in a beaker of boiling water. Note the time that the test tube was immersed and the time of the precipitation. After 20 min, remove the test tube from the hot water bath and set it aside to cool. A small amount of the liquid and solid is poured on a watch glass. Tip the watch glass from side to side to spread out the crystals, and absorb some of the mother liquid with a piece of filter paper, taking care not to crush or break up the clumps of crystals. Examine the crystals under a low-power microscope (about 80-100×), and compare with photomicrographs. The formation of tarry products due to oxidation of the phenylhydrazine may be prevented by the addition of 0.15 mL of saturated sodium bisulfite solution. This should be done before heating if it is desired to isolate the osazone and determine its melting point.

Positive Test

Carbohydrates and 1,2-diols give a positive test.

Complications

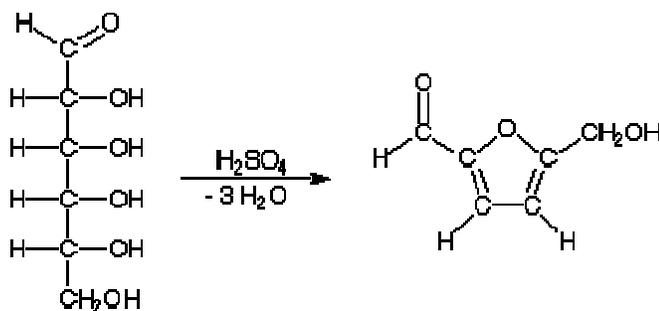
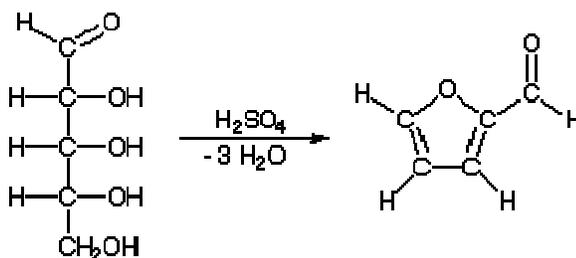
The times required for the formation of the osazones can be a valuable aid in distinguishing among various sugars. The following figures are the times required for the osazone to precipitate from the hot solution: fructose, 2 min; glucose, 4-5 min; xylose, 7 min; arabinose, 10 min; galactose, 15-19 min; raffinose, 60 min; lactose, osazone soluble in hot water; maltose, osazone soluble in hot water; mannose, 0.5 min (hydrazone); sucrose, 30 min (owing to hydrolysis and formation of glucosazone). Osazone formation involves hydrazone formation at C-1 of an aldose (or C-2 of a ketose) and oxidation of C-2 (or C-1) of an alcohol group to a ketone (or an aldehyde). The new carbonyl group is also converted to a hydrazone. It has been suggested that the reaction stops here (rather than further oxidation at C-3, etc.) because of hydrogen-bonding stabilization of the osazone.

Molisch Test (Carbohydrates)

All carbohydrates. Monosaccharides give a rapid positive test. Disaccharides and polysaccharides react slower.

Reactions:

The test reagent dehydrates pentoses to form furfural (bottom reaction) and dehydrates hexoses to form 5-hydroxymethyl furfural (bottom reaction). The furfurals further react with α -naphthol present in the test reagent to produce a purple product (reaction not shown).



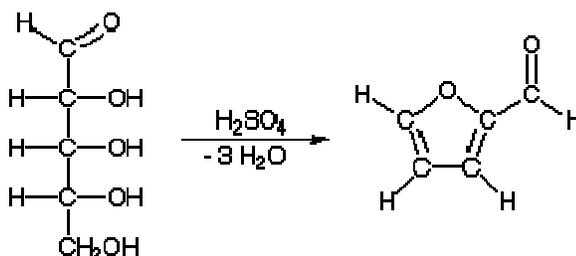
Prepare Molisch's reagent by dissolving 0.5 g reagent grade α -naphthol in 10 mL of 95% ethanol. Store the reagent, protected from light, at room temperature. To test for carbohydrates, add 0.02 mL of the reagent to 1 mL of 0.1% carbohydrate (1 mg/mL) solution in a small test tube. After mixing, tilt the tube and carefully add without mixing, 0.5 mL of concentrated sulfuric acid by pouring it down the side of the tube. (Use a glass Pasteur pipette to add the H_2SO_4 : **do not** use a mechanical pipettor with concentrated acids.) A red-violet layer at the interface between the acid (bottom) and aqueous (upper) layers is a positive test for carbohydrates. (Reminder: Always add acid to water.)

A positive test is indicated by:

The formation of a purple product at the interface of the two layers.

Bial's Test (Pentoses)

The test reagent dehydrates pentoses to form furfural. Furfural further reacts with orcinol and the iron ion present in the test reagent to produce a bluish product (reaction not shown).



Prepare Bial's reagent by dissolving 0.3 g reagent-grade orcinol and 0.05 g ferric chloride in 100 mL of concentrated (12 M) HCl. Store the reagent protected from light. To test for pentoses, add 0.05 mL of 0.1% carbohydrate solution in water to 1 mL of Bial's reagent, (Use a glass Pasteur pipette to add the Bial's reagent: **do not** use a mechanical pipettor with concentrated acids.) and heat the solution in a boiling water bath for 2 minutes.* A blue-green color indicates pentoses or nucleotides containing pentoses; a yellow-green color indicates hexoses, and disaccharides are yellow.

Diphenylamine ((ϕ)₂NH) Test (2-Deoxy sugars)

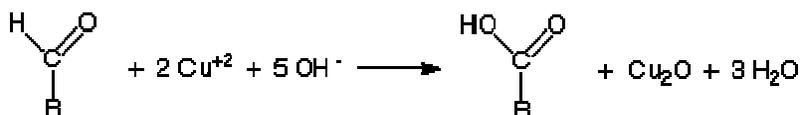
Prepare the reagent by dissolving 0.1 g of diphenylamine in 10 mL of glacial acetic acid and adding 0.25 mL of sulfuric acid. To test for 2-deoxy sugars, add 0.6 mL of a 0.01% carbohydrate solution to 1 mL of the diphenylamine reagent and heat in a boiling-water bath for 10 minutes.* If 2-deoxy sugars are present a blue-green color is produced.

Resorcinol (Seliwanoff's) Test (Ketohehexoses)

Prepare Seliwanoff's reagent by dissolving 0.05 g of reagent-grade resorcinol in 100 mL of 3 M HCl. Store protected from light. To test for ketohehexoses, add 0.1 mL of a 1% carbohydrate solution in water to 1 mL of the reagent, and heat the solution in a boiling water bath for 5 minutes.* A deep red colored precipitate within 5 minute indicates ketohehexoses. Sucrose gives a positive ketohehexose test because of partial hydrolysis to glucose and fructose. Other sugars give a red color upon prolonged heating.

Benedict's Test (Reducing sugars)

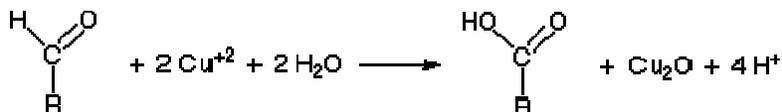
Dissolve 1.73 g trisodium citrate (dihydrate) and 1.0 g anhydrous sodium carbonate in 8 mL of warm dH₂O (Solution A). Dissolve copper sulfate (pentahydrate) (1.73 g) separately in 20 mL of dH₂O (Solution B). Immediately before using, prepare Benedict's reagent by mixing 0.8 mL of Solution A with 0.2 mL of Solution B. To test for reducing sugars, add 0.2 mL of a 1% carbohydrate solution to 1 mL of Benedict's reagent and heat in a boiling water bath for 5 minutes. A brick-red precipitate indicates a positive test for reducing sugars.



Barfoed's Test (Reducing monosaccharides)

Reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide.

Prepare Barfoed's reagent by dissolving 0.66 g cupric acetate (monohydrate) and 0.18 mL glacial acetic acid in 10 mL of dH₂O. To test for reducing monosaccharides, add 0.3 mL of 1% carbohydrate solution to 0.6 mL of Barfoed's reagent and heat in a boiling-water bath for 5 minutes,* then cool to room temperature. A copious amount of brick-red precipitate indicates a reducing monosaccharide. Some hydrolysis of disaccharides may lead to trace precipitates.



Iodine Test (Starch/Amylose)

A few drops of 0.01 M iodine in 0.12 M KI are added to a 1% solution of the carbohydrate in question. The immediate formation of a vivid blue color indicates amylose.

Glucose Test

Prepare the assay reagent by dissolving a capsule (Sigma 510-6) containing 500 units of glucose oxidase and 100 units of horseradish peroxidase with buffer salts in 100 mL of distilled water, and adding 1.6 mL of 2.5 mg/mL o-dianisidine dihydrochloride (Sigma 510-50). Combine 0.1 mL of a sample solution containing 0.25 to 3 mg/mL of carbohydrate with 1 mL of the assay reagent. A brown color within 30 minutes indicates glucose.

* Heat in a fume hood.

Qualitative Tests for Carbohydrates

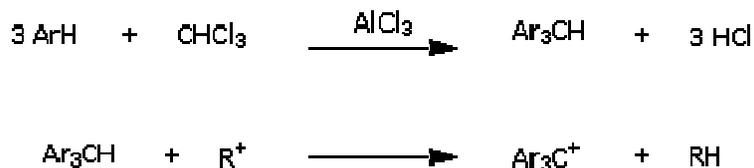
Sugar	Molisch	Bial	(ϕ) ₂ NH	G.O. [§]	Resorcinol	Benedict	Barfoed	Iodine
Ribose	+ (r/v)	(bl/gr)	-	-	-	+(r ppt)	+ (r ppt)	-
DNA*	+ (r/v)	(y)	(bl/gr)	-	-	+(r ppt)	+ (r ppt)	-
Fructose	+ (r/v)	(y/gr)	-	-	+ (r)	+(r ppt)	+ (r ppt)	-
Galactose	+ (r/v)	(y/gr)	-	-	-	+(r ppt)	+ (r ppt)	-
Glucose	+ (r/v)	(y/gr)	-	+(br)	-	+(r ppt)	+ (r ppt)	-
Sucrose	+ (r/v)	(y)	-	-	+ (r)	-	-	-
Lactose	+ (r/v)	(y)	-	-	-	+(r ppt)	-	-
Maltose	+ (r/v)	(y)	-	-	-	+(r ppt)	-	-
Amylose	+ (r/v)	(y)	-	-	-	-	-	+ (bl)
Glycogen	+ (r/v)	(y)	-	-	-	-	-	+ (br)

+ = positive; - = negative; bl = blue; br = brown; gr = green; r = red; v = violet; y = yellow; ppt = precipitate

* To test for DNA, the sample is first hydrolyzed in 10% trichloroacetic acid at 95°C for 10 minutes then diluted with two volumes of water before assaying with the diphenylamine test.

Chloroform and Aluminum Chloride

Aromatic Compounds



Procedure

To 1 mL of dry chloroform in a test tube add 0.05 mL or 0.05g of the unknown compound. Mix thoroughly, and incline the test tube so as to moisten the wall. Then add 0.5g of anhydrous aluminum chloride so that some of the powder strikes the side of the test tube. Note the color of the powder on the side, as well as the solution. Discussion. The colors produced by the reaction of aromatic compounds with chloroform and aluminum chloride are quite characteristic. Aliphatic compounds, which are insoluble in sulfuric acid, give no color or only a very light yellow. Typical colors produced are the following.

Positive Test

Formation of a colored carbocation is a positive test.

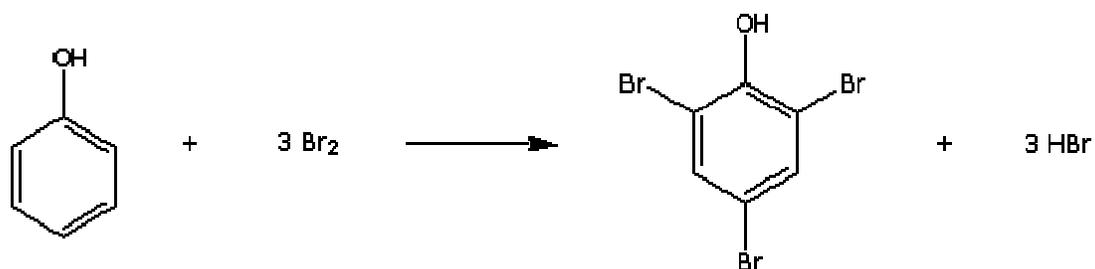
Compound	Color
benzene and its homologs	orange to red
aryl halides	orange to red
naphthalene	blue
biphenyl	purple
phenanthrene	purple
anthracene	green

Complications

Aromatic esters, ketones, amines, and other oxygen- or nitrogen-containing compounds may also give blue or green colors.

Bromine Water

Phenol



Procedure

Dissolve 0.05 g of unknown in 5 mL of water. Add bromine water drop by drop until the bromine color is no longer discharged.

Positive Test

Discharging of the bromine color or formation of a white precipitate is a positive test.

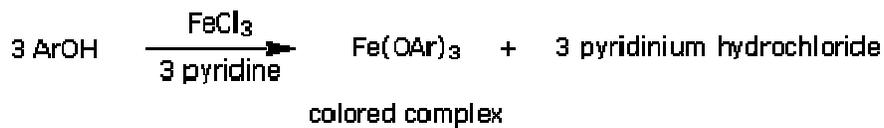
Complications

Mercaptans react readily.

HBr is generated, but will not be observed since the reagent is aqueous.

Ferric Chloride - Pyridine Test

Phenol



Procedure

In hood: Add 2 drops or 20 mg of unknown to 1 mL of pure chloroform in a clean, dry test tube. Stir the solution. If the unknown does not dissolve, even partially, add 2 mL more chloroform and warm gently. Cool to 25°C and add 1 drop of 1% solution of anhydrous ferric chloride in chloroform followed by 2 drops of pyridine. Shake the test tube and note the color produced immediately.

Positive Test

The appearance of blue, violet, purple, green, or red-brown color is a positive test.

Complications

Not all phenols or enols give positive results.

Most oximes, hydroxamic acids, and sulfinic acids give a positive test.

THE PREPARATION OF DERIVATIVES OF ORGANIC COMPOUNDS

The preliminary examination and group classification tests indicate the particular class (functional group) to which an unknown organic compound may belong. Further characterisation and identification depends on the selection and preparation of a suitable solid derivative and accurate determination of its melting point (best, between 90 - 150).

The following table lists some of the classes of organic compounds and a selection of derivatives that may be prepared to characterise them. Check with the tables of melting points in Vogel which derivatives are most suitable for the characterisation of your particular compound.

CLASS OF COMPOUND	DERIVATIVES
1. ALCOHOLS	3,5-dinitrobenzoate
2. PHENOLS	benzoate, acetate, bromo-derivative
3. ALDEHYDES AND KETONES	semicarbazone, 2,4-dinitrophenyl-hydrazone, oxime
4. ACIDS	anilide, amide, p-toluidide.
5. AMINES	benzoyl, acetyl and sulphonamide derivatives

METHODS FOR THE PREPARATION OF DERIVATIVES

ALCOHOLS

(i) 3,5-Dinitrobenzoates

3,5-Dinitrobenzoyl chloride is usually partially hydrolysed and should be prepared in the pure state by heating gently a mixture of 3,5-dinitrobenzoic acid (1 g) and phosphorus pentachloride (1.5 g) in a dry test tube, until it liquifies (5 min).* The liquid is poured on a dry watch glass and allowed to solidify. The phosphoryl chlorides are removed by pressing the solid with a spatula on a wad of filter paper. The residual acid chloride is suitable for immediate use in the preparation of the derivatives.

*Work under fume hood. Fumes are irritating to the eyes and nose.

The 3,5-dinitrobenzoyl chloride is mixed with the alcohol (0.5 - 1 mL) in a loosely corked dry test tube and heated on a steam bath for about 10 min. Secondary and tertiary alcohols require up to 30 min. On cooling add 10 mL sodium hydrogen carbonate solution, stir until the ester crystallises out, and filter at the pump. Wash with a little carbonate solution, water and suck dry. Recrystallise from the minimum hot ethanol or light petroleum. Cool slowly to avoid the formation of oily droplets of your ester.

PHENOLS

(i) Benzoates (Schotten-Baumann method).

To the phenol (0.5 g) is added 5% sodium hydroxide (10 mL) in a well-corked boiling tube or a small conical flask. Benzoyl chloride (2 mL) is added in small quantities at a time, and the mixture shaken vigorously with occasional cooling under the tap or in ice-water. After 15 min the solid benzoate separates out: the solution should be alkaline at the end of the reaction; if not alkaline, or if the product is oily, add a solid pellet of sodium hydroxide and shake again. Collect the benzoate, wash thoroughly with cold water, and recrystallise from alcohol or light petroleum.

(ii) Acetates

Acetates of many simple phenols are liquids; however, this is a suitable derivative for polyhydric and substituted phenols. The phenol (0.5 g) is dissolved in 10% sodium hydroxide solution and an equal quantity of crushed ice is added, followed by acetic anhydride (2 mL). The mixture is vigorously shaken in a stoppered test tube until the acetate separates. The product is filtered and recrystallised from alcohol.

(iii) Bromo derivatives

The phenol (0.3 g) is suspended in dilute hydrochloric (10 mL) and bromine water added dropwise until no more decolourisation occurs. The bromo derivative which precipitates out is filtered off and recrystallised from alcohol.

ALDEHYDES AND KETONES

(i) Semicarbazones

Dissolve semicarbazide hydrochloride (1 g) and sodium acetate (1.5 g) in water (8 - 10 mL), add the aldehyde or ketone (0.3 mL) and shake. Shake the mixture for a few minutes and then cool in ice-water. Filter off the crystals, wash with a little cold water and recrystallise from methanol or ethanol.

(ii) 2,4-Dinitrophenylhydrazones

Suspend 0.25 g of 2,4-dinitrophenylhydrazine in 5 mL of methanol and add 0.5 mL of concentrated sulphuric acid cautiously. Filter the warm solution and add a solution of 0.2 g of the carbonyl compound in 1 mL of methanol. Recrystallise the derivative from methanol, ethanol or ethyl acetate.

(iii) Oximes

Hydroxylamine hydrochloride (0.5 g) is dissolved in water (2 mL). 10% sodium hydroxide (2 mL) and the carbonyl compound (0.2 - 0.3 g) dissolved in alcohol (1 - 2 mL) are added, the mixture warmed on a steam bath for 10 min and then cooled in ice. Crystallisation is induced by scratching the sides of the test tube with a glass rod. The oximes may be crystallised from alcohol.

ACIDS

(i) Amides, anilides and p-toluidides

The acid (0.5 g) is refluxed with thionyl chloride (2 - 3 mL) in a fume cupboard for about 30 mins.* It is advisable to place a plug of cotton wool in the top of the reflux condenser to exclude moisture. The condenser is removed and the excess of thionyl chloride is distilled off (b.p. 78). The acid chloride thus produced is treated with concentrated ammonia solution (5 mL) or aniline (0.5 - 1 mL) or p-toluidine (0.5 - 1 g), when the solid derivative separates out. It is collected and recrystallised from alcohol adding decolourising charcoal if found necessary.

*Alternately use PCl_5 to form the acid chloride.

AMINES

(i) Acetyl derivatives (acetamides)

Reflux gently in a small dry flask under a dry condenser the amine (1 g) with acetic anhydride (3 mL) for 15 min. Cool the reaction mixture and pour into 20 mL cold water. Boil to decompose the excess acetic anhydride. Cool and filter by suction the insoluble derivative. Recrystallise from ethanol.

(ii) Benzoyl derivatives (benzamides)

Suspend 1 g of the amine in 20 mL of 5% aqueous sodium hydroxide in a well-corked flask, and add 2 mL benzoyl chloride (fume hood!), about 0.5 mL at a time, with constant shaking. Shake vigorously for 5 - 10 min until the odour of the benzoyl chloride has disappeared. Ensure that the mixture remains alkaline. Filter off the solid derivative, wash with a little cold water and recrystallise from ethanol.

(iii) Benzenesulphonamides

To 1 g of the amine in 20 mL of 5% sodium hydroxide solution in a well-corked flask add 1 mL benzenesulphonyl chloride (fume hood!). Shake the mixture until the odour of the sulphonyl chloride disappears. Check that the solution is alkaline. Acidify if necessary to obtain the precipitated derivative. Concentrated hydrochloric acid added dropwise should be used. Filter the product, wash with a little cold water and suck dry. Recrystallise from ethanol.