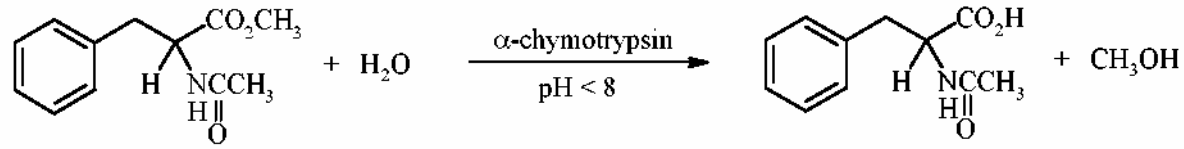
PRACTICAL PROBLEMS

PROBLEM 1 (Practical)

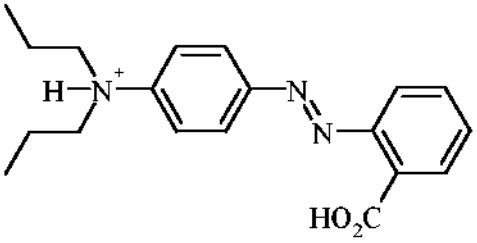
Enzymatic Hydrolysis of Methyl *N*-Acetyl-phenylalaninate

α-Chymotrypsin, a protease enzyme recognizing derivatives of natural α-amino acids, catalyses the hydrolysis of esters. In this experiment the enzymatic hydrolysis of racemic methyl N-acetyl-phenylalaninate **A** is investigated (Scheme).



A **B**

The rate of formation of *N*-acetyl-phenylalanine **B** can be monitored by titration with 0.100 M NaOH in the presence of propyl red as a pH indicator.



Propyl red (protonated form)
At pH < 5: pink; at pH > 6: yellow

Procedure

Note: the required amount of α-chymotrypsin will be supplied in a sample vial by the laboratory assistant on request.

Racemic methyl *N*-acetyl-phenylalaninate **A** [500 mg, the exact mass (± 1 mg) is indicated on the label of the vial marked as NacPheOMe] is transferred quantitatively into a 50 cm^3 Erlenmeyer flask and dissolved in methanol (~ 2.5 cm^3). Subsequently, propyl red (0.02 % solution in ethanol; 4 drops) is added. The kinetic experiment is started by adding α-chymotrypsin (10.0 cm^3 of a 0.05 % solution in distilled water) in one portion (*start the stopwatch*).

When the reaction mixture turns pink, it is immediately titrated with 0.100 M NaOH

until the colour changes to yellow. When the pink colour reappears, add just enough titrant to restore the pale yellow colour, swirling the flask continually during the addition. You only need to record the reading on the burette every 5 minutes. (*Note: at the beginning colour changes occur very frequently.*)

Monitor the reaction for 75 minutes. A graph showing the amounts of NaOH consumed in cm^3 versus time is constructed, in order to visualize the kinetic course of this enzymatic reaction.

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SOLUTION

The competitors were expected to show on the answer sheet the following data, tables, graphs or calculations:

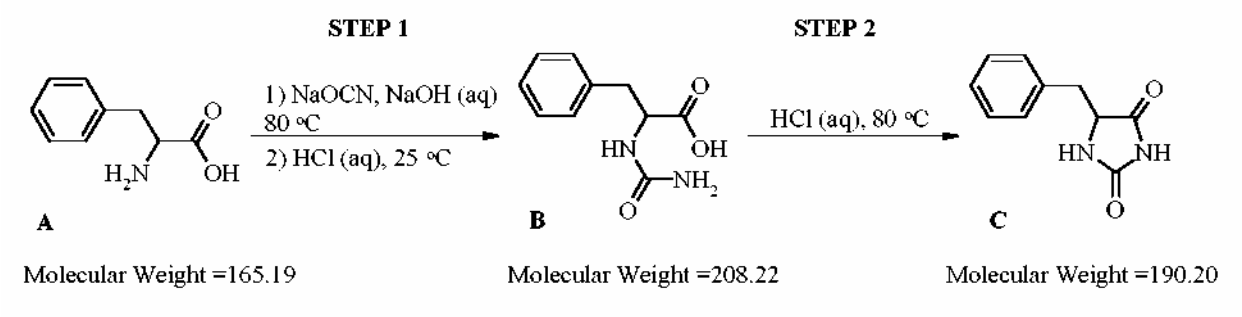
1. Amount of the starting racemic methyl *N*-acetyl-phenylalaninate **A** (in mg and mmol).
2. Table with time measured and the total consumption of NaOH in cm^3.
3. A graph of the total consumption of NaOH vs time.
4. Calculation of the amount of 0.100 M NaOH consumed in this experiment in mmol.
5. Calculation of the degree of hydrolysis of methyl *N*-acetyl-(*R,S*)-phenylalaninate **A** in mol %.

PROBLEM 2 (Practical)

Synthesis of Benzylhydantoin

Introduction

α-Amino acids are the building blocks for peptides and proteins. They are also frequently used as starting material for the synthesis of pharmaceuticals. In this experiment natural *S*-phenylalanine **A** is converted in two steps into benzylhydantoin **C**, which is a useful intermediate for the preparation of various physiologically active derivatives.



Procedure

STEP 1

*Retain a tiny amount of starting material **A** for the TLC analysis (see below).* A long-necked round-bottomed flask is charged with (*S*)-phenylalanine **A** (500 mg, 3 mmol, the exact amount is indicated on the label of the vial), sodium cyanate (300 mg, 4.6 mmol), water (3 cm^3) and a stirring bar. Two drops of aqueous sodium hydroxide (1 M) are added to the stirred suspension. The flask is equipped with a condenser (distillation column) and the reaction mixture is heated to 80 ^oC on a sand bath while stirring magnetically.

Important

In order to reach the appropriate temperature in time and not lose too much time, start the electric heating of the sand bath immediately at the beginning of this experiment. Check the temperature of the sand bath regularly and carefully with a thermometer.

After heating the reaction mixture at 80 ^oC for at least 30 minutes, the resulting clear solution is cooled to room temperature and poured into a small Erlenmeyer flask. Rinse the round-bottomed flask with a little water. The solution is acidified by dropwise addition

of hydrochloric acid (4 M) to pH < 3 with magnetic stirring. Some water is added to the resulting white suspension in order to facilitate stirring.

The white precipitate is then filtered off by suction, washed with ample water (on the filter) and then washed twice with a small amount of di-isopropyl ether to remove most of the adhering water. The urea derivative **B** is left on the filter under suction for at least 3 minutes to remove as much solvent as possible.

A small amount of the obtained urea derivative **B** is retained for TLC-analysis later.

STEP 2

The urea derivative **B** is now transferred into a long-necked round-bottomed flask and hydrochloric acid (4 M, 3 cm^3) is added. A stirring bar is introduced and the suspension is stirred thoroughly whilst heating at 80 ^oC on a sand bath. A clear solution is obtained. After a reaction time of 30 minutes, the reaction mixture, which may already contain some precipitate, is cooled to room temperature. The obtained suspension is filtered by suction, washed thoroughly with water and finally washed twice with a small amount of di-isopropyl ether. The product is left on the filter under suction for at least 3 minutes. It is then collected on a filter paper and dried in the air for at least 30 minutes.

The final product **C**, its precursor **B** and starting material **A** (see above) are subjected to TLC-analysis. For this purpose small amounts of either compound are dissolved in a tiny amount of pure acetone. Small samples of these solutions are applied to a TLC plate, using the supplied capillary tubes. The analysis is carried out with two TLC plates in one run. The TLC-plates are developed with a solution of 2% formic acid in ethyl acetate as the eluent. After the elution the TLC-plates are analysed using a UV-lamp. The starting line, solvent front and the UV-active spots are clearly marked with a pencil. Copy the diagram in the box on the answer sheet. The R_f values are determined. Finally, the TLC-plate with the best analysis is wrapped in parafilm and placed in a plastic bag with a sealing strip.

The final product **C** is transferred into a sample vial of which the empty weight has been pre-determined (weight is indicated on the label). Weigh the vial with product and calculate the yield of the product **C**.

The examination committee will check the quality of the benzylhydantoin that you have prepared by determining its melting point using an automatic melting point apparatus.

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SOLUTION

The competitors were expected to show the following data, tables, graphs or calculations on the answer sheet:

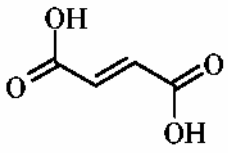
1. Mass of your starting material **A** (see label on the vial) in mg.
2. Amount of benzylhydantoin **C** obtained.
3. Calculation of the yield of benzylhydantoin **C** in %.
4. R_f value of urea derivative **B.**
5. R_f value of benzylhydantoin **C.**
6. A copy of the TLC diagram with indication of the front base line of the solvent.
7. Conclusions from the TLC analysis:
   Compound **B**: is pure; contains some **A**; contains several contaminants.
   Compound **C**: is pure; contains some **B**; contains some **A** and **B**; contains several contaminants.
8. Appearance of benzylhydantoin **C**: white colour, yellowish colour, sticky, crystalline, powder.
9. Melting point of benzylhydantoin **C** was determined by the examination committee.
10. The TLC plate was necessary to pack in an envelope and leave it to a laboratory superviser.

PROBLEM 3 (Practical)

Determination of Iron in Iron Pills

Introduction

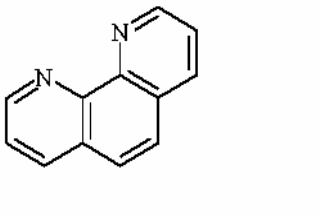
Iron is an essential component of hemoglobin, transporting oxygen in the blood to all parts of the body. It also plays a vital role in many metabolic reactions. Iron deficiency can cause anaemia resulting from low levels of hemoglobin in the blood. Iron deficiency is the most widespread mineral nutritional deficiency worldwide. One way to reduce iron shortage is by treatment with iron pills. The active ingredient in the iron pill to be examined, is iron(II) present as iron(II) fumarate. Besides this organic iron(II) compound the pill contains other compounds such as binding agents. The structure of fumaric acid is:



Fumaric acid

Iron(II) and 1,10-phenanthroline form an orange/red coloured complex $[(C_{12}H_8N_2)_3Fe]^{2+}$. The absorbance of this complex, determined at 510 nm in a buffer solution (pH=8) is a measure for the iron content of the iron pill. Since 1,10-phenanthroline only binds to iron(II) and iron(II) is readily oxidized to iron(III), hydroxylammonium chloride is added to reduce all iron(III) to iron(II). A simplified reaction scheme is:

$$2\ NH_2OH + 4\ Fe^{3+} \rightarrow N_2O + 4\ H^+ + H_2O + 4\ Fe^{2+}$$



1,10-Phenanthroline

Procedure

The weight of the iron pill is determined with an accuracy of 1 mg using a balance. The pill is carefully pulverized in a mortar and transferred quantitatively into a 100 cm^3 beaker with the aid of a small amount of distilled water. Hydrochloric acid (5 cm^3, 4 M) is added. The content of the beaker is heated up to approximately 60 ℃ on a hotplate. The solution turns a yellow colour.

The beaker is then placed in an ultrasonic bath for at least 5 minutes. The beaker is kept in place by styrofoam. The suspension is filtered by suction using a Hirsch funnel containing a small layer of moistened hi-flow filter aid pressed onto the filter. The hi-flow filter aid is washed with ample distilled water. The filtrate is carefully transferred into a volumetric flask (250 cm^3) and the final volume adjusted by adding distilled water and with regular mixing. An amount of 10 cm^3 is pipetted from this solution and transferred into a volumetric flask of 100 cm^3. Again the volume is adjusted with distilled water while mixing the content of the flask.

From this solution, 10 cm^3 is pipetted and transferred into a volumetric flask of 100 cm^3. Subsequently, 1,10-phenanthroline solution (10 cm^3) and hydroxylammonium chloride solution (1 cm^3) are added. Then the volume is adjusted with buffer solution (pH 8).

The absorbance of this solution is measured with a spectrophotometer at 510 nm against water as a blank in a 1.000 cm cell.

Calculate the amount of iron in the iron pill on basis of the known molar absorptivity (extinction coefficient, ε) of the iron(II)phenanthroline complex at 510 nm. The molar absorptivity of the iron(II)phenanthroline complex at 510 nm is 11100 $M^{-1}cm^{-1}$.

Important

In order to eliminate deviations in absorbance typically connected to the spectrophotometer used, a correction factor is denoted on the spectrophotometer you will be using for your experiment. The absorbance observed must be multiplied by this factor in order to obtain the correct absorbance of the solution of the iron complex.

SOLUTION

The competitors were expected to show the following data, tables, graphs or calculations:

1. Weight of the iron pill in mg.
2. Reading of the spectrophotometer and corrected absorbance.
3. Calculation of the concentration of iron(II)phenanthroline complex in the cell in mmol dm^{-3}.
4. Calculation of the total amount of iron(II) in the pill in mg.
5. Calculation of the iron content of the pill in weight %.