Coenzyme Synthesis of Benzoin

Coenzyme chemistry
Benzoin condensation

In this experiment, a benzoin condensation of benzaldehyde will be carried out with a biological coenzyme, thiamine hydrochloride, as the catalyst:

![Reaction diagram]

The mechanistic information needed for understanding how thiamine accomplishes this reaction is given in the essay that precedes this experiment. The conversion of benzaldehyde to benzoin can also be accomplished with cyanide ion, an inorganic reagent, as the catalyst (see Question 7).

SPECIAL INSTRUCTIONS
This experiment may be conducted concurrently with another experiment. It involves a few minutes at the beginning of a first laboratory period for mixing reagents and is finished in a subsequent laboratory period. The remaining portion of the period may be used for another experiment.

WASTE DISPOSAL
Pour all the aqueous solutions produced in this experiment into a waste container for aqueous waste. The ethanolic mixtures obtained from the crystallization of crude benzoin should be poured into a waste container designated for nonhalogenated waste.

NOTES TO THE INSTRUCTOR
It is essential that the benzaldehyde used in this experiment be pure. Benzaldehyde is easily oxidized in air to benzoic acid. Even when benzaldehyde appears free of benzoic acid by infrared spectroscopy, you should check the purity of your benzaldehyde and thiamine by following the instructions given in the first paragraph of the Procedure (Reaction Mixture section). When the benzaldehyde is pure, the solution will be nearly filled with solid benzoin after two days (you may need to scratch the inside of the flask to induce crystallization). If no solid appears, or very little appears, then there is a problem with the

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purity of the benzaldehyde. If possible, use a newly opened bottle that has been purchased recently. However, it is essential that you check both the old and new benzaldehyde before doing the laboratory experiment.

We have found that the following procedure does an adequate job of purifying benzaldehyde. The procedure does not require distillation of benzaldehyde. Shake the benzaldehyde in a separatory funnel with an equal volume of 5% aqueous sodium carbonate solution. Shake gently, and occasionally open the stopcock of the funnel to vent carbon dioxide gas. An emulsion forms that may take two to three hours to separate. It is helpful to stir the mixture occasionally during this period to help break the emulsion. Remove the lower sodium carbonate layer, including any remaining emulsion. Add about ¼ volume of water to the benzaldehyde, and shake the mixture gently so as to avoid an emulsion. Remove the cloudy lower organic layer, and dry the benzaldehyde with calcium chloride until the next day. Any remaining cloudiness is removed by gravity filtration through fluted filter paper. The resulting clear purified benzaldehyde should be suitable for this experiment without vacuum distillation. You must check the purified benzaldehyde to see if it is suitable for the experiment by following the instructions in the first paragraph of the Procedure.

It is advisable to use a fresh bottle of thiamine hydrochloride, which should be stored in the refrigerator. Fresh thiamine does not seem to be as important as pure benzaldehyde for success in this experiment.

**PROCEDURE**

**Reaction Mixture.** Add 0.30 g of thiamine hydrochloride to a 25-mL Erlenmeyer flask. Dissolve the solid in 0.45 mL of water by swirling the flask. Add 3.0 mL of 95% ethanol, and swirl the solution until it is homogeneous. To this solution add 0.90 mL of an aqueous sodium hydroxide solution and swirl the flask until the bright yellow color fades to a pale yellow color. Weigh the flask and solution, add 0.90 mL of benzaldehyde, and reweigh the flask to determine an accurate weight of benzaldehyde introduced to the flask. Swirl the contents of the flask until it is homogeneous. Stopper the flask, and let it stand in a dark place for at least 2 days.

**Isolation of Crude Benzoin.** If after 2 days crystals have not formed, initiate crystallization by scratching the inside of the flask with a glass stirring rod. Allow about 5 minutes for the crystals of benzoin to form fully. Place the flask, with crystals, into an ice bath for 5-10 minutes. If for some reason the product separates as an oil, it may be helpful to scratch the flask with a glass rod or seed the mixture by allowing a small amount of solution to dry on the end of a glass rod and then placing this into the mixture. Cool the mixture in an ice bath before filtering. Break up the crystalline mass with a spatula, swirl the flask rapidly, and quickly transfer the benzoin to a Hirsch funnel under vacuum.

Wash the crystals with three 1.0-mL portions of ice-cold water. Allow the benzoin to dry in the Hirsch funnel by drawing air through the crystals for about 5 minutes. Transfer the benzoin to a watch glass, and allow it to dry in air for one day. The product may be dried in a few minutes in an oven set at about 100°C.

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2 Dissolve 8.0 g of NaOH in 100 mL water.
Yield Calculation and Melting-Point Determination. Weigh the benzoin and calculate the percentage yield. Determine the melting point (pure benzoin melts between 134 and 135°C). Since your crude benzoin will normally melt between 129 and 132°C, the benzoin should be crystallized before conversion to benzin (Experiment 33) or benzilic acid (Experiment 34).

Crystallization of Benzoin. Purify the crude benzoin by crystallization from hot 95% ethanol (use 0.8 mL of alcohol/0.1 g of crude benzoin) using a 10-mL Erlenmeyer flask for the crystallization. On cooling in an ice bath, collect the crystals on a Hirsch funnel. Determine the melting point of the product.

Spectroscopy. Determine the infrared spectrum of the benzoin as a KBr pellet.

QUESTIONS
1. The infrared spectrum of benzoin and benzaldehyde are given in this experiment. Interpret the principal peaks in the ir spectra.
2. Why is sodium hydroxide added to the solution of thiamine hydrochloride?
3. Using the information given in the essay that precedes this experiment; formulate a complete mechanism for the thiamine-catalyzed conversion of benzaldehyde to benzoin.
4. How do you think the appropriate enzyme would have affected the reaction (degree of completion, yield, stereochemistry)?
5. What modifications of conditions would be appropriate if the enzyme were to be used?
6. Draw a mechanism for the cyanide-catalyzed conversion of benzaldehyde to benzoin. The intermediate, shown in brackets, is thought to be involved in the mechanism.
Benzil

Oxidation
Crystallization

In this experiment, an \( \alpha \)-diketone benzil, is prepared by the oxidation of an \( \alpha \)-hydroxyketone, benzoin (Experiment 32). This oxidation can be done easily with mild oxidizing agents such as Fehling’s solution (alkaline cupric tartrate complex) or with copper sulfate in pyridine. In this experiment, the oxidation is performed with nitric acid.

SPECIAL INSTRUCTIONS

Nitric acid should be dispensed in a good hood to avoid the choking odor of this substance. The vapors will irritate your eyes. Avoid contact with your skin. During the reaction, considerable amounts of noxious nitrogen oxide gases are evolved. Be sure to run the reaction in a good fume hood.

WASTE DISPOSAL

The aqueous nitric acid wastes should be poured into the aqueous waste container. The ethanolic wastes from the crystallization should be poured into the nonhalogenated waste container.

PROCEDURE

Reaction Mixture. Place 0.30 g of benzoin (Experiment 32) in a 5-mL conical vial and add 1.5 mL of concentrated nitric acid. Add a spin vane, and attach an air condenser. In a hood, set up the apparatus for heating in a hot water bath. Heat the mixture in a hot water bath at about 70°C for one hour, with stirring. Avoid heating the mixture above this temperature to reduce the possibility of forming a by-product. During the one-hour heating period, nitrogen oxide gases (red) will be evolved. If it appears that gases are still being evolved after one hour, continue heating for another 15 minutes, but then discontinue heating at that time.

Isolation of Crude Product. Cool the mixture for a few minutes, and detach the air condenser. With a Pasteur pipet, transfer the reaction mixture to a beaker containing 4 mL of ice cold water. Rinse the conical vial and spin vane with a small amount of water. Cool the mixture in an ice bath until crystals have formed. If the material o ils out rather than crystallizes, scratch the oil vigorously with a spatula until it does crystallize completely. Collect the crude product on a Hirsch funnel under vacuum

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3 At higher temperatures, some 4-nitrobenzil will also be formed along with benzil.
(Technique 4, Section 4.3, and Fig. 4.5, p. 551). Wash it well with cold water (about 5 mL). Continue drawing air through the solid mass on the Hirsch funnel to help dry the solid. Weigh the solid.

**Crystallization of Product.** Purify the solid by dissolving it in hot 95% ethanol in a small Erlenmeyer flask (about 5 mL per 0.5 gram of product) using a hot plate as the heating source. Be careful to not melt the solid on the hot plate. You can avoid melting the benzil by occasionally lifting the flask from the hot plate and swirling the contents of the flask. You want the solid to dissolve in the hot solvent, rather than melt. You will obtain better crystals if you add a little extra solvent after it dissolves completely. Remove the flask from the hot plate, and allow the solution to cool slowly. As the solution cools, seed it with a solid product that forms on a spatula after the spatula is dipped into the solution. The solution may become supersaturated unless this is done, and crystallization will occur too rapidly. Yellow crystals are formed. Cool the mixture in an ice bath to complete the crystallization. Collect the product on a Hirsch funnel, under vacuum. Rinse the flask with small amounts (about 1 mL total) of ice-cold 95% ethanol to complete the transfer of product to the Hirsch funnel. Continue drawing air through the crystals on the Hirsch funnel by suction for about 5 minutes. Then remove the crystals and air-dry them.

**Yield Calculation and Melting-Point Determination.** Weigh the dry benzil, and calculate the percentage yield. Determine the melting point. The melting point of pure benzil is 95°C. Submit the benzil to the instructor unless it is to be used to prepare benzilic acid (Experiment 34). At the instructor’s option, obtain the infrared spectrum of benzil in KBr. Compare it to the spectrum shown in this experiment. Also, compare it with the spectrum of benzoin shown on page 309. What differences do you notice?

**Experiment 34**

**BenziIic Acid**

Anionic Rearrangement

In this experiment, benzilic acid will be prepared by causing the rearrangement of the α-diketone benzil. Preparation of benzil is described in Experiment 33. The rearrangement of benzil proceeds in the following way:

![Chemical Reaction Diagram]

The driving force for the reaction is provided by the formation of a stable carboxylate salt (potassium benzilate). Once this salt is produced, acidification yields benzilic acid. The reaction can generally be...
used to convert aromatic α-diketones to aromatic α-hydroxyacids. Other compounds, however, also will undergo benzilic acid type of rearrangement (see Questions).

**SPECIAL INSTRUCTIONS**

This experiment works best with pure benzil. The benzil prepared in Experiment 33 is usually of sufficient purity after it has been crystallized.

**WASTE DISPOSAL**

Pour all the aqueous filtrates into the waste bottle designated for aqueous waste. Ethanolic filtrate should be put in the nonhalogenated organic waste bottle.

**PROCEDURE**

**Running the Reaction.** Add 0.100 g of benzil and 0.30 mL of 95% ethanol to a 3-mL conical vial. Place a spin vane in the vial, and attach an air condenser. Heat the mixture with an aluminum block (90 - 100°C) while stirring until the benzil has dissolved. Using a 9-inch Pasteur pipet, add dropwise 0.25 mL of an aqueous potassium hydroxide solution\(^4\) downward through the condenser into the vial. Gently boil the mixture (aluminum block about 110°C) while stirring for 15 minutes. The mixture will be blue-black in color. As the reaction proceeds, the color will turn to brown, and the solid should dissolve completely. Solid potassium benzilate may form during the reaction period. At the end of the heating period, remove the assembly from the aluminum block and allow it to cool for 1-2 minutes.

**Crystallization of Potassium Benzilate.** Detach the air condenser when the apparatus is cool enough to handle. Transfer the reaction mixture, which may contain some solid, with a Pasteur pipet into a 10-mL beaker. Allow the mixture to cool to room temperature and then cool in an ice-water bath for about 15 minutes until crystallization is complete. It may be necessary to scratch the inside of the beaker with a glass stirring rod to induce crystallization. Crystallization is complete when virtually the entire mixture has solidified. Collect the crystals on a Hirsch funnel by vacuum filtration and wash the crystals thoroughly with three 1-mL portions of ice-cold 95% ethanol. The solvent should remove most of the color from the crystals.

Transfer the solid, which is mainly potassium benzilate, to a 10-mL Erlenmeyer flask containing 3 mL of hot water. Stir the mixture until all the solid has dissolved or until it appears that the remaining solid will not dissolve. Any remaining solid will likely form a fine suspension. **If solid still remains in the flask,** filter the mixture in the following manner. Place about 0.5 g of Celite (Filter Aid) in a beaker with about 5 mL of water. Stir the mixture vigorously and then pour the contents into a Hirsch funnel (with filter paper) or a small Buchner funnel while applying a *gentle vacuum*, as in a *vacuum* filtration. Be careful not to let the Celite dry completely. This procedure will cause a thin layer of Celite to be deposited on the filter paper. Discard the water that passes through this filter. Pass the mixture containing potassium benzilate through this filter, using *very gentle* suction. The filtrate should be clear.

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\(^4\) The aqueous potassium hydroxide solution should be prepared for the class by dissolving 2.75 g of potassium hydroxide in 6.0 mL of water. This will provide enough solution for 20 students, assuming little solution is wasted.
Transfer the filtrate to a 10-mL Erlenmeyer flask. If no solid remains in the flask, the filtration step may be omitted. In either case, proceed to the next step.

**Formation of Benzilic Acid.** With stirring, add dropwise 0.5 mL of 1 M hydrochloric acid to the solution of potassium benzilate. As the solution becomes acidic, solid benzilic acid will precipitate. The pH should be about 2; if it is higher than this, add a few more drops of acid and check the pH again. Allow the mixture to cool to room temperature, and then complete the cooling in an ice bath. Collect the benzilic acid by vacuum filtration using a Hirsch funnel. Wash the crystals thoroughly with 3-4 mL of water to remove salts and remove the wash water by drawing air through the filter. Dry the product thoroughly by allowing it to stand until the next laboratory period.

**Melting Point and Crystallization of Benzilic Acid.** Determine the melting point of the product. Pure benzilic acid melts at 150 °C. If necessary, crystallize the product from hot water using a Craig tube (Technique 5, Section 5.4, and Fig. 5.5, p. 566). If some impurities remain undissolved, filter the mixture using the following procedure. It will be necessary to keep the mixture hot during this filtration step. Transfer the hot mixture to a test tube with a Pasteur pipet. Clean the Craig tube, and filter the mixture by transferring it back to the Craig tube with a filter-tip pipet. Cool the solution, and induce crystallization, if necessary. Allow the mixture to stand at room temperature until crystallization is complete (about 15 minutes). Cool the mixture in an ice bath, and collect the crystals by centrifugation. Determine the melting point of the crystallized product. At the instructor’s option, determine the infrared spectrum of the benzilic acid in potassium bromide. Calculate the percentage yield. Submit the sample to your laboratory instructor in a labeled vial.

**QUESTIONS**

1. Show how to prepare the following compounds, starting from the appropriate aldehyde (see Experiments 32 and 33).

![Diagram of compounds](image)

2. Give the mechanisms for the following transformations:
3. Interpret the infrared spectrum of benzilic acid.