A GREENER, BIOCATALYTIC BENZOIN SYNTHESIS
Kenyon College – CHEM 234 – Organic Chemistry Lab II
Adapted from Mircoscale Organic Laboratory, 3rd ed. by Mayo, Pike, and Trumper

CYANIDE ION AND THIAMINE CATALYZED BENZOIN CONDENSATION

The reaction of two moles of benzaldehyde to form a new carbon-carbon bond is known as the benzoin condensation. Two rather different catalysts promote this reaction; namely, cyanide ion and the vitamin thiamine. Both catalysts function in similar fashion in this reaction. In many ways the benzoin condensation is a unique reaction for benzaldehyde. Benzaldehyde does not possess an β-hydrogen, therefore, benzaldehyde cannot form enolate (enol) reaction intermediates. This precludes intermolecular aldol condensation reactions. Second, the electronic character of the phenyl ring is perfectly suited for the benzoin condensation. Electron-donating substituents on the phenyl ring inhibit benzoin condensation because the carbanion intermediate is destabilized. Conversely, electron-withdrawing groups on the phenyl ring stabilize the analogous carbanion and subsequent nucleophilic addition reaction will not occur.

One approach to the synthesis of benzoin by condensation involves the use of cyanide ion (See Mayo et. al.). Cyanide is a strong nucleophilic agent. Addition of the cyanide ion to the aldehyde carbonyl forms a stable cyano hydrin. Mandelonitrile, the product of the addition, can be isolated as a stable liquid. Under basic conditions however, mandelonitrile loses a proton from C-1 carbon giving a resonance-stabilized carbanion. This carbanion, in turn, functions as a nucleophile and attacks a second molecule of benzaldehyde. This condensation intermediate experiences proton transfer and loses the cyanide ion producing benzoin. Evidence for this mechanism lies in the failure of 4-nitrobenzaldehyde to undergo the same reaction. The nitro group reduces the nucleophilic character of the resonance-stabilized anion. On the other hand, a strong electron-donating group in the 4-position of the phenyl ring makes the loss of the proton from the cyano hydrin very difficult, thus 4-N,N-dimethylaminobenzaldehyde also does not undergo the benzoin condensation.

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{O} & \quad \text{H} \\
\text{2 H} & \quad \text{CN}^- \\
\text{catalyst} & \quad \text{base} \\
\text{OH} & \quad \text{H} \\
\text{phenyl} & \quad \text{phenyl}
\end{align*}
\]

Figure 1. Formation of Benzoin by use of Cyanide Ion.

Altenatively, a number of biochemical reactions bear close similarity to the benzoin condensation. Obviously, biological systems will not be catalyzed by the highly toxic cyanide ion. In the 1960’s, Ronald Breslow, of Columbia University, proposed that vitamin B₁, thiamine hydrochloride, served as a catalyst in biochemical acyloin condensations. Specifically, coenzyme thiamine pyrophosphate was identified as an active catalyst. The resonance-stabilized conjugate base of the thiazolium ion (thiamine)

\[\text{H}O\text{H}^{-}\text{CN}^{-}\text{H}\]

and the resonance-stabilized carbanion that forms, are key intermediates in this reaction. Like cyanide ion, the thiazolium ion has the right balance of nucleophilicity, ability to stabilize the intermediate anion, and leaving group properties. Thiamine is an essential vitamin that must be provided in the diet. Its presence in the diet prevents beriberi, a nervous system disease.

![Figure 2. Coenzyme Thiamine Pyrophosphate.](image)

**Thiamine as a Coenzyme**

Vitamin B1, thiamine (actually thiamine pyrophosphate) is a universal coenzyme present in all living systems. It was originally discovered as a required nutritional factor (vitamin) in humans by its link with the disease beriberi. Symptoms for this disease include pain and paralysis of the extremities, emaciation, or swelling of the body. The disease is most common in the developing countries in Asia.

Thiamine serves as a coenzyme (defined later) for three important types of enzymatic reactions:

1) Nonoxidative Decarboxylations of α-Keto Acids

\[
\text{R-CH}_{2}\text{COOH} \xrightarrow{\text{Vitamin B}_1} \text{R-CH}_2\text{H} + \text{CO}_2
\]

2) Oxidative Decarboxylations of α-Keto Acids

\[
\text{R-CH}_{2}\text{COOH} \xrightarrow{\text{Vitamin B}_1} \text{R-CHOH} + \text{CO}_2
\]

3) Formation of Acyloins (α-Hydroxy Ketones)

\[
\text{R-CH}_{2}\text{COOH} + \text{R-CH}_2\text{H} \xrightarrow{\text{Vitamin B}_1} \text{R-CHOH} + \text{CO}_2
\]
Most biochemical processes involve a series of elementary organic chemical reactions that are carried out under special enzymatic conditions. Most steps of ubiquitous metabolic pathways can be explained by mechanistic organic chemistry. Although simple organic reactions serve as models for biological reactions, they are often modified ingeniously through the intervention of biocatalyst. When proteins molecules intervene in a reaction they influence the reaction by either stabilizing the transition state of a reaction, or by making the reaction more selective, and/or making the reactions more stereospecific. If any of these events characterize the influence of the protein on a reaction, then the protein is called an enzyme. One further characteristic of an enzyme is that they can bring about chemical reactions under a narrow pH range. Reactions in the chemistry laboratory utilize highly acidic to highly basic reaction conditions. Enzymatic reactions, however, must utilize a pH ranging from 4-9.

From an organic chemical perspective, the most important part of the entire thiamine molecule is the central thiazole ring. This ring constitutes the reactive portion of the coenzyme. The other structural features of thiamine, although important in a biological sense, are not involved in the catalytic chemistry of thiamine. Undoubtedly the pyridine ring and the pyrophosphate group have important ancillary functions, such as enabling the coenzyme to make the correct attachment to its associated protein molecule.

Bioorganic experiments with the model compound 3,4-dimethylthiazolium bromide illustrate that the thiazolium ring catalyzes all of expected acyloin condensations. For example, it was found that the model compound rapidly exchanged the C-2 proton for deuterium in D₂O solution. At a pD of 7 (No pH here!), this proton was completely exchanged in seconds! This indicates that C-2 proton is more acidic than one would have expected. The hydrogen at C-2 is easily removed because the conjugate base is highly stabilized. Stabilization, in this case, arise from the presence of positive and negative formal charges on adjacent atoms. This chemical arrangement is called an ylide structure.

![Figure 3. Deuterium Exchange in 3,4-Dimethylthiazolium Bromide.](image)
The sulfur atom in the thiazolium ring plays an important role in stabilizing the ylide structure. For instance, the rate of deuterium exchange in 1,3-dimethylimidazolium ion is much slower than the exchange rate of the thiazolium ion. Sulfur, being in the third row of the periodic chart, has d-orbitals available for bonding to adjacent atoms. Thus, it has fewer geometrical restrictions than carbon and nitrogen and can form carbon-sulfur bonds in situations in which carbon and nitrogen normally would not. These bonding situations can delocalize the negative charge and thereby stabilize the conjugate base.

![Figure 4. Slow Deuterium Exchange for 1,3-Dimethylimidazolium Bromide](image)

**Function of a Coenzyme**

In biological terminology, thiamine is a coenzyme. It must bind to an enzyme before the enzyme is activated. The same enzyme binds the substrate. The coenzyme reacts with the substrate while they are both bound to the enzyme (a large protein). Without the coenzyme thiamine, no chemical reaction would occur. The coenzyme is the chemical reagent. The protein molecule (the enzyme) mediates the reaction by controlling stereocritical, energetic, and entropic factors, but, in this case, it is nonessential to the overall result. A special name is given to coenzymes that are essential to the nutrition of an organism. They are called vitamins. Many biological reactions are of this type, in which a chemical reagent (coenzyme) and a substrate are bound to an enzyme for reaction and, after the reaction, are again released into the medium.

**REFERENCES**

SYNTHESIS OF BENZOIN WITH THIAMINE

**Reaction Mixture.** Add 0.67 g of thiamine hydrochloride to a 25-ml Erlenmeyer flask. Dissolve the solid in 1.0 ml of water by swirling the flask. Add 6.7 ml of 95% ethanol and swirl the solution until it is homogeneous. To this solution, add 2.0 ml of an aqueous 8% sodium hydroxide solution (8.0 g NaOH in 100 ml water, provided) and swirl the flask until the bright yellow color fades to a pale yellow color. Add 2.0 ml of benzaldehyde and swirl the contents of the flask until it is homogeneous. Stopper the flask and allow it to 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 ~2-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.

**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 an appropriately sized Erlenmeyer flask for the crystallization. Upon cooling in an ice bath, collect the crystals on a Hirsch funnel. Try to maximize your isolated yield, both crude and recrystallized. It is suggested that you try to obtain a second crop of crystals to increase your yield of benzoin. After the recrystallization of benzoin, you should evaporate the ethanol until the solution is almost cloudy while hot. Upon cooling a second crop of benzoin can be isolated. Transfer the benzoin to a watch glass and allow it to dry in an oven set at about 100° C for a few minutes.

**Yield Calculation and Melting Point Determination.** Use 2-3 mg of air-dried purified benzoin to obtain a melting point.

**Spectroscopy.** Record an IR spectrum of your benzoin sample. Grind ~3 mg of product in ~100 mg of KBr.