Experiment 1. The Determination of an Equilibrium Constant by NMR spectroscopy

When left to stand in solution, unsymmetrical azines, \( R^1 \text{CH}=\text{N} \equiv \text{N}=\text{CHR}^2 \), disproportionate, and an equilibrium mixture is reached which contains the original unsymmetrical azine, and the two symmetrical azines, \( R^1 \text{CH}=\text{N} \equiv \text{N} \equiv \text{CHR}^1 \) and \( R^2 \text{CH}=\text{N} \equiv \text{N}=\text{CHR}^2 \). This experiment illustrates the use of NMR spectroscopy to determine the equilibrium constant, \( K_E \), for the disproportionation of the unsymmetrical azine of benzaldehyde and acetophenone, \( M \), into the symmetrical azines \( A \) and \( B \) of acetophenone and benzaldehyde.

The equilibrium is given by:

\[
K_E = \frac{[A][B]}{[M]^2}
\]

where \([A]\), \([B]\) and \([M]\) are the concentrations of \( A \), \( B \) and \( M \) respectively at equilibrium. The reaction is set up in an NMR tube, and NMR spectroscopy is used to determine the concentrations of the various compounds in the equilibrium mixture.

Since the equilibrium can be reached from either direction, the experiment can be conducted in several ways:

- you can prepare a sample of the mixed azine, \( M \), and then observe its disproportionation by NMR spectroscopy;
- you can prepare both single azines, \( A \) and \( B \), make an equimolar mixture of the two, and observe the formation of the mixed azine, \( M \), by NMR spectroscopy;
- you can prepare one of the single azines, \( A \), and then make an equimolar mixture with the single azine, \( B \), which has been prepared by a fellow student.
The symmetrical azines \( \text{A} \) and \( \text{B} \) are synthesized from hydrazine hydrate and acetophenone and benzaldehyde, respectively. The reaction involving benzaldehyde proceeds readily, although the acetophenone reaction requires the presence of an acid. The preparation of the mixed unsymmetrical azine \( \text{M} \) is slightly more time consuming.

\[
\begin{align*}
\text{Ph}_2\text{O} & \xrightarrow{\text{H}_2\text{NNH}_2} \text{Ph} - \text{N} - \text{N} - \text{Ph} \\
\text{Ph}_2\text{O} & \xrightarrow{\text{H}_2\text{NNH}_2} \text{Ph} - \text{N} - \text{N} - \text{Ph} \\
\text{Ph}_2\text{O} & \xrightarrow{\text{H}_2\text{NNH}_2} \text{Ph} - \text{N} - \text{N} - \text{Ph}
\end{align*}
\]

### Materials

1. **Preparation of acetophenone azine**
   - Acetophenone (FW 120.1) 6mL, 6.2g (51mmol)  
   - Hydrazine hydrate (FW 32.0) 1mL (ca.20mmol)  
   - Ethanol  
   - Hydrochloric acid (conc.)

2. **Preparation of benzaldehyde azine**
   - Benzaldehyde (FW 106.1) 5mL, 5.2g (49mmol)  
   - Hydrazine hydrate (FW 32.0) 1mL (ca.20mmol)  
   - Ethanol

3. **Preparation of mixed acetophenone benzaldehyde azine**
   - Acetophenone (FW 120.1) 3mL, 3.1g (25mmol)  
   - Ethanoic acid (glacial)  
   - Hydrazine hydrate (FW 32.0) 2.5mL (ca.50mmol)  
   - Diethyl ether  
   - Benzaldehyde (FW 106.1) 2.5mL, 2.6g (25mmol)  
   - Ethanol
**Procedure**

1. **Preparation of acetophenone azine**
   Dissolve the hydrazine hydrate in 7 mL ethanol in a 25 mL flask. Add 1 mL concentrated hydrochloric acid (add slowly and use care), swirl the flask, and add the acetophenone dropwise from a Pasteur pipette. Heat the mixture on a steam bath for 15 min. Filter off the yellow product with suction, and recrystallize it from 95% ethanol. Record the yield and mp of your product.

2. **Preparation of benzaldehyde azine**
   Dissolve the hydrazine hydrate in 7 mL ethanol in a 25 mL Erlenmeyer flask. Swirl the flask, and add the benzaldehyde dropwise from a Pasteur pipette. Cool the mixture in ice, collect the product by suction filtration, and recrystallize it from 95% ethanol. Record the yield and mp of your product.

3. **Preparation of mixed acetophenone benzaldehyde azine**
   Place the hydrazine hydrate in a 25 mL Erlenmeyer flask. In another vessel, dissolve the acetophenone in a mixture of 0.5 mL glacial ethanoic acid and 1 mL ethanol, and add this mixture dropwise to the hydrazine hydrate. Heat the mixture for 10 min on a steam bath, and after cooling, dissolve the mixture in 30 mL diethyl ether. Transfer the ether solution to a separatory funnel and wash it with 2 x 10 mL portions of water. Dry the ether layer over anhydrous MgSO4, and filter off the spent drying agent. Transfer the filtrate to a 50 mL round-bottomed flask, and add the benzaldehyde dropwise from a Pasteur pipette. Allow the mixture to stand for 10 min at room temperature (during which time it may become cloudy), and then evaporate to dryness on the rotary evaporator. Recrystallize the residue from 95% ethanol. Record the yield and mp of your product.

4. **Equilibration studies and determination of KE by NMR spectroscopy**
   Make up the solution for the NMR studies as follows:
   1. Weigh out exactly equimolar (ca. 0.1mmol) quantities of acetophenone azine and benzaldehyde azine, mix them and dissolve in ca. 0.6 mL deuterochloroform. OR
   2. Weigh out about 40mg (ca. 0.2mmol) of the mixed azine, and dissolve it in ca. 0.6 mL deuterochloroform.

   As soon as the relevant NMR solution has been prepared, transfer it to an NMR tube, and record the NMR spectrum immediately. Set the mixture in the NMR tube aside, ideally until next week’s laboratory period, to allow the reaction
to reach equilibrium. If a full week is not available, then the equilibration can be achieved in 2–3h by the addition of 1 drop glacial ethanoic acid to the NMR tube. Occasionally the reaction mixture remains essentially unchanged even after a week; to preclude this, a drop of acid can be added at the start so that equilibrium is achieved quickly. Record a second NMR spectrum once the mixture has equilibrated.

The spectrum of the mixed azine, $M$, shows singlets for the methyl and methine hydrogens at about $\delta 2.6$ and $8.4$, respectively. The spectrum of acetophenone azine, $A$, shows a singlet for its methyl group at about $\delta 2.4$, whereas the benzaldehyde azine, $B$, shows a singlet at about $\delta 8.7$ for the methine hydrogen. The areas of these peaks (obtained by integration) is proportional to the number of protons and the concentration of compound responsible for the signal. Hence the relative concentrations of each species at equilibrium can be determined, and hence the equilibrium constant calculated. Interpret the results.

Questions:

1. Propose a mechanism for azine formation from acetophenone and hydrazine?
2. What product would you expect if acetophenone was reacted with an excess of hydrazine?
3. Propose a mechanism for the disproportionation of $M$. 
Experiment 2. Stereospecific Reduction of Benzoin with Sodium Borohydride; Determination of the Stereochemistry by NMR Spectroscopy

The stereochemistry of the reduction cyclic ketones with sodium borohydride is reasonably well understood with various torsional and steric effects playing important roles determining the relative energies of transition states. The stereochemical course of ketone reductions can be influenced by the presence of hydroxyl groups close to the carbonyl function. This experiment illustrates the stereoselective reduction of benzoin using sodium borohydride as a reducing agent, followed by the conversion of the resulting 1,2-diol into its acetonide (isopropylidene/acetal) derivative catalyzed by anhydrous iron(III) chloride, a reaction commonly used for the protection of 1,2-diols during a synthetic sequence when strong bases are used as reagents. Nuclear magnetic resonance (NMR) spectroscopic analysis of the acetonide permits the determination of its relative stereochemistry and hence that of the diol.
Materials
1. Preparation of 1,2-diphenylethane-1,2-diol
   - benzoin (FW 212.3) 2.00g (9.4mmol) irritant
   - sodium borohydride (FW 37.8) 0.40g (10.6mmol) corrosive, flammable
   - ethanol
   - light petroleum (bp 60–80ºC)
   - hydrochloric acid (6 M)

2. Preparation of acetonide derivate
   - acetone (pure)
   - iron(III) chloride (anhydrous) 0.30g corrosive, hygroscopic
   - dichloromethane
   - light petroleum (bp 40–60ºC)
   - potassium carbonate solution (10%)

Procedure

1. Preparation of 1,2-diphenylethane-1,2-diol
   Dissolve the benzoin in 20 mL of ethanol in a 100 mL Erlenmeyer flask. Stir the solution magnetically, and add the sodium borohydride in small portions over 5 min using a spatula. If necessary, rinse in the last traces of sodium borohydride with 5mL of ethanol. Stir the mixture at room temperature for a further 20 min, and then cool it in an ice bath whilst adding 30 mL of water followed by 1 mL of 6 M hydrochloric acid. Add a further 10 mL of water, and stir the mixture for a further 20 min. Collect the product by suction for 30 min, and record the yield. This material is sufficiently pure to be used, so set aside 1.00 g of the product to be left drying until the next period for use in the next stage. Recrystallize the remainder (ca.0.50 g) from petroleum (bp 60–80ºC). record the mp and IR spectrum of the product after one recrystallization. Record an IR spectrum of benzoin for comparison.

2. Preparation of acetonide derivative (2,2-dimethyl-4,5-diphenyl-1,3-dioxolane)
   Dissolve 1.00 g of the diol in 30 mL of pure acetone, and add the anhydrous iron(III) chloride. Heat the mixture under reflux with a calcium chloride guard tube for 20 min, and then allow it to cool to room temperature. Pour the mixture into a 100 mL beaker containing 40 mL water, and add 10 mL potassium carbonate solution. Transfer the mixture to a 250 mL separatory funnel and extract with 3 x 20 mL portions of dichloromethane. Wash the combined organic extracts with 25 mL water, and then dry them over MgSO4. Evaporate the solvent on the
rotary evaporator, and purify the crude acetonide by dissolving it in 15 mL boiling petroleum (bp 40–60°C), and filtering whilst hot to remove any unreacted diol. Concentrate the filtrate to a volume of 3–5 mL, and then cool the solution in ice, whereupon the acetonide crystallizes out. Collect the product by suction filtration, and wash it with a little ice-cold petroleum. Dry the product by suction at the filter pump for 10 min. Record the yield, mp and IR spectrum of the product. Record the NMR spectrum (CDCl₃) of your purified material for assignment of stereochemistry.

Questions:

1. Discuss the NMR spectrum of the acetonide derivative; assign its stereochemistry, and hence that of the diols.
2. Discuss the mechanism and stereochemistry of the reduction of benzoin; propose a transition state for the reduction which accounts for the stereochemistry.
3. Propose a mechanism for acetonide formation. What is the role of the FeCl₃?
4. Compare and contrast the IR spectra of benzoin, the diol and the acetonide; Assign peaks to the important group frequencies of the functional groups.