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## Preparation of Acetate Derivatives of Alcohols

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### *Introduction*

Hydroxyl groups can be reacted with acetic anhydride to form an acetate derivative (ethyl ester) that is suitable for injection into the GC/MS. Pyridine acts as a basic catalyst to increase the reactivity of sterically hindered hydroxyl groups. The reaction is rapid and simple to perform, the reagents and byproducts are all volatile so no sample extraction is necessary, and the derivatized compounds are indefinitely stable when kept dry and in the freezer. The primary drawback to this method is that only alcohol groups are derivatized. This procedure is commonly used for the analysis of sterols, hopanols, *n*-alkanols, hydroxy-fatty acids, and others.

### *Materials*

- Acetic anhydride
- Anhydrous pyridine
- Methanol (GC grade)
- DCM (GC grade)
- 50  $\mu$ L syringe
- heating block (70°C)
- GC vials

### *Special Hazards and Warnings*

- ☒ Pyridine is toxic, carcinogenic, and smells truly horrible. Keep it in the hood and off your hands and clothes.
- ☒ Capped vials can sometimes burst while being heated. Keep the hood sash lowered while samples are heating, and wear safety glasses.

### *Procedure*

1. Transfer sample to a GC vial in an appropriate solvent (DCM, ether). Samples should be carefully dried prior to this step. Add any internal standard.
2. Add 20  $\mu$ L acetic anhydride and 20  $\mu$ L pyridine (**note A**) to the sample. This amount is sufficient for a sample containing <100  $\mu$ g of derivatizable material in ~100  $\mu$ L of solvent. If your sample is bigger, you will need to scale up the amount of reagent being added, or take a smaller aliquot of your sample for the reaction.
3. Cap the vial tightly and put in the heating block at 70°C for ~20 minutes. The heating step is simply to ensure that the reaction goes to completion, so the the exact time

and temperature are not critical. You should not need to heat samples for any longer than 30 minutes.

4. Let the sample cool to room temperature, then inject on the GC/MS.
5. Clean all syringes that come in contact with the reagents thoroughly, first using methanol then DCM.

### **Notes**

**A.** Pyridine is added as a catalyst for the reaction. Be sure to use anhydrous pyridine, as the normal stuff will contain lots of water and will cause a poor reaction yield.

**B.** If you are trying to derivatize hydroxyl groups on hydroxy-fatty acids, you need to methylate the carboxyl groups first. Otherwise the acid catalyst used in the methylation reaction will hydrolyze the acetate-hydroxyl ester.

**C.** If you are derivatizing compounds for isotopic analysis, you will need to use acetic anhydride with a known  $\delta D$  and/or  $\delta^{13}C$  value. We have a special (hidden!) stock of these reagents.

### **Troubleshooting**

The most common symptom is that the yield of derivatized product is low, or zero. Generally this is caused either by using old (or degraded) acetic anhydride, or because your reaction was too wet. First try opening a new ampoule of acetic anhydride, add it to the same sample, and see if that does the trick. If it doesn't, then the next thing to try is drying your sample, reagents, and glassware. Filter your sample through a column of anhydrous sodium sulfate to remove any water. Get a fresh batch of anhydrous pyridine. Put all your glassware and syringes in a drying oven for several hours before using it. We find these latter steps are rarely necessary in Pasadena where its pretty dry, but they can be essential in more humid climates