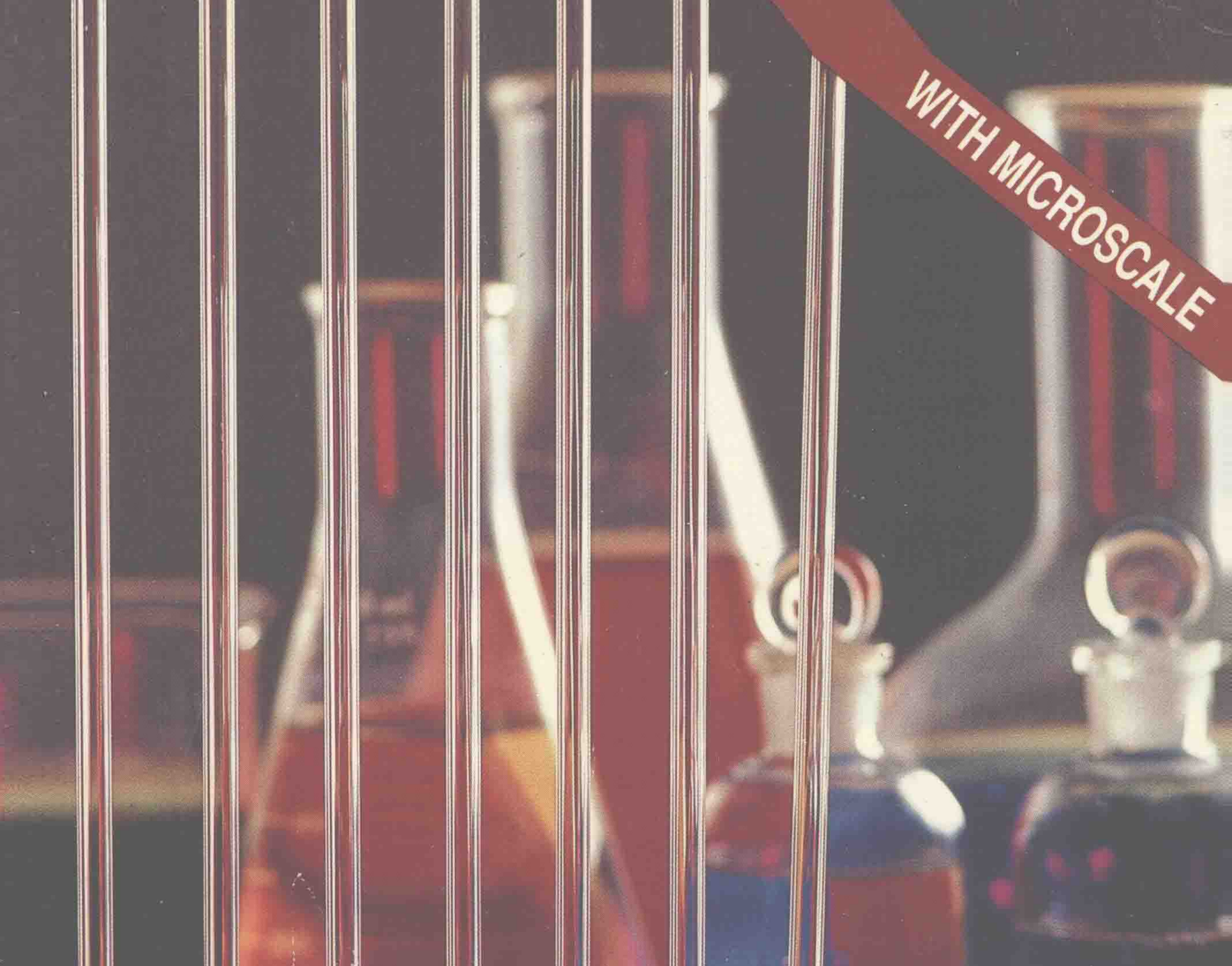

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With Contributions by John A. Landgrebe

ORGANIC LABORATORY TECHNIQUES

SECOND EDITION

Organic Laboratory Techniques

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Preface

Organic Laboratory Techniques, second edition, is a supplemental text for the introductory organic laboratory course in which the experiments are supplied by the instructor or in which the students work independently. In this text, the standard laboratory techniques are described, along with suggestions for experiments.

Since the techniques presented here are used in laboratory work by students of biochemistry, molecular biology, and cell biology, this text could be used in selected life science laboratory courses as well. It is also my hope that *Organic Laboratory Techniques* finds its way into the professional library of every science student.

The general order of presentation is isolation and purification techniques, refractive index and chromatography, setting up and carrying out reactions, infrared and NMR spectroscopy, and use of the chemical literature. The order in which the techniques are used is flexible. For example, a student could use Technique 11 (refractive index) along with distillation (Techniques 5–8).

Each technique includes a brief theoretical discussion. Because many students in this course have not yet had a course in physical chemistry, we have kept these discussions fairly general. The steps used in each technique, along with common problems that might arise, are discussed in detail. Supplemental or related procedures, suggested experiments, and suggested readings are included for many of the techniques. Each chapter ends with a set of study problems that primarily stress the practical aspects of the technique.

We have emphasized safety throughout all the techniques. Safety notes are included with each technique where appropriate. Appendix III ("Toxicology of Organic Compounds") is included to alert the student to the possible toxicities of even common compounds.

This second edition has expanded or added information on the following topics.

Safety and Waste Disposal: The introduction to the text on safety has been expanded to include a discussion of locating chemical safety information. Emphasis has been placed on chemical container labels and on Material Safety Data Sheets (MSDS). Appendix IV has been added to deal with the disposal of chemical wastes from student laboratories.

Microscale: A chapter (Technique 10) has been added for those instructors who wish to include microscale experiments. The basic microscale procedures—material transfer, crystallization, distillation, and extraction—have been included. The discussion of these techniques is not exhaustive. The intent is to provide the instructor with a nucleus of information upon which to build.

Additional chromatographic techniques: Flash chromatography and an expanded discussion of HPLC have been added to the chapter on column chromatography (Technique 13).

Spectroscopy: All theory and spectral interpretation has been removed from the text. This material, for the most part, is redundant, since these topics are covered in lecture texts. In addition, there are several short laboratory texts that specialize in these topics. (See the references given at the end of Technique 16.) The space has been used to present an expanded discussion on sampling techniques for spectroscopy (IR, NMR, and UV/visible). In addition, the chapter now covers some instrumental aspects of NMR spectroscopy: ringing, spinning sidebands, and high– and low–field strength spectrometers. The NMR section also includes a discussion of shift reagents and deuterium.

Miscellaneous changes: The one experiment, a Grignard reaction, found in the first edition has been dropped. The reason for this deletion is, in part, to focus text on laboratory techniques only. Other changes include additional background on acid–base reactions used in chemical extraction (Technique 3) and an increased emphasis on on-line computer searches in Technique 17 ("Introduction to the Chemical Literature"). Last, but not least, I hope the numerous small changes made throughout the text have improved its readability.

I am indebted to Joyce Brockwell, Northwestern University; Patricia Feist, University of Colorado-Boulder; Paul McMaster, College of the Holy Cross; James Pavlik, Worcester Polytechnic Institute; and Jan Simek, California Polytechnic State University who have provided extensive and extraordinarily useful comments. I am particularly indebted to John Landgrebe for his help with the chapter on microscale techniques and for figures and sections scattered throughout the text.

Ralph J. Fessenden

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Introduction to the Organic Laboratory

1 Safety in the Laboratory

Organic chemistry is an experimental science. Our understanding of organic chemistry is mainly the result of laboratory observation and testing. For this reason, the laboratory is an important part of a student's education in organic chemistry.

Because of the nature of organic compounds, the organic chemistry laboratory is generally more dangerous than the inorganic chemistry laboratory. Many organic compounds are volatile and flammable. Some can cause chemical burns; many are toxic. Some can cause lung damage, some can lead to cirrhosis of the liver, and some are *carcinogenic* (cancer causing). Yet organic chemists generally live as long as the rest of the population because they have learned to be careful. When working in an organic laboratory, you must always think in terms of safety.

Summary of Safety Rules

It may happen that you are confronted with a laboratory accident and cannot remember exactly what to do. In such a situation, just remember the following:

In the case of a spill: WASH!
In the case of a fire: GET OUT!

In either case, your instructor or someone in a calmer frame of mind can then decide how to handle the situation.

A. Personal Safety

(1) Using Common Sense

Most laboratory safety precautions are nothing more than common sense. The laboratory is not the place for horseplay. Do not work alone in the laboratory. Do not perform unauthorized experiments. Do not sniff, inhale, touch, or taste organic compounds, and do not pipet them by mouth. Wipe up all spilled chemicals, using copious amounts of water to wash up spilled acids and bases. Neutralize residual spilled acid with sodium bicarbonate and spilled base with diluted acetic acid. Do not put dangerous chemicals in the waste crock—the janitor may become injured. Do not pour chemicals down the sink—the environment will be injured. Instead, use the containers provided for chemical disposal.

When working in the laboratory, wear suitable clothing. Jeans and a shirt with rolled-up sleeves, plus a rubber lab apron or cloth lab coat, are ideal. Do not wear your best clothing—laboratory attire usually acquires many small holes from acid splatters and may also develop a distinctive aroma. Loose sleeves can sweep flasks from the laboratory bench, and they present the added hazard of easily catching on fire. Long hair should be tied back. Broken glass sometimes litters the floor of a laboratory; therefore, always wear shoes. Sandals are inadequate because they do not protect the feet from spills. Wash your hands frequently, and always wash them before leaving the laboratory, even to go to the rest room.

Because of the danger of fires, smoking is prohibited in laboratories. Because of the danger of chemical contamination, food and drink also have no place in the laboratory. On the first day of class, familiarize yourself with the locations of the fire extinguishers, fire blanket, eyewash fountain, and shower.

(2) Safety Glasses

Chemicals splashed in the eyes can lead to blindness; therefore, it is imperative that you wear **safety glasses,** or better yet, **safety goggles.** Wear them *at all times*, even if you are merely adding notes to your laboratory notebook or washing dishes. You could be an innocent victim of your lab partner's mistake, who might inadvertently splash a corrosive chemical in your direction. In the case of particularly hazardous manipulations, you should wear a **full-face shield** (similar to a welder's face shield). Your instructor will tell you when this is necessary.

Contact lenses should not be worn, even under safety glasses. The reason for this rule is that contact lenses cannot always be removed quickly if a chemical gets into the eye. A person administering first aid by washing your eye might not even realize that you are wearing contact lenses. In addition, "soft" contact lenses can absorb harmful vapors. If contact lenses are absolutely necessary, properly fitted goggles must be worn. Also, inform your laboratory instructor and neighbors that you are wearing contact lenses.

B. Laboratory Accidents

(1) Chemicals in the Eyes

If a chemical does get into your eye, flush it with gently flowing water for 15 minutes. Do not try to neutralize an acid or base in the eye. Because of the natural tendency for the eyelids to shut when something is in the eye, *they must be held open during the washing*. If there is no eyewash fountain in the laboratory, a piece of rubber tubing attached to a faucet is a good substitute. Do not take time to put together a fountain if you have something in your eye, however! Either splash your eye (held open) with water from the faucet immediately or lie down on the floor and have someone else pour a gentle stream of water into your eye. *Time* is important. The sooner you can wash a chemical out of your eye, the less the damage will be.

After the eye has been flushed, medical treatment is strongly advised. For any corrosive chemical, such as sodium hydroxide, prompt medical attention is imperative!

(2) Chemical Burns

Any chemical (whether water-soluble or not) spilled onto the skin should be washed off immediately with soap and water. The detergent action of the soap and the mechanical action of washing remove most substances, even insoluble ones. If the chemical is a strong acid or base, rinse the splashed area of the skin with *lots and lots of cool water*. Strong acids on the skin usually cause a painful stinging. Strong bases usually do not cause pain, but they are extremely harmful to tissue. Always wash carefully after using a strong base.

If chemicals are spilled on a large area of the body, wash them off in the safety shower. If the chemicals are corrosive or can be absorbed through the skin, remove contaminated clothing so that the skin can be flushed thoroughly. If chemical burns result, the victim should seek medical attention.

(3) Heat Burns

Minor burns from hot flasks, glass tubing, and the like are not uncommon occurrences in the laboratory. The only treatment needed for a very minor burn is holding it under cold water for 5–10 minutes. A painkilling lotion may then be applied. To prevent minor burns, keep a pair of inexpensive, loose-fitting cotton gloves in your laboratory locker to use when you must handle hot beakers, tubing, or flasks.

A person with a serious burn, as from burned clothing, is likely to go into shock. He or she should be made to lie down on the floor and kept warm with the fire blanket or with a coat. Then, an ambulance should be called. Except to extinguish flames or to remove harmful chemicals, do not wash a serious burn and do not apply any ointments. However, cold compresses on a burned area will help dissipate heat.

(4) Cuts

Minor cuts from broken glassware are another common occurrence in the laboratory. These cuts should be flushed thoroughly with cold water to remove any chemicals or slivers of glass. A pressure bandage can be used to stop any bleeding.

Major cuts and heavy bleeding are a more serious matter. The injured person should lie down and be kept warm in case of shock. A pressure bandage (such as a folded, clean dish towel) should be applied over the wound and the injured area elevated slightly, if possible. An ambulance should be called immediately.

The use of a tourniquet is no longer advised. Experience has shown that cutting off all circulation to a limb may result in gangrene.

(5) Inhalation of Toxic Substances

A person who has inhaled vapors of an irritating or toxic substance should be removed immediately to fresh air. If breathing stops, artificial respiration should be administered and an emergency medical vehicle called.

C. Laboratory Fires

(1) Avoiding Fires

Most fires in the laboratory can be prevented by the use of common sense. Before lighting a match or burner, check the area for flammable solvents. Solvent fumes are heavier than air and can travel along a benchtop or a drainage trough in the bench. These heavy flammable fumes can remain in sinks or wastebaskets for days. While it is indeed true that a flammable solvent should not have been discarded in the sink or wastebasket, it is always possible that some inconsiderate fool has done so. Therefore, do not discard hot matches, even if extinguished, or any other hot substances in sinks or wastebaskets.

Whenever you use a flammable solvent, extinguish all flames in the vicinity beforehand. Always cap solvent bottles when not actually in use. Do not boil away flammable solvents from a mixture except in the fume hood. Place solvent-soaked filter paper in the fume hood to dry before discarding it in a waste container. Spilled solvent should not be allowed simply to evaporate. If a solvent is spilled, clean it up immediately with paper towels, which should be placed in the hood to dry.

Solvents should never be poured into a drainage trough (which is for water only). Because of environmental concerns, solvents should be disposed of only in containers provided for solvent disposal. In general, these disposal containers are located in the fume hood in the laboratory.

(2) Extinguishing Fires

In case of even a small fire, tell your neighbors to leave the area and notify the instructor. A fire confined to a flask or beaker can be smothered with a watch glass or large beaker placed over the flaming vessel. (Try not to drop a flaming flask—this will splatter burning liquid and glass over the area.) All burners in the vicinity of a fire should be extinguished, and all containers of flammable materials should be removed to a safe place in case the fire spreads.

For all but the smallest fire, the laboratory should be cleared of people. It is better to say loudly, "Clear the room," than to scream "Fire!" in a panicky voice. If you *hear* such a shout, do not stand around to see what is happening, but stop whatever you are doing and walk immediately and purposefully toward the nearest clear exit.

Many organic solvents float on water; therefore, water may serve only to spread a chemical fire. Some substances, like sodium metal, explode on contact with water. For these reasons, water should not be used to extinguish a laboratory fire; instead, a *carbon dioxide* or *powder fire extinguisher* should be used.

If a fire extinguisher is needed, it is best to clear the laboratory and allow the instructor to handle the extinguisher. Even so, you should acquaint yourself with the location, classification, and operation of the fire extinguishers on the first day of class. Inspect the fire extinguishers. Find the sealing wire (indicating that the extinguisher is fully charged) and the pin that is used to break this sealing wire when the extinguisher is needed.

Fire extinguishers usually spray their contents with great force. To avoid blowing flaming liquid and broken glass around the room, aim toward the base and to the side of any burning equipment, not directly toward the fire. Once a fire extinguisher has been used, it will need recharging before it is again operable. Therefore, any use of a fire extinguisher must be reported to the instructor.

(3) Extinguishing Burning Clothing

If your clothing catches fire, walk (do not run) to the shower if it is close by. If the shower is not near, lie down, roll to extinguish the flames, and call for help.

A clothing fire may be extinguished by having the person roll in a fire blanket. The rolling motion is important because a fire can still burn under the blanket. Wet towels can also be used to extinguish burning clothing. A burned person should be treated for shock (kept quiet and warm). Medical attention should be sought.

D. Handling Chemicals

(1) Acids and Bases

To prevent acid splatters, *always add concentrated acids to water* (*never add water to acids*). Concentrated sulfuric acid (H_2SO_4) should be added to ice water or crushed ice because of the heat generated by the mixing. Do not pour acids down the drain without first diluting them (by adding them to large amounts of water) and then neutralizing them. Strong bases should also be diluted and neutralized before discarding. If you splash an acid or strong base on your skin, wash with copious amounts of water, as described in the section on chemical burns. Concentrated hydrochloric acid (HCl) and glacial acetic acid (CH_3CO_2H) present the added hazard of extremely irritating vapors. These two acids should be used only in the fume hood.

Sodium hydroxide (NaOH) is caustic and can eat away living tissue. As a solid (usually pellets), it is deliquescent; a pellet that is dropped and ignored will form a dangerous pool of concentrated NaOH. Pick up spilled pellets while wearing plastic gloves or by using a piece of paper, neutralize them, and then flush the neutralized mixture down the drain with large amounts of water.

Aqueous ammonia ("ammonium hydroxide") emits ammonia (NH_3) vapors and thus should be used only in the fume hood.

(2) Solvents

Organic solvents present the double hazard of flammability and toxicity (both short-term and cumulative). (Table III.1, page 240, in Appendix III lists the toxic levels and allowable limits of some common solvents.) *Diethyl ether* ($CH_3CH_2OCH_2CH_3$) and *petroleum ether* (a mixture of alkanes) are both very volatile (have low boiling points) and extremely flammable. These two solvents should never be used in the vicinity of a flame, and they should be boiled only in the hood. *Carbon disulfide* (CS_2), which is now rarely used in the organic laboratory, is uniquely hazardous. Its ignition temperature is under 100°, the boiling point of water; therefore, fires can result even from its contact with a steam pipe. *Benzene* (C_6H_6) is flammable and also extremely toxic. It can be absorbed through the skin, and long-term exposure is thought to cause cancer. Benzene should be used as a solvent only when absolutely necessary (and then handled with great care to avoid inhalation, splashes on the skin, or fire). In most cases *toluene* can be substituted for benzene. Although toluene is flammable, it is less toxic than benzene.

Most halogenated hydrocarbons, such as *carbon tetrachloride* (CCl_4) and *chloroform* ($CHCl_3$), are toxic, and some are carcinogenic. Halogenated hydrocarbons tend to accumulate in the fatty tissues in living systems, instead of