Experiment 7:
Extraction of Usnic Acid from Lichen

Objectives

The purpose of this experiment is to

1. isolate an enantiomer of usnic acid, a natural antibacterial organic, optically active compound with a very high specific rotation, found in a native species of lichen called 'Old Man's Beard' (*Usnea* sp.). **Note:** Lichens are fungi/algae symbionts, where the fungus provides a physical support structure and micronutrients for the algal cells while the algal cells provide the fungus with nutrients derived from photosynthesis.

2. learn the technique of liquid solid extraction used in this experiment and the method of two-solvent recrystallization.

3. determine the specific rotation of the optically active product using a polarimeter, thereby exposing the student to the fundamentals of polarimetry.

Introduction to Natural Products Extractions and Polarimetry

Compounds that contain a carbon atom which is bonded to four different atoms or groups are said to be **chiral** and can exist in two **enantiomeric** forms. Molecular models of these **enantiomers**, or **optical isomers**, are mirror images of one another (see Figure 7.1). Enantiomers have identical physical properties, except that one will rotate plane polarized light to the right, while the other rotates plane polarized light to the left.

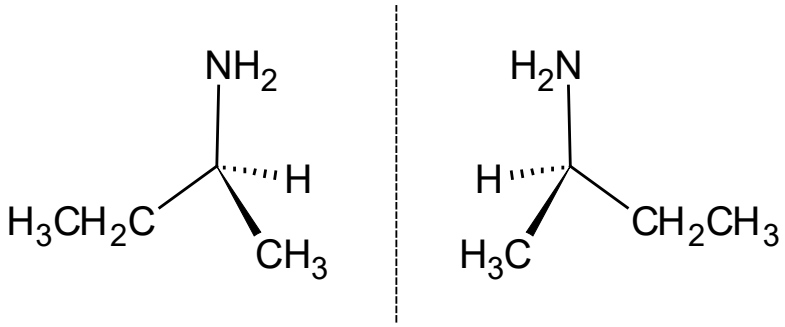


Figure 7.1. Line/wedge Diagrams of the two enantiomers of *sec*-butylamine

Because of the similarity in their properties, pairs of enantiomers would not be separated by the methods used in earlier experiments in this course: e.g., distillation, extraction or recrystallization. One common method of separating enantiomers (i.e., of **resolving a racemic mixture**) is to react the mixture with an optically active reagent so that a pair of **diastereomers** (i.e., stereoisomers that are not mirror images of each other) is formed. In general, diastereomers do differ from one another in their physical properties and can often be separated on the basis of one such property (e.g., solubility in a given solvent). To accomplish this, you would have to react a racemic mixture of (±)-*sec*-butylamine with (+)-tartaric acid to produce two diastereoisomeric salts:

(+)-*sec*-butylamine + (+)-tartaric acid → (+)-amine-(+)-acid salt
(−)-*sec*-butylamine + (+)-tartaric acid → (−)-amine-(+)-acid salt

These two salts can then be separated by repeated crystallizations from water—the salt formed from the (+)-*sec*-butylamine being the least soluble of the two. The salt from the (+)-*sec*-butylamine will be isolated, and the pure (+)-amine regenerated by treating the salt with a strong base.

Another and much simpler way to obtain a pure enantiomer is to find a source which is essentially pure. In this experiment you will attempt to isolate (+)- or (−)-usnic acid using a common method for extracting organic compounds from natural sources. Generally, a particular lichen will contain only one of the enantiomers of usnic acid, *R* or *S*.

In Experiment 5, we learned the technique **liquid-liquid extraction** for the separation of a mixture of organic solids based on solubility in aqueous versus non-aqueous solvents and acid-base chemistry. In this experiment, another type of extraction method, **solid-liquid extraction**, is used to separate and recover an organic compound (usnic acid) from a complex solid mixture (lichen). The purity of the recrystallized **'chiral'**, (optically active), product is then assessed using polarimetry.

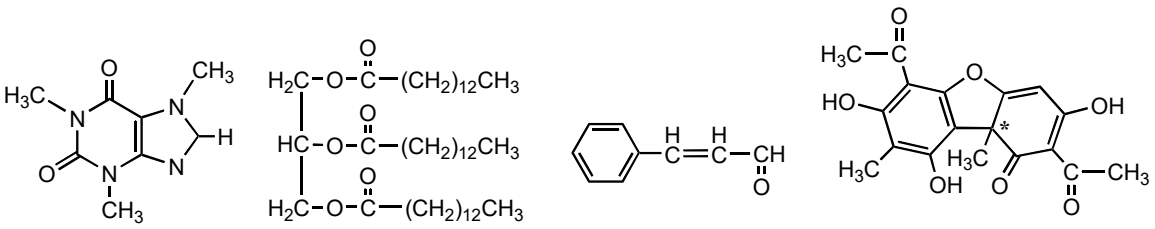
Solid-Liquid Extraction Procedure:

There are only 4 steps involved in performing a solid liquid extraction.

1. Add the unknown mixture and extraction solvent to a vessel.
2. Allow time for the extraction to take place.
3. Gravity filter to remove the unwanted source material.
4. Remove the solvent to concentrate the desired extracted solute.

Background Information

Natural products are of very high interest to chemists. Well-known natural products include caffeine, trimyristin, and cinnamaldehyde.



caffeine trimyristin cinnamaldehyde **usnic acid**

Caffeine can be extracted from tea leaves (2-3% w/w) using boiling water, while trimyristin can be extracted from nutmeg (2-4% w/w) using dichloromethane, and cinnamaldehyde can be extracted from cinnamon using steam distillation. In this experiment, acetone is used to extract usnic acid from the lichen, 'Old Mans Beard'.

After the usnic acid ($C_{18}H_{16}O_7$) is extracted and concentrated, the product is recrystallized, weighed and then a specific amount placed in the polarimeter and the specific optical rotation determined.

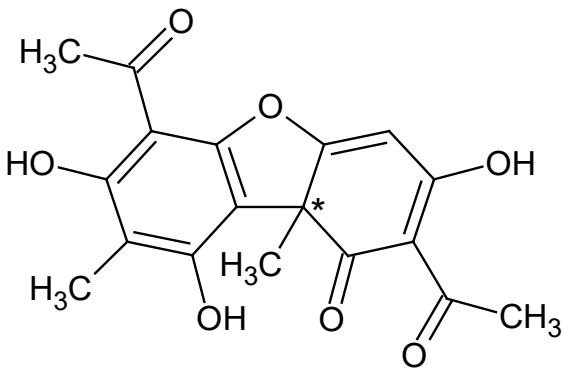


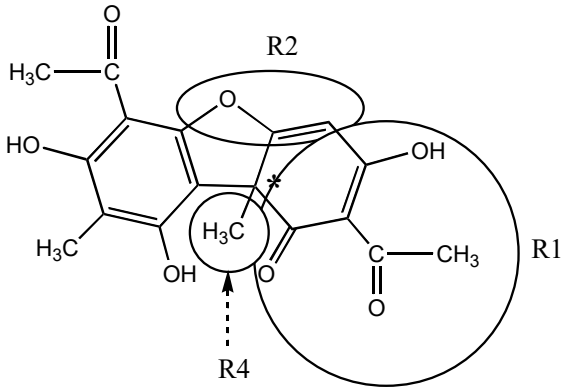
Figure 7.2. Structure of usnic acid (* = chiral or stereogenic C)

Assigning *R* or *S* Designations

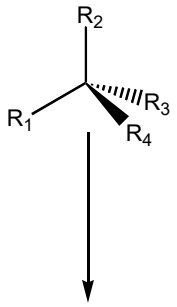
When given a large complicated molecule, especially with ring systems, we advise that you simplify the molecule into arbitrary portions surrounding the chiral centre (see below for R1, R2, R3, R4). Once this is done, follow the Cahn-Ingold-Prelog Sequence Rules to determine *R* or *S* configurations:

usnic acid

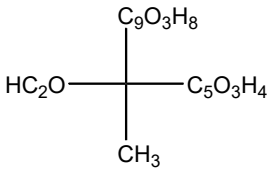
Uncircled atoms =R3



R1 = $C_5O_3H_4$
R2 = C_2OH
R3 = $C_9O_3H_8$
R4 = CH_3
+Chiral C = C
$C_{18}O_7H_{16}$



must orient so that R4 is



Fisher projection of usnic acid
(R or S designation not given)

Cahn-Ingold-Prelog Sequence Rules

1. Rank atoms attached to stereogenic C in order of atomic #, High 1, Low 4. (e.g., Br > Cl > O > N > C > H)
2. If decision cannot be reached, look at second atom of substituent, etc.
3. Multiple bonded 'C's are equivalent to the same # of single bonded atoms.
4. Mentally orient the molecule so that the lowest priority group (R4) is pointing directly back, away from you.

Note: Usnic acid has only one chiral center, and therefore only 2 enantiomers.

Chemicals, Equipment, Utilities Required

All glassware used for solid-liquid extraction must be clean and free of any organic contamination.

Chemicals	Equipment	Utilities
lichen (dried and crushed), reagent & HPLC grade acetone, ethanol, L-tartaric acid, distilled water, tetrahydrofuran, ice.	-stirrer-hot Plate, lab jack, retort stands, utility clamps -polarimeter -melting-point apparatus -hazardous waste disposal containers (in fume hood)	-115V electrical, -water aspirator -air-line

More on Polarimetry

You should already be familiar with the concept of **plane-polarized light** from the theory component of the course. In a **polarimeter**, plane-polarized light of a single wavelength is passed through a sample which, if it is 'optically active', will rotate the plane of polarization in one direction or the other. The light then encounters a rotatable Nicol prism through which it cannot pass until it has been rotated back to its original plane of polarization. Instead of rotating the light back to its original plan of polarization, it is simpler to rotate the Nicol prism so that the plane polarized light may pass through. The angle (and direction) through which the prism must be rotated to allow the maximum amount of light to pass is then measured and recorded as the **observed rotation**, α (see Figure 7.3).

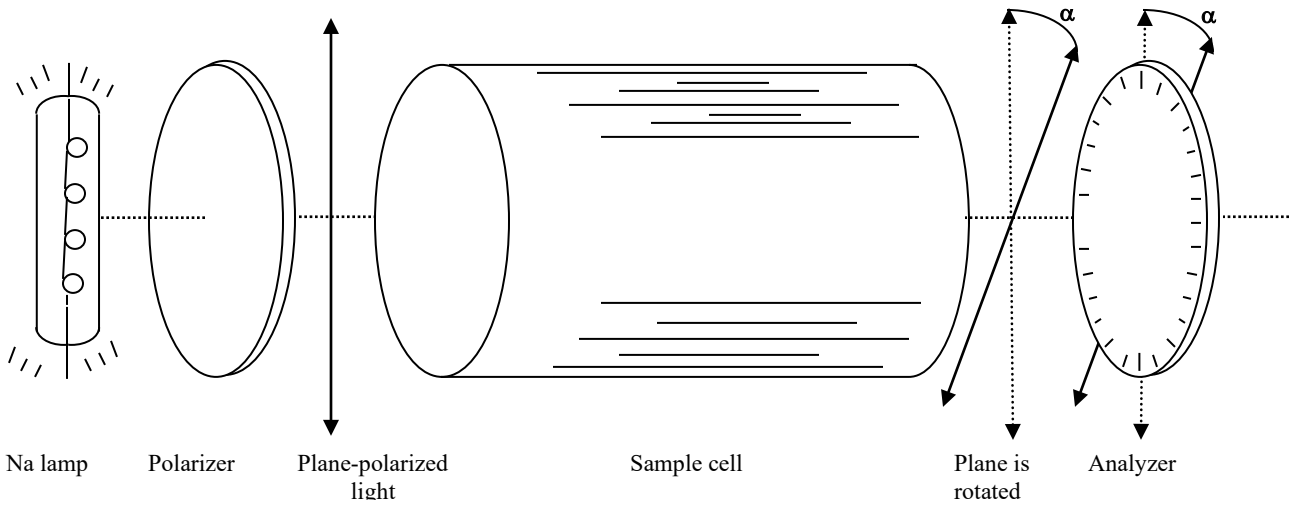


Figure 7.3. Schematic representation of a polarimeter

To be able to make meaningful comparisons between results obtained by different groups of workers, instead of reporting observed rotations, chemists usually report the results of polarimetry measurements in the form of **specific rotation**, $[\alpha]_D^{20}$ where

$$[\alpha]_D^{20} = \frac{\alpha}{\mathrm{L} \times \mathrm{d}}$$ for a liquid, and

$$[\alpha]_D^{20} = \frac{\alpha}{\mathrm{L} \times \mathrm{c}}$$ for a solution.

In the above equations, the superscript (20) indicates the temperature at which the measurements were made and the subscript (D) indicates that the measurements were made using the D line obtained from a sodium lamp (i.e., a wavelength of 589.3 nm). The observed rotation is represented by α, and the length of the sample tube (in dm) is presented by L. When the measurement is made on a liquid, it is necessary to know the density of the liquid, d, in g mL^{-1}. When using a solution, the concentration of the solution, c, must be included in the calculation using the units g mL^{-1}. To be complete, the specific rotation must include a sign to indicate the direction of the rotation:

(+) for rotation to the right (dextrorotatory)
(–) for rotation to the left (levorotatory).

Finally, the "optical purity" is a comparison of the optical rotation of a pure sample of unknown stereochemistry versus the optical rotation of a sample of pure enantiomer. It is expressed as a percentage. If the sample only rotates plane-polarized light half as much as expected, the optical purity is 50%.

$$\%\ optical\ purity = \frac{specific\ rotation\ of\ mixture}{specific\ rotation\ of\ pure\ enantiomer} \times 100$$