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Infrared Analysis of Piperine in Black Pepper

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Background

The infrared (IR) region of electromagnetic radiation extends from 0.75 μm (the upper end of the visible spectrum) to 400 μm (the microwave region). The 2.5–16 μm region (or in wave numbers, 4,000–625 cm^{-1}) provides the most useful chemical information. Instrument design for this region is simple and cost-effective.

When IR radiation is absorbed by molecules, bond deformations occur, with the bonds either stretching or bending. Stretching occurs when the excitation causes frequency of vibration changes in the bonded atoms along the bond axis, and bending involves atoms moving out of the bonding axis. The amount of energy required to cause stretching or bending depends on the masses of the atoms or groups of atoms and on the hybridization of the atoms making up the bond.

Many functional groups, such as carbonyls (aldehydes and ketones), hydroxyls (alcohols), and double bonds, give rise to characteristic absorptions in the “group frequency” region of the IR spectrum (4,000–1,500 cm^{-1}). Bond vibrations in OH, NH, and CH bonds occur in the 3,600–2,800 cm^{-1} range. Carbonyls, SH bonds, amino acids, triple bonds, and cumulative double bonds absorb in the 2,850–1,850 cm^{-1} range. The 1,500–650 cm^{-1} region is called the fingerprint region. Spectra tend to be very complex in this region, and identification of all of the bands is impossible since many bond deformation and group vibrations occur here. These bands are more characteristic of the particular molecule being studied than the bands that appear in the group frequency region. A direct comparison of two spectra in this region provides positive structural identification.

No general procedure exists for evaluating spectra. IR absorptions tend to be very complex. A complete vibrational analysis can be carried out for the total molecule using computers, but even the most advanced computers are successful only with highly symmetric, simpler molecules. Alternatively, the spectrum of interest can be sorted by comparison to spectral libraries. Tables for the characteristic bond and group frequencies can be used to help construct a possible structural formula. In this experiment, the spectrum of a known compound will be obtained and then compared to a table of expected absorption bands for the molecule’s various groups and bonds.

Piperine 1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine $\text{C}_{17}\text{H}_{19}\text{NO}_3$ (see Figure 1) can be isolated from black pepper (*Piper nigrum*) and other *Piper* species. Black pepper contains 6–9% piperine by weight. Piperine is tasteless, but its stereoisomer, chavicine, is the active ingredient in black pepper that provides its characteristic taste. Loss of pungency during storage of black pepper is attributed to the slow isomerization of chavicine into piperine.

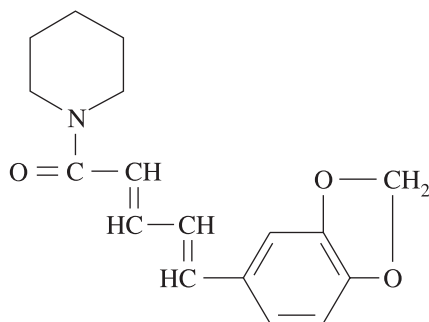


Figure 1: Piperine

Piperine is extracted from black pepper by ethanol using a soxhlet extraction apparatus. The piperine is purified by recrystallization and then characterized by IR spectroscopy.

Materials

- soxhlet extractor
- black pepper (Generic pepper does not work as well as major brands.)
- 95% ethanol
- 10% alcoholic potassium hydroxide (KOH) in 95% ethanol
- Whatman #1 filter paper
- agate mortar and pestle
- drying oven
- desiccator
- IR-grade potassium bromide (KBr)
- Wig-L-bug and accessories (stainless steel mixing vial and mixing ball)
- KBr die
- hydraulic lab press
- KBr pellet holder
- IR spectrophotometer
- 50°C water bath

Safety, Handling, and Disposal

It is your responsibility to specifically follow your institution's standard operating procedures (SOPs) and all local, state, and national guidelines on safe handling and storage of all chemicals and equipment you may use in this activity. This includes determining and using the appropriate personal protective equipment (e.g., goggles, gloves, apron). If you are at any time unsure about an SOP or other regulation, check with your instructor.

Procedure

1. Grind 10 g black pepper to a fine powder with a mortar and pestle.
2. Extract the finely ground pepper with 200 mL 95% ethanol in a soxhlet extraction apparatus for 4 hours. (The ground pepper is placed in the thimble and the ethanol in the round-bottomed flask.)
3. Allow the solution to cool and filter through Whatman #1 filter paper.
4. Concentrate the solution *in vacuo* on a 50°C water bath to remove most of the ethanol solvent. The final volume should be about 5 mL.

5. Add 10 mL 10% alcoholic KOH to the residue and let stand 1 hour.
6. Decant the solution from the insoluble residue.
7. Allow the alcoholic solution to stand undisturbed overnight; long yellow needles of piperine will be deposited. (The crystals may take 24–48 hours to form.) The yield is approximately 0.3 g.
8. Collect the yellow needles by vacuum filtration and wash with a minimum volume of 95% ethanol.
9. Allow the crystals to air dry. Weigh them and determine the melting point. (It should be 125–126°C.)

Preparation of KBr Pellet for IR Analysis

1. Dry IR-grade KBr in a drying oven for at least 1 hour.
2. Grind approximately 15 mg of the piperine needles in an agate mortar and pestle for 20 minutes. Make sure the crystals are kept dry and are thoroughly ground to remove any reflective surfaces and reduce particle size.
3. Allow the KBr to cool in a desiccator. Weigh out 300 mg and place in a stainless steel mixing vial with a stainless steel mixing ball.
4. Carefully weigh 3 mg of the finely ground piperine and place in the vial with the KBr.
5. Shake the vial on a Wig-L-bug for 60 seconds to thoroughly mix.
6. Place the KBr-piperine mixture into an evacuable die on a hydraulic laboratory press. Press *in vacuo* at 15,000 pounds of pressure for 6 minutes.
7. Release the pressure, remove the die from the press, disassemble the die, and remove the KBr pellet. Place the KBr pellet in a pellet holder and put it into the sample beam of an IR spectrophotometer.
8. Run the spectrum of the pellet from 4,000–600 cm^{-1} .
9. Using the following table of expected absorption bands, look for the corresponding bands on the IR spectrum of piperine.

Table of Expected Absorption Bands for Piperine in KBr	
Type of Phenomenon	Wave Number (cm ⁻¹)
aromatic C-H stretching	3,000
symmetric and asymmetric stretching of C=C (diene)	1,635; 1,608
aromatic stretching of C=C (benzene ring)	1,608; 1,580; 1,495
stretching of -CO-N	1,635
methylenedioxy group:	
asymmetric and symmetric CH ₂ stretching, aliphatic C-H stretching	2,925; 2,840
CH ₂ bending	1,450
asymmetrical stretching =C-O-C	1,250; 1,190
symmetrical stretching =C-O-C	1,030
C-O stretching (most characteristic)	930
in-plane bending of phenyl C-H	1,132
C-H bending of <i>trans</i> -CH=CH-	995
out-of-plane C-H bending 1,2,4-trisubstituted phenyl (two adjacent hydrogen atoms)	850; 830; 805

References

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