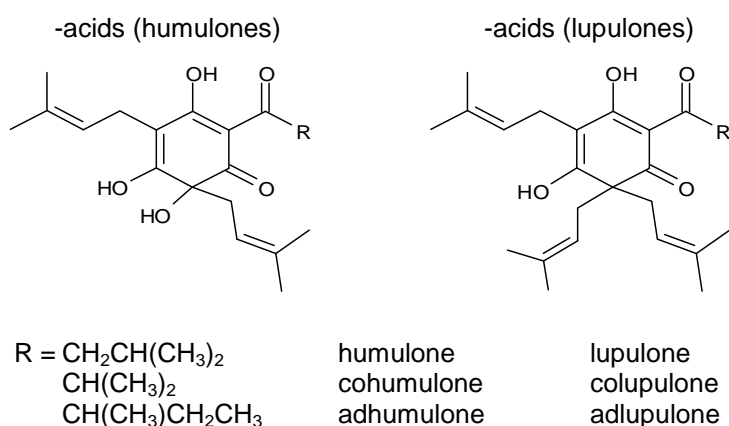


Analysis of α - and β -Acids in Hops by RP-HPLC

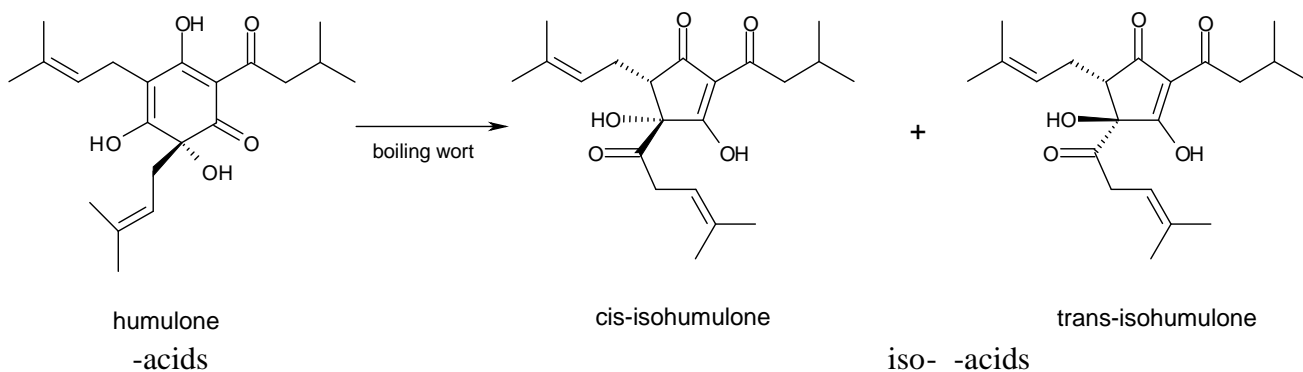
Adapted from Travis M. Danenhowter, Leyna J. Force, Kenneth J. Petersen, and Thomas A. Betts*: Journal of Chemical Education, 2008, Vol. 85, No. 7, 954-955

Introduction

Hops are used to impart bitter flavor and aroma to beer. Within the cones of the female hop plant are substances generally described as α - and β -acids. The predominant α -acids (humulone, cohumulone, and adhumulone) are precursors to the bitter compounds that balance the sweetness of the malted barley.¹ The corresponding β -acids (lupulone, colupulone, and adlupulone) contribute significantly less to overall bitterness than α -acids.



Hops are often added as the mashed malted barley (or malt extract) is boiled in water. During this boiling process the modestly bitter α -acids undergo thermal isomerization to form extremely bitter iso- α -acids.



The more soluble and stable iso- α -acids contribute the greatest extent to the bitter flavor of beer. Beta-acids are oxidized during the boil, rather than isomerized². These oxidation products of the β -acids do influence taste and aroma, but to a much lesser extent than the α -acids. It is important for brewers not only to understand the character of different hop varieties (Cascade, Hallertauer, Fuggles, Saaz, etc.) add to brewed products, but also to predict the ultimate bitterness

of a batch of beer. The bitterness of a brew depends primarily on the concentration of α -acids contained in the hops, the amount of hops used, and the length of time the hops are boiled.

In this experiment we will use high performance liquid chromatography (HPLC) to determine the α - and β -acid concentrations in several hop samples. External standards will be prepared from an international calibration extract commonly used in the brewing industry (ICE-2).

Procedure

HPLC System

Column: 4.0 x 125 mm Agilent Hypersil octadecylsilane (C-18)
Mobile Phase: 85% Methanol/15% Water, acidified with 0.025% v/v formic acid
(Filtered and degassed)
Flow Rate: 0.8 mL/min.
Detector: Absorbance at 326 nm
Injection. Volume 50 μ L

Extraction Solvent: 85% Methanol/15% Water, acidified with 0.025% v/v formic acid

Other: 0.45 μ m nylon syringe filters, 50ml volumetric flasks (5), 25ml volumetric flask, beakers (100mL, 250 mL), magnetic stirrer, medium porosity filter paper, vacuum filtration system.

Hazard Warning

The extraction solvent is flammable and toxic. Use caution when dispensing this solution, and keep it away from ignition sources. Clean up any spills immediately. Methanolic HPLC waste must be disposed of in accordance with federal, state and local environmental regulations.

Preparation of Samples

Begin by preparing **three** commercial hop samples until steps 1, 2, given below. While the samples are stirring, proceed to the preparation of standards.

1. Crush hops with a mortar and pestle. Weigh accurately an approximately 0.25 g sample of hops. If using pellets place hops in a 100- or 150-mL beaker. If loose hop cones are used a 250-mL beaker may be necessary.
2. Add approximately 20 mL of extraction solvent. Stir the solution for one hour using a magnetic stir bar.

Proceed to the Preparation of ICE-2 Standard while the hop samples are stirring.

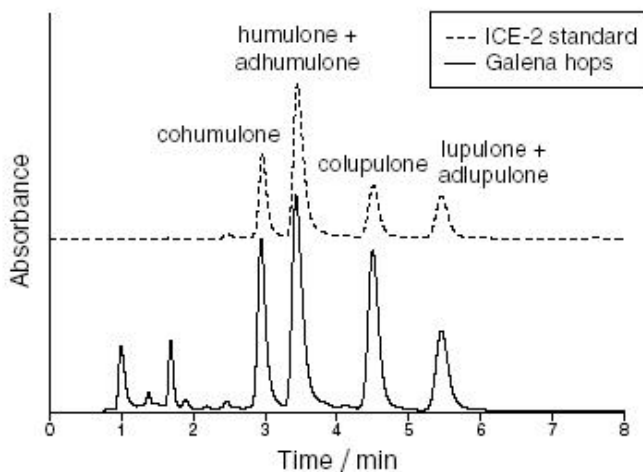
3. Vacuum filter the solution through medium porosity filter paper and collect *all* of the filtrate. Be sure to *quantitatively* transfer all the solids and solution from the beaker into the funnel.
4. Using a polyethylene disposable pipette, rinse the solid on the filter paper with approximately 10 mL of extraction solvent in 2-mL increments.
5. Quantitatively transfer the filtrate to a 50-mL volumetric flask and dilute to mark using extraction solvent.

6. Filter a small volume of solution from the preceding step, using a 0.45 μ m nylon syringe filter.
7. Inject the filtered samples into the HPLC according to instructions provided by your laboratory instructor. Record a chromatogram for each sample.

Preparation of the ICE-2 Standard

1. Weigh *accurately* about 0.15 g sample of ICE-2 standard and place into a small beaker. (This may have been prepared ahead of time by your lab instructor.)
2. Add extraction solvent to dissolve the standard.
3. Quantitatively transfer the solution to a 25-mL volumetric flask and dilute to volume using extraction solvent.
4. Using the composition information provided with the ICE-2 standard, determine the concentration of cohumulone in your diluted standard in units of mg/mL.
5. Filter a portion of this solution (enough to prepare the working standards) using a 0.45 μ m nylon syringe filter.
6. Prepare a set of 5 calibration standards with concentrations of cohumulone between 0.0 and 0.3 mg/ml. Suggested method; dilute 3.00mL of solution from step 5 in a 10.00mL volumetric flask, calculate the concentration of this solution. (say = c_1 mg/mL)
7. Dilute 2.50mL of solution from step 5 in a 10.00mL volumetric flask; calculate the concentration of this solution. (say = c_2 mg/mL). Dilute 2.00mL of solution from step 5 in a 10.00mL volumetric flask; calculate the concentration of this solution. (say = c_3 mg/mL). Dilute 1.50mL of solution from step 5 in a 10.00mL volumetric flask; calculate the concentration of this solution. (say = c_4 mg/mL). Dilute 1.00mL of solution from step 5 in a 10.00mL volumetric flask; calculate the concentration of this solution. (say = c_5 mg/mL).
8. Using the information provided with the ICE-2 standard, determine the concentrations of all other α - and β -acids in mg/mL in all of your working standards (adhumulone + humulone, colupulone, and lupulone + adlupulone).
9. Inject the standards into the HPLC. Record a chromatogram for each standard.

Data Analysis



There are four major peaks in the chromatogram of the standard which are due to six α - and β -acids. Under these separation conditions cohumulone elutes first, followed by a peak comprised of co-eluting humulone and adhumulone, then colupulone, and finally co-eluting lupulone and adlupulone.

Create a table of retention times and peak areas for all four of the α - and β -acid peaks in all chromatograms. Using the peak areas from the standard chromatograms, create four calibration curves (peak area vs. concentration in mg/mL): cohumulone, adhumulone + humulone, colupulone, and adlupulone + lupulone.

Calibration Data:

cohumulone		adhumulone + humulone		colupulone		adlupulone + lupulone	
C mg/mL	Peak area	C mg/mL	Peak area	C mg/mL	Peak area	C mg/mL	Peak area

Calibration Results:

Calibration curve	cohumulone	adhumulone + humulone	colupulone	adlupulone + lupulone
slope (mL/mg)				
intercept				

Determine the concentrations (or combined concentrations for adhumulone + humulone and adlupulone + lupulone) in mg/mL of the α - and β -acids in the hop extracts using their respective calibration curves. Then calculate the weight % of the α - and β -acids in the original hops samples. Add the concentrations of the α -acids (cohumulone plus adhumulone + humulone) and β -acids (colupulone plus adlupulone + lupulone), and report the total α -acid and total β -acid concentrations (% w/w) in the hop samples. (Work out the statistics.)

Results:

Sample	cohumulone/ % w/w	adhumulone + humulone/ % w/w	colupulone/ % w/w	adlupulone + lupulone/ % w/w	Total α -acids/ % w/w	Total β -acids/ % w/w
1						
2						
3						

Literature Cited

¹ Keukelerie, J.D.; Ooms, G.; Heyerick, A.; Roldan-Ruiz, I.; Bockstaele, E.V.; Keukelerie, D.D. *J. Agric. Food Chem.* **2003**, *51*, 4436-4441.

² Pyle, N. Norm Pyle's Hop FAQ. <http://realbeer.com/hops/FAQ.html> (accessed 1-3-07).

⁴ Harris, D.C. *Quantitative Chemical Analysis*, 6th Edition; W.H. Freeman and Co.: New York, 2003: pp 734-739.