Spring 2019

Synthesis and Analysis of 6-acetyl-1,2,3,4-tetrahydropyridine, a Major Contributor to 'Mousy' Off-Flavor in Sour and Wild Beers

Liesel Krout
University of Southern Maine

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Liesel Krout[1], Lucille Benedict[1], Meg Hausman[1], Zach Bodah[2]

[1]Department of Chemistry, Quality Control Collaboratory, University of Southern Maine, Portland Maine

[2]Allagash Brewing Company, Portland Maine

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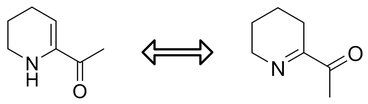
Introduction

Unexpected tastes can negatively impact consumer experience in the beer industry. One such taste, a 'mousy' off-flavor, is diminishing the quality of sour and wild beers throughout the brewing community.

The unpleasant flavor is a result of the combined presence of three organic compounds, one of which is 6-acetyl-1,2,3,4-tetrahydropyridine (ATHP). Because of its unpredictable nature, brewers must wait for the compound to disappear before distribution, costing them valuable time and revenue. Previous research on these compounds was primarily focused on their presence in red wine but lacked analogous study in beer. The factors responsible for formation, reasons for persistence, and degradation timelines of the compounds were unknown.

This project attempts to synthesize and extract ATHP, the greatest contributor to the 'mousy' flavor.



Ultimate Goals

1. Perfect a method for synthesis of ATHP in order to obtain high yields of pure ATHP. This will allow for more precise and accurate quantification methods that are essential for kinetic experiments and compound analysis.
2. Understand the degradation pathways and kinetics of ATHP, and use that knowledge to encourage faster degradation on a large scale, at breweries.
3. Conduct analogous research on ETHP and APY and determine reasons for their formation to prevent their presence from occurring at all in the first place.

Acknowledgements

This project was funded in part by Allagash Brewing Company and Maine Economic Improvement Fund.

Sources

(1) Costello, Peter James. *Formation of mousy off-flavor in wine by lactic acid bacteria.* Doctoral Thesis, The University of Adelaide (Australian Wine Research Institute), **1998**.

(2) Hunter, Irving R.; Walden, Mayo K. (1971). United States Patent 3,620,771

(3) Romano, A.; Perello, M.C.; de Revel, G.; Lonvaud-Funel, A. Growth and volatile compound production by Brettanomyces/Dekkera bruxellensis in red wine. *J Appl Microbiol.* **2008**. *104,* 1577-1585.

(4) Snowdon, Eleanor M.; Bowyer, Michael C.; Grbin, Paul R.; Bowyer, Paul K. Mousy Off-Flavor: A Review. *J Agric Food Chem.* **2006**. *54,* 6465-6474.

Synthesis Methods

Method 1 - Low Yield of Impure ATHP

4:2:2 mix of sodium bisulfite, l-proline, dihydroxyacetone ground into fine powder

Bake at 94℃ for 30 min until orange and foamy

Basified, and extracted with diethyl ether

GC-MS Method for Compound Identification

Instrument: Thermo Scientific Trace GC Ultra and ISQ Series

Oven: Hold 60℃ for 6.00 min, Ramp 5℃/min until 220℃, hold for 2.00 min, ramp 20℃/min until 300℃, hold for 3.00 min

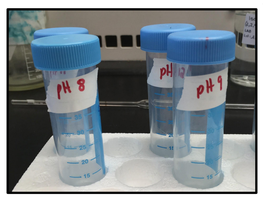
Injection: Splitless, with a 1.2 mL/min flow rate

Detector: Starts collecting data at 10.00 min

Extraction Method

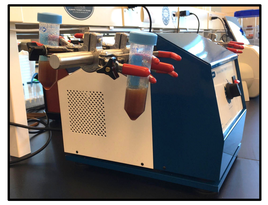
Synthesize ATHP with the first two steps mentioned above

Add orange cake substance to a pint of fresh beer

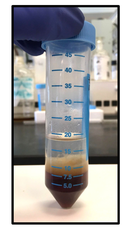


Add to centrifuge tube:
-5.0 mL of spiked beer
-internal standard (4-acetylpyridine) of desired amount
- 3.0 M NaOH dropwise to desired pH

Add 10.0 mL iso-octane, then shake 20 min



Centrifuge at 1000 rcf, for 5 min intervals until layers separate



Dry 2.0 mL of organic layer over anhydrous Na_2SO_4

Dilute to 10.0 mL and analyze via GC-MS

Identification via GC-MS

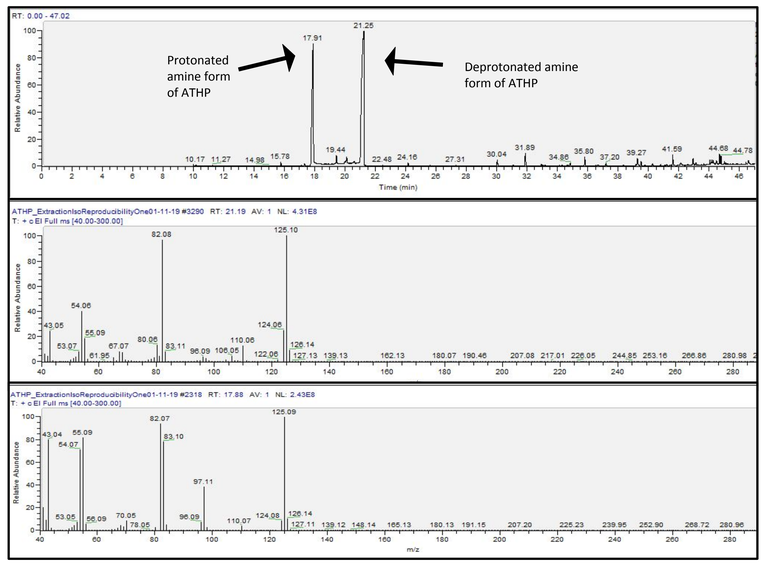


Figure 1a - The GC-MS data at left results from highly basic (pH > 14) extraction with iso-octane.

Figure 1b - This MS data is from the peak at 21.25 min (deprotonated amine form of ATHP)

Figure 1c - This MS data is from the peak above at 17.91 min (protonated amine form of ATHP)

Results

- Synthesis with the method described above results in <0.1% yields.
- Extraction of ATHP was qualitatively successful in beer spiked with ATHP. Refer to **Figure 1** at left.
- The internal standard, 4-acetylpyridine, exhibits a downward trend in peak area (in GC data) as the pH of the extracted solution is increased.
- As the pH of the solution (prior to extraction) was increased, the ability to detect both forms of ATHP increased. There was no detectable peak below a pH of 12.0

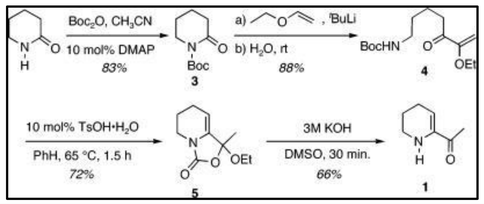


Figure 2 - Proposed reaction for next synthesis attempt.