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Analysis of Deuterated Standards for Determination of Humulones and Their Derivatives in Beer

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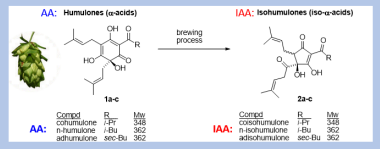
Introduction

Hop plants, *humulus lupulus*, are used in the beer brewing process to contribute aroma, bitterness and product stability. Quantifying the bittering compounds, isomerized and oxidized humulones (iso-humulone and humulinones, respectively, and their homologs), are of importance for both quality and promotional purposes. Previous methods demonstrated ability to separate and measure the homologs (co-, ad-, and n-) of humulone, iso-humulone and humulinone species in beer samples using LC-MS and HPLC with UV detection.[1,2] The measurements obtained were relative amounts and were consistent with the expected hop characteristics based on the style of beer (expected bitterness, strength of hop aroma). However, in order to quantify the species present, a stable isotope dilution mass spectrometry (SIDA-MS) method is being developed using deuterated iso-humulone (figure 5). In SIDA-MS, an internal standard is introduced to a sample that varies from the target analyte in isotopic composition only. This minimizes matrix effects and differences in ionization efficiency.

Figure 1. Structures of humulone and iso-humulone.

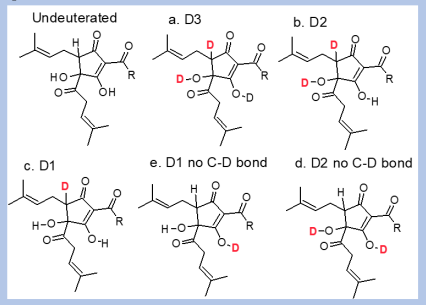


Methods

- The separation, purification and isomerization of the humulones from commercial hop extract (Hopsteiner®, New York, New York) was carried out by acyloin rearrangement of humulone in the presence of NaOD in deuterated solvent using $MgSO_4$ as a catalyst (Figure 6).
- A deuterated species was synthesized ([n-^{2}H]iso-co-humulone) in D_2O with $MgSO_4$ and NaOD from the the purified co-humulone and analyzed using a Bruker Maxis Plus qTOF (Bruker, Billerica, MA) at multiple time points (Figure 3) in both positive and negative ionization modes.
- A purified sample of n-humulone (93%) was used to test linearity of the signal response with concentration (Figure 5).
- NMR analysis was performed on the co- and n-humulone samples using a Bruker Avance 300 Mhz Spectrometer (Bruker, Billerica, MA) (Figure 7).

Results

Figure 2. The variety of possible structures during the deuteration process. The isomerization is carried out in D_2O which allows the 2H to be incorporated



Results Continued

Table 1. The possible m/z species identifiable in the mass spectra

	Possible ^{2}H Varieties	
m/z	Neg (deprotonated)	Pos (sodiated)
374.2017		D3 (a)
373.1954		D2 (b), D2 (d, no C-D)
372.1892		D1 (c), D1 (e, no C-D)
371.1829		Undeuterated
349.1984	D3 (a), D2 minus H (b)	
348.1921	D2 minus D (b), D1 minus H (c), (d, no C-D)	
347.1858	Undeuterated, D1 minus D (c), (e, no C-D),	

Figure 3. The percentage of deuterated species detected in the same sample over time.

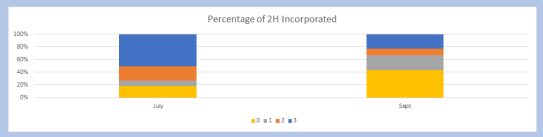


Figure 4. Spectrum of the [n-^{2}H]iso-co-humulone showing the four peaks: a, undeuterated; b, M+1^{2}H; c, M+2^{2}H; d, M+3^{2}H

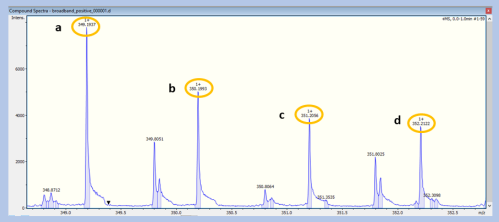


Figure 5. A graph of the concentration of ad-humulone to the signal.

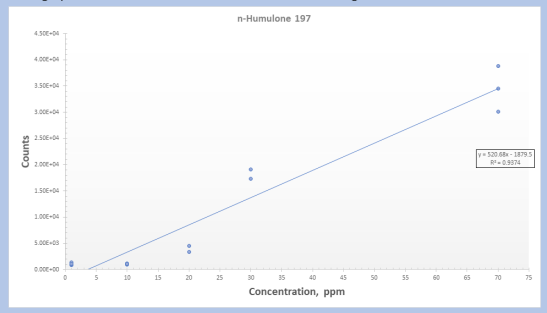
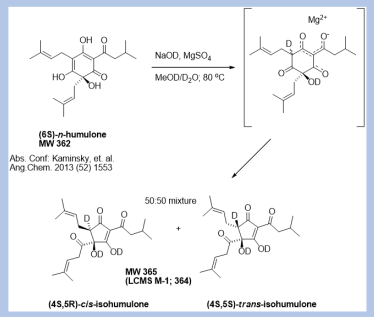
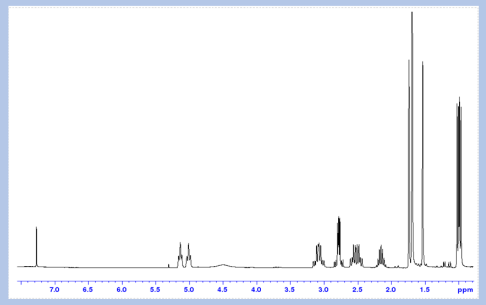


Figure 6. Synthesis of deuterated analog of cis/trans-isohumulone.



Results Continued

Figure 7. NMR spectrum of the purified n-humulone sample, 190-3.



Analysis of 190-3

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (s, 0.23H), 5.14 (d, 1H, J_{HH} = 6.8 Hz), 5.01 (d, 1H, J_{HH} = 6.8 Hz), 4.53 (s, 0.24H), 3.08 (m, 2H, J_{HH} = 7.8 Hz), 2.77 (m, 2H, J_{HH} = 2.4 Hz), 2.52 (m, 2H, J_{HH} = 7.2 Hz), 2.15 (sep, 1H, J_{HH} = 6.5 Hz), 1.73 (s, 3H), 1.69 (s, 6H), 1.53 (s, 3H), 1.01 (d, 3H, J_{HH} = 6.7 Hz), 0.97 (d, 3H, J_{HH} = 6.7 Hz).

Discussion

- Analysis showed multiple [^{2}H]iso-co-humulone species with zero to three ^{2}H incorporation including a C-^{2}H bond. However, the time comparison showed ^{2}H - ^{1}H exchange between the various ^{2}H species (Figure 3).
- In negative mode the multiple [^{2}H]iso-co-humulone collapsed into three signals, while four species were able to be distinguished in positive mode.
- Based on Selective purification of different homologs as source material for the deuterated standard may be done with high degree of purity. The NMR for the n-humulone is shown here (Figure 6).
- In a plot measuring signal for n-humulone over the relevant concentration range exhibits a linear response with and R^2 value of 0.937.

Conclusion and Future Work

- One species, the [5-^{2}H]iso-ad-humulone with the C-^{2}H will be the target for deuteration going forward. The C-^{2}H, bond will not exchange during analysis like the O-^{2}H will and will be clearly distinguishable in the mass spectrum.
- The n-humulone is the most abundant homolog and the isolated and purified species will be the precursor for the internal standard going forward.
- Determine the most efficient deuteration method for synthesizing the isotopically labeled standard, [5-^{2}H]iso-n-humulone.
- Develop response factors and calibration curves for the [5-^{2}H]iso-n-humulone.
- Assess the standard using beer as the matrix.

References

1. B. Hamper, K. Zawatzky, V. Zhang, C. Welch. "Rapid Determination of Humulones and Isohumulones in Beers Using MISER LC-MS Analysis," J. Am. Soc. Brew. Chem., **75**, 333 – 338 (2017).
2. B. Hamper, N. Viriyasiri, A. Boland, *Comparison of Hop Derived Humulone Constituents in Beer Using UV-VIS, HPLC, and LC-MS*, Undergraduate Research Symposium, University of Missouri-St. Louis, St. Louis, MO, 2019
3. C. Cervino, S. Asam, D. Knopp, M. Rychlik, R. Niessner. "Use of Isotope-Labeled Alfatoxins for LC-MS/MS Stable Isotope Dilution Analysis of Foods." J. Agric. Food Chem, **56**, 1873 – 1879 (2008