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The Synthesis of Deuterated Isohumulones for Use as Internal Standards in LC-MS Stable Isotope Dilution Assays

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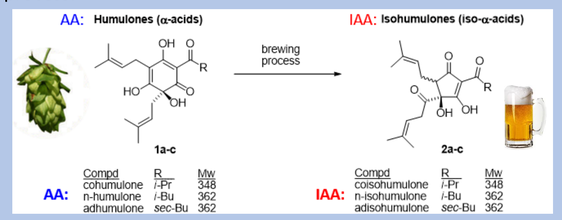
Introduction

Humulones are compounds that are prevalent in the hops flowers (Humulus Lupulus) used in beer brewing. These compounds undergo isomerization during the brewing process, and the resulting isohumulones are considered to be the primary contributors to the bitter flavors present in beer. As such, quantifying their presence, and the relative presence of their homologs(n-, co-, ad-), is of great importance in the characterization of beer. In this effort, one of the homologs of humulone (co) was isolated before being subsequently isomerized and deuterated for the purpose of analyzing beer by stable isotope dilution assay mass spectrometry(SIDA-MS). The addition of this stable isotopically substituted isohumulone as an internal standard can potentially allow the comparative quantification of humulones, isohumulones, and oxidized humulinones present in a beer sample.

Figure 1. Structures of humulone and the iso-humulones formed during the brewing process.



Methods

- Cohumulone was initially separated from a mixture of α-acids(present as a PDA salts) using C_{18} reverse-phase chromatography.
- Isomerization of cohumulone was carried out by acyloin rearrangement in the presence of NaOD in deuterated solvent using $MgSO_4$ as a catalyst.
- NMR analysis was performed on the cohumulone and isocohumulone samples using a Bruker Avance 300 MHz Spectrometer(^{1}H, ^{13}C), while 2-D NMR was carried out using a Bruker Avance 700 MHz spectrometer.
- The *cis-trans* isomers were separated through crystallization with β-cyclodextrin, and separation was confirmed using an Agilent 1200 Infinity Series HPLC.

Results

Figure 2. The preparation process used for the isolation of co-humulone from PDA salt mixture of α-acids.

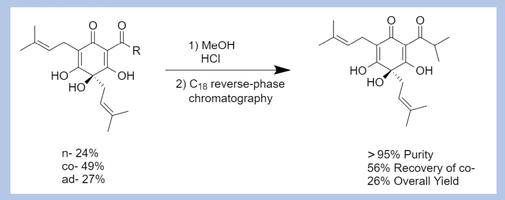
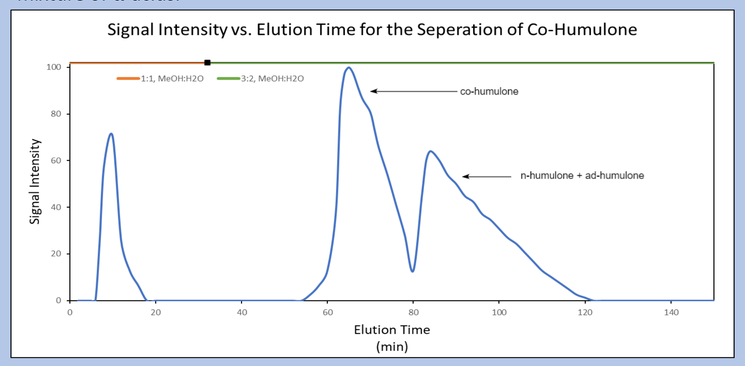
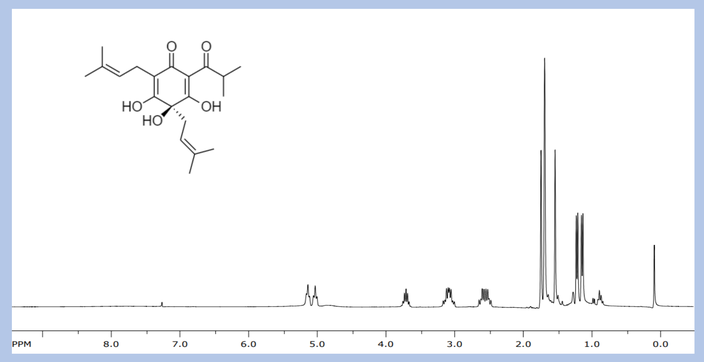


Figure 3. The chromatogram for the separation of co-humulone from PDA salt mixture of α-acids.



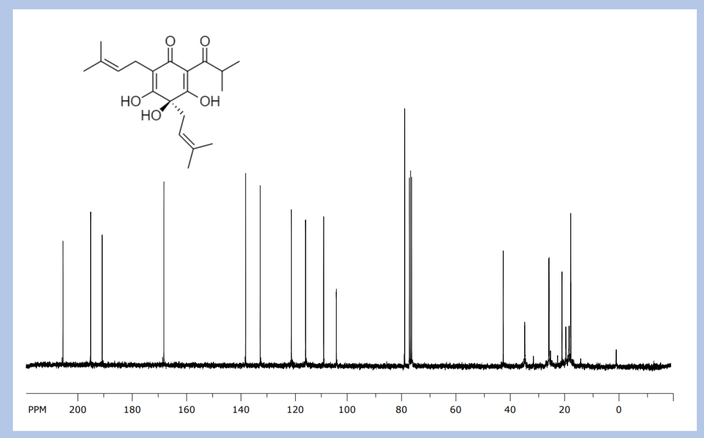
Results Continued

Figure 4. The ^{1}H NMR spectrum of cohumulone.



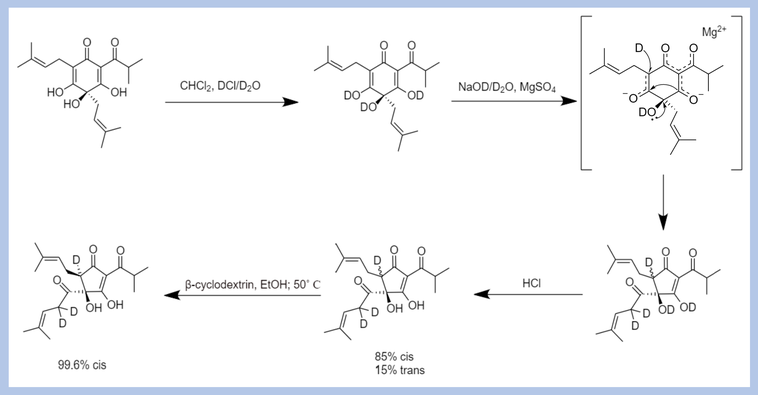
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.14 (t, 1H, J_{HH} = 6.8 Hz), 5.03 (t, 1H, J_{HH} = 7.6 Hz), 3.70 (sep, 1H, J_{HH} = 6.7 Hz), 3.08 (ddd, 2H, J_{HH} = 27.0, 14.4, 7.3 Hz), 2.55 (ddd, 2H, J_{HH} = 29.9, 13.7, 8.0 Hz), 1.73 (s, 3H), 1.68 (s, 6H), 1.52 (s, 3H), 1.20 (d, 3H, J_{HH} = 6.8 Hz), 1.13 (d, 3H, J_{HH} = 6.7 Hz) ppm.

Figure 5. The ^{13}C spectrum of cohumulone.



^{13}C NMR (75 MHz, $CDCl_3$) δ 205.5, 195.3, 191.0, 168.2, 138.0, 132.6, 121.1, 115.8, 109.1, 79.2, 77.5, 77.1, 76.6, 42.8, 34.8, 25.8, 21.1, 19.6, 18.4, 17.8 ppm

Figure 6. The process and proposed mechanism for the isomerization, deuteration, and isolation of [3-^{2}H]*cis*-coisohumulone from cohumulone.



Results Continued

Figure 7. The 2-D NMR spectrum of coisohumulone.

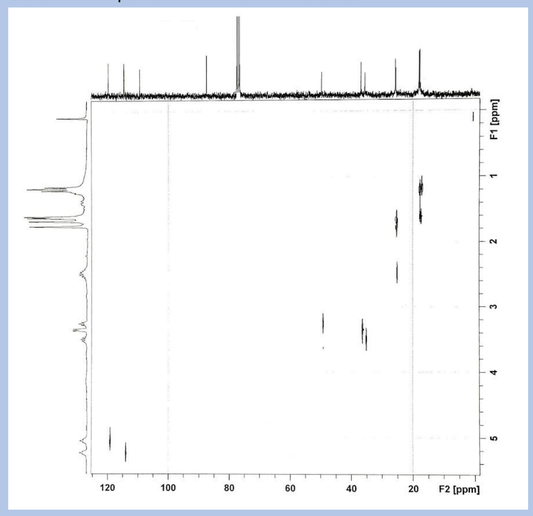
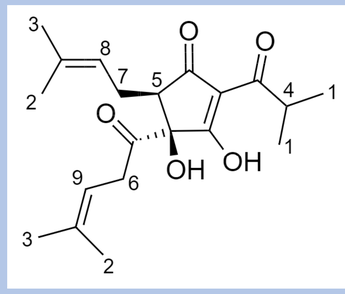


Table 1. The assignments of the 2-D NMR resonances to structure of coisohumulone.



Assignment	1H NMR Chemical Shift (ppm)	13C Correlation (ppm)
1	1.13 1.16	17
2,3	1.57 1.59 1.64 1.72	18-25
4	2.45	25
5	3.22	49.7
6	3.32	36.9
7	3.46	35.6
8,9	5.02 5.20	119.7 114.5

Conclusion

- Selective purification of different homologs as source material for the deuterated standard may be achieved with high degree of purity.
- The process employed for deuteration and isomerization may be successfully carried out with a high yield.
- Separation of *cis-trans* isomers may be done with a high degree of purity.

Future Work

- Analyze deuterated species by 2-D NMR to determine exact placement of deuterium in compound.
- Develop response factors and calibration curves for the [3-^{2}H]coisohumulone.
- Assessing 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