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Jeremy Leker & John Paul Maye

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Discovery of Acetohumulone and Acetolupulone a New Hop Alpha Acid and Beta Acid

Jeremy Leker and John Paul Maye

S.S. Steiner, Inc, New York, NY

ABSTRACT

Hops, *Humulus lupulus*, contain two major organic acids, humulones, also known as alpha acids, and lupulones, also known as beta acids. These two organic acids are composed of a mixture of three major homologs referred to as co-, n-, and ad-. There are also three minor homologs known as post-, pre- and adpre- however these minor hop acid homologs are found in dry hops at very low concentrations, usually less than 0.2%. Today, we report the discovery of a fourth minor homolog for alpha acids and beta acids found in hops called aceto-.

KEYWORDS

Humulone; lupulone; alpha acids; beta acids; co-; n-; ad-; post-; pre-; adpre-; aceto-

Introduction

Hops play a major role in the brewing of beer contributing bitterness, aroma, foam, antibacterial, and antioxidant protection to the beer. Brewers are primarily interested in alpha acids given it thermally isomerizes to isoalpha acids when boiled and is the main bittering substance in beer. Although beta acids are the most antibacterial of all the hop acids, they are typically not found in most beers due to their non-polarity and low solubility in beer, however, they are found in low concentrations in hazy beers such as New England IPA.[1]

Although brewers knew for centuries that hops contributed bitterness to beer,[2] it was not until 1953 that Rigby and Bethune discovered and proved that alpha acids thermally isomerize into isoalpha acids and that isoalpha acids are primarily responsible for beer's bitterness.[3] They also correctly identified the molecular structure of the three major homologs, co-, n-, and ad-.[3] A few years later in 1958, Verzele discovered a new minor alpha acid, posthumulone[4] and in 1962, Rillaers and Verzele discovered another new minor alpha acid, prehumulone.[5] In 2004, Zhang et al. identified a new minor alpha acid and beta acid adprehumulone and adprelupone.[6] Today, we report the discovery of a new minor alpha acid and beta acid found in hops called acetohumulone and acetolupulone.

Results and discussion

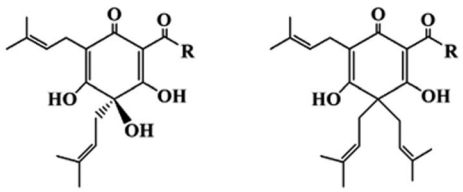
Figure 1 shows the molecular structure of all the major and minor alpha acids and beta acids known to date.

What differentiates the homologs of alpha acids and beta acids is the acyl group or side chain R. It has been proposed that the aromatic core, phloroglucinol, is derived from the condensation of acyl-CoA and three molecules of malonyl-CoA via valerophenone synthase (VPS).[7] It has also been proposed that the acyl-CoA is derived from hydrophobic amino acids.[8] The amino acid leucine can explain the isobutyl side chain found in n-humulone and n-lupulone, valine for the isopropyl side chain for cohumulone and colupulone, and iso-leucine for the sec-butyl side chain for adhumulone and adlupulone. The amino acid alanine can explain the side chains for acetohumulone and acetolupulone. However, there are no coding or proteinogenic amino acids that can explain the side chain homologs for pre- (isopentyl), post- (ethyl) or adpre- (2-methylbutane) humulones and lupulones. Thus, another mechanism is likely, or these side chains come from non-coding or non-proteinogenic amino acids. After condensation, the acylphloroglucinol (PIVP) undergoes two prenylations with dimethylallyl diphosphate (DMAPP) to form deoxyhumulone. It is important to note that the side chains, R, are formed during the early stages of biosynthesis and before the last step, oxidation (which forms alpha acids) or prenylation (which forms beta acids). Therefore, if one discovers a new homolog for alpha acids, there should be a corresponding homolog for beta acids and vice-versa (Figure 2).

While preparing a new HPLC calibration standard, beta acid-dicyclohexylamine (Beta Acid-DCHA), a small peak was noted in the HPLC trace that eluted at 4.492 min, just before the post-lupulone peak at 6.278 min (see HPLC Trace 1). The HPLC method used was Analytica-EBC 7.8 (ASBC Methods of Analysis Section 7 Hops, Method 7.8).

The HPLC used has a diode array detector, which allows one to look at the UV spectra of each peak (Figure 3). Because the UV spectra of the new peak had the same

CONTACT John Paul Maye jmaye@hopsteiner.com



R	Alpha Acids	Beta Acids
$CH(CH_3)_2$	cohumulone	colupulone
$CH_2CH(CH_3)_2$	n-humulone	n-lupulone
$CH(CH_3)CH_2CH_3$	adhumulone	adlupulone
CH_2CH_3	posthumulone	postlupulone
$CH_2CH_2CH(CH_3)_2$	prehumulone	prelupulone
$CH_2CH(CH_3)CH_2CH_3$	adprehumulone	adprelupulone
CH_3	acetohumulone	acetolupulone

Figure 1. Major and minor alpha acids and beta acids.

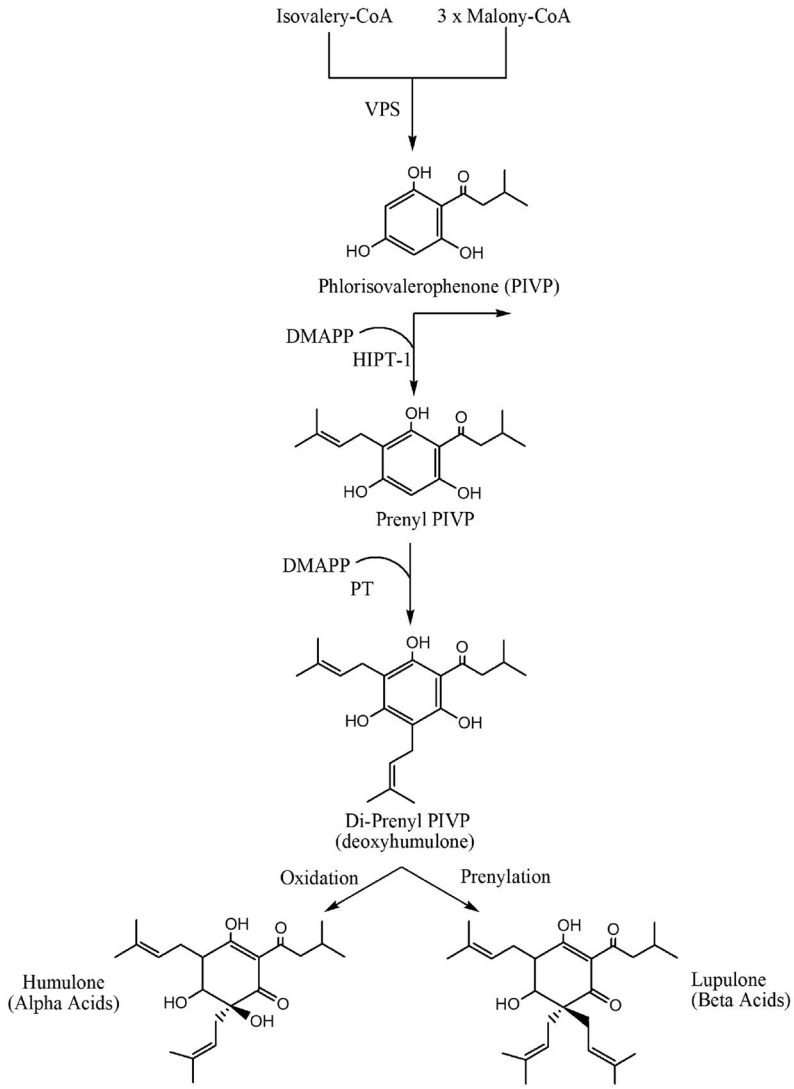
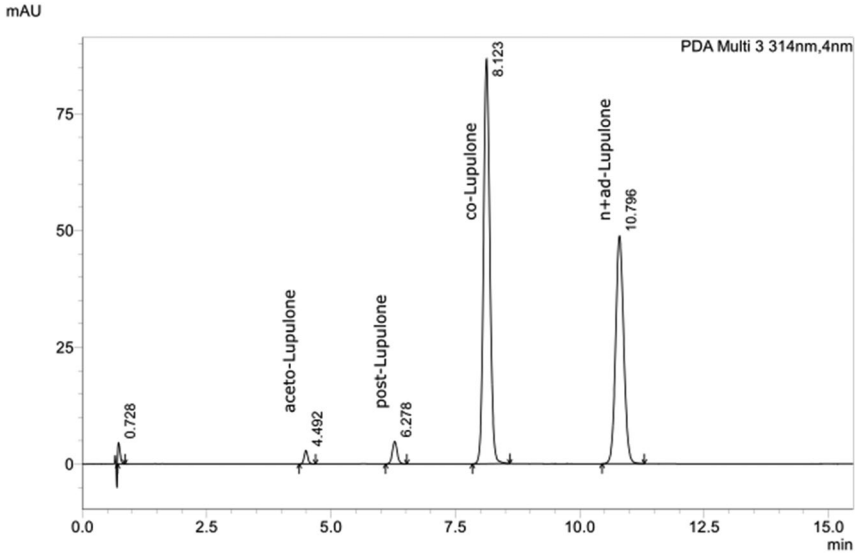


Figure 2. Biosynthetic pathway of humulone and lupulone.



HPLC Trace 1. Reverse phase HPLC trace of beta acids-DCHA HPLC calibration standard.

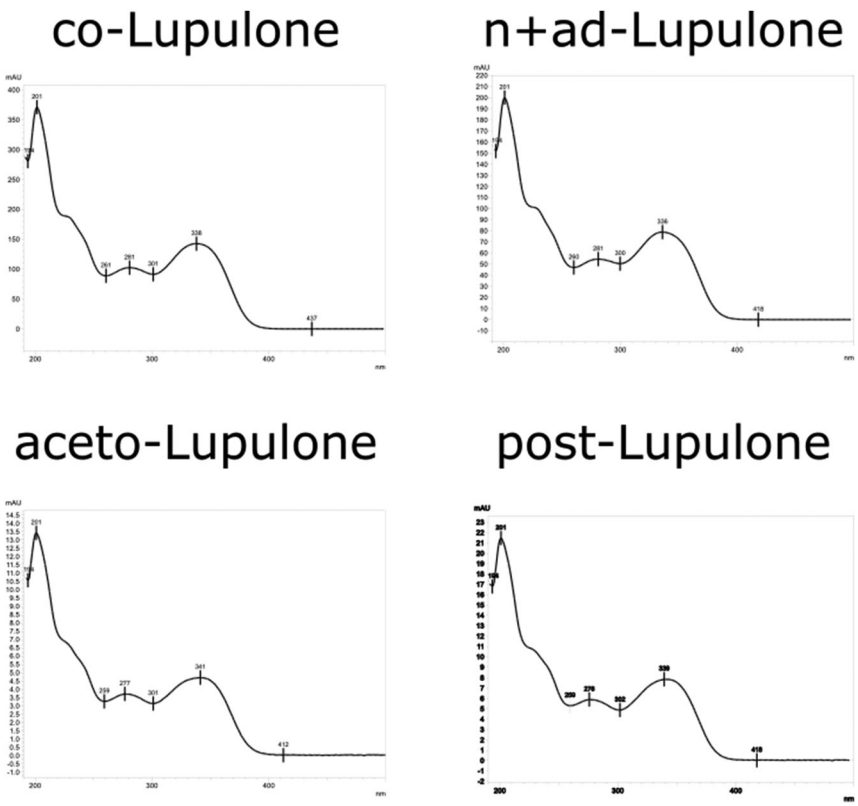


Figure 3. UV Spectra of co-, n + ad, aceto, and post- lupulones.

spectra profile as the other beta acid compounds, it was suspected that it could be a new unknown beta acid.

In addition, since this new compound eluted before post-lupulone (ethyl homolog) on our reverse phase HPLC method, it was surmised that the side chain R for this new beta acid was most like methyl. In 2020, Decuyper et al. published the synthesis of novel beta acids, including one where the side chain R is methyl, which he called acetolupulone.[9] To confirm that the new peak was acetolupulone, we synthesized acetolupulone following the Decuyper et al. method.[9] After synthesizing acetolupulone, it was treated with dicyclohexylamine to produce an HPLC calibration

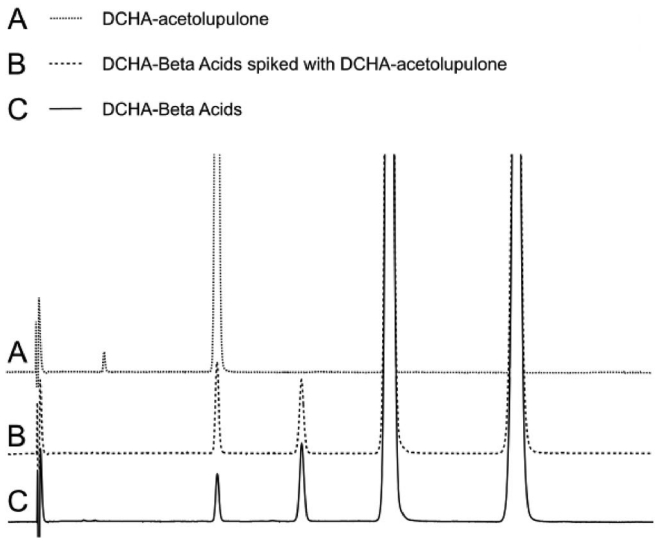
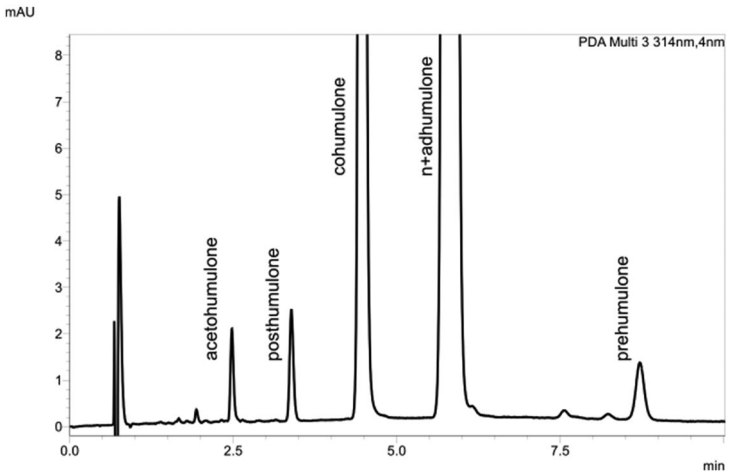


Figure 4. Coinjection of DCHA-Acetolupulone with DCHA-Beta Acids Standard.



HPLC Trace 2. Reverse phase HPLC trace of alpha acids.

standard for acetolupulone, acetolupulone-DCHA. When acetolupulone-DCHA was injected into the HPLC, it eluted at the same exact time as the new minor beta acid peak in our beta acid-DCHA standard and it also had the same UV spectra as the new peak (Figure 4).

Below is an HPLC trace of alpha acids containing a new peak before the post-humulone peak (see HPLC Trace 2). Again, the new peak had the same UV spectra as the other alpha acids (Figure 5).

To confirm the molecular structure of acetohumulone, the synthesis of acetohumulone as reported in the patent of Sigg-Grutter and Wild was followed.[10] The laboratory prepared acetohumulone was treated with dicyclohexylamine to make an HPLC calibration standard for this new minor alpha acid, acetohumulone-DCHA. Again, the acetohumulone-DCHA standard co-eluted at the same time as the new peak and had the same UV Spectra confirming that this new minor alpha acid is acetohumulone (Figure 6).

To better understand how common acetohumulone and the other minor alpha acid homologs are found in hops, a number of hop varieties as well as their corresponding CO_2 hop extracts were analyzed by HPLC to measure their concentration (Table 1). One can see that the concentrations of these minor homologs were quite small, with acetohumulone being the smallest.

The concentration of the minor beta acid homologs in a number of different hop varieties, as well as their corresponding CO_2 hop extracts, were measured by HPLC (Table 2). Again, the concentrations of these minor beta acid homologs were very small.

Conclusions

Sixty-four years after the discovery of the first minor alpha acid and beta acid, we report the discovery of a fourth minor alpha acid and beta acid, acetohumulone and acetolupulone. As analytical instrumentation improves with time, it is likely additional minor alpha acids and beta acids will be discovered in hops.

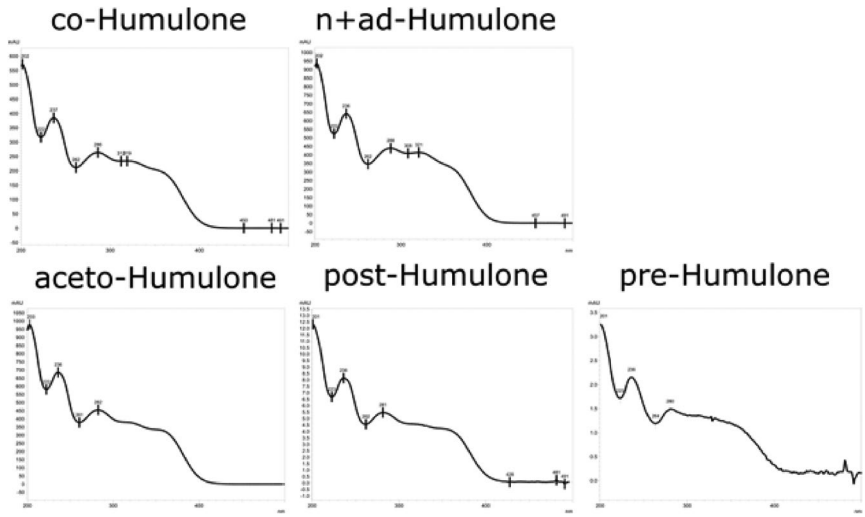


Figure 5. UV Spectra of co-, n + ad, aceto-, post-, and pre- humulones.

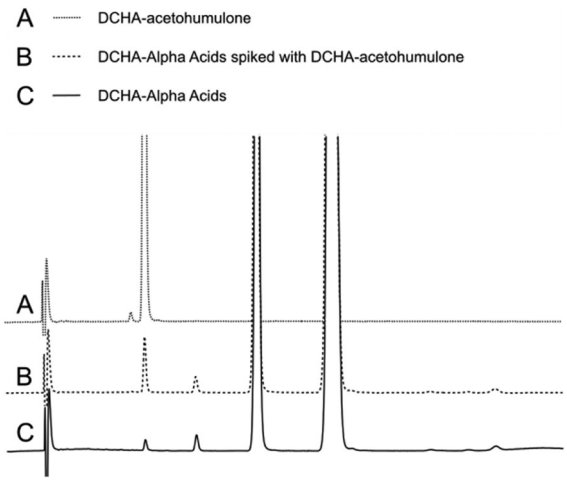


Figure 6. Coinjection of DCHA-Acetohumulone with DCHA-Alpha Acids.

Table 1. Concentration of minor alpha acid homologues in hops and hop extract versus the total alpha acid concentration.

Type	Variety	Aceto- humulone (%)	Post- humulone (%)	Adpre- humulone (%)	Pre- humulone (%)	Total Alpha (%)
Pellets	Eureka	0.014	0.123	0.126	0.037	18.2
CO_2 Extract	Eureka	0.045	0.403	0.474	0.131	59.5
Pellets	Calypso	0.00095	0.036	0.098	0.068	9.7
CO_2 Extract	Calypso	0.0065	0.205	0.512	0.380	51.9
Pellets	Lotus	0.0031	0.119	0.103	0.229	12.2
CO_2 Extract	Lotus	0.0088	0.480	0.442	1.128	54.8
Pellets	Sultana	0.0002	0.035	0.081	0.044	8.8
CO_2 Extract	Sultana	0.0318	0.318	0.885	0.300	58.8
Pellets	Lemon Drop	0.0011	0.049	0.131	0.053	4.3
CO_2 Extract	Lemon Drop	0.0024	0.291	1.001	0.361	35.2

(Total Alpha % = co + n+ad)

Table 2. Concentration of minor beta acid homologues in hops and hop extract versus the total beta acid concentration.

Type	Variety	Aceto- lupulone (%)	Post- lupulone (%)	Pre- lupulone (%)	Total Beta (%)
Pellets	Eureka	0.023	0.037	0.014	4.3
CO_2 Extract	Eureka	0.090	0.223	0.049	41.5
Pellets	Lemon Drop	0.020	0.103	0.061	3.8
CO_2 Extract	Lemon Drop	0.294	0.672	0.665	37.6
Pellets	Calypso	0.030	0.022	0.049	4.1
CO_2 Extract	Calypso	0.137	0.138	0.209	31.9
Pellets	Lotus	0.035	0.125	0.070	5.0
CO_2 Extract	Lotus	0.148	0.645	0.053	36.3
Pellets	Sultana	0.023	0.019	0.019	3.4
CO_2 Extract	Sultana	0.071	0.182	0.178	45.5

(Total Beta %= co + n+ad)

ORCID

John Paul Maye http://orcid.org/0000-0002-1261-8954

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