Investigation of Synthetic Strategies for the Full Synthesis of a Homologue of the Hop α-Acid, α-Humulone

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I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code, and to maintain the integrity of myself and the college community as a whole.

Pledge_____

Acknowledgements

Dr. Persichini: Thank you for all you have done for me over the past three years. From employing me last summer, to the immense amount of help you have given me in the research process, to sparking my interest in organic chemistry in the first place, you have been a major part of my positive experience at Allegheny College.

My Parents: Thank you for supporting me in everything I have done. I owe all of my life's accomplishments to the endless hours of hard work you have put into supporting my brothers and me and the valuable example you set for us day-in and day-out.

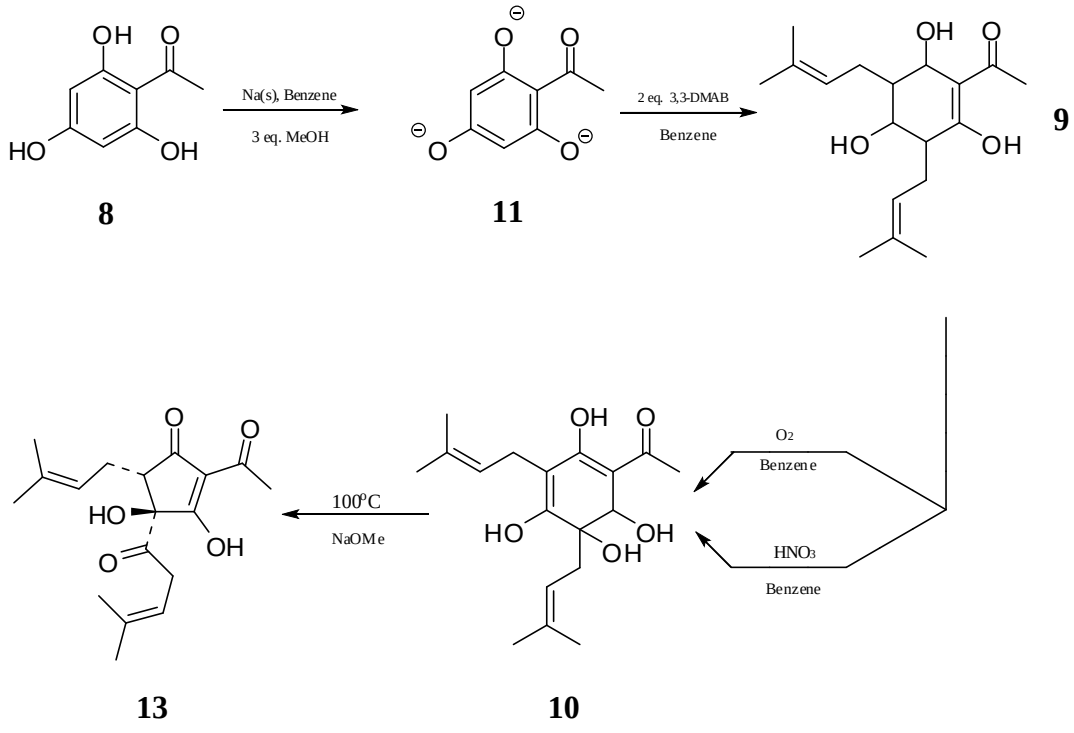
My Fellow Lab-mates: It has been fun working with you guys the last two semesters. I wish all of you well in your future endeavors.

297 Church Street: You six have made my time at Allegheny College great beyond belief. It is because of you guys I was able to find our collective living arrangement, the ungodly J.Y. known as Highwater Manor, livable and the stress of Allegheny College bearable.

Bob Caskey: Whether it be shooting the breeze or accomplishing something productive, I would have been lost without your assistance.

Abstract

Over the past few years, there has been a noted increase food prices. Because of this, the brewing industry has been affected by the rising price of hops (*Humulus lupulus*), which contain α-acids, known as α-humulones **1**. A versatile molecule in the pharmaceutical arena, when α-humulone isomerizes during the brewing process, it rearranges to the beer bittering agent, iso-α-humulone **2**. Because of improved analytical techniques used in the extraction of α-humulone **1** from the hop cone, attempted syntheses of α-humulone ceased in the late 1950's. As a result of the current economic times, we seek to integrate techniques from the historic syntheses of α-humulone **6** with more modern techniques to devise a contemporary synthesis of an α-humulone homologue **10**.



Introduction

The major historical application of the female hop plant (*Humulus lupulus*) is its use in the bittering of beer. Hops have been a major ingredient in beer dating back 7000 years to Ancient Egypt and other areas of the Middle East. The selection of finer hop varieties has been a long and ongoing botanical process.[1] Today, the hop plant is valued worldwide for its role in the brewing process, specifically the inclusion of bitter flavor from hop acids, known as α-humulones, as well as the ability of the addition of hops to impart unique aroma components that come from the volatile essential oils of the hop cone.[2] Hops, like most other food products in the world, are becoming more expensive[3] increasing the economic viability of research into synthetic methods for not only the production of organic hop components, but also the production of synthetic components of other foods.

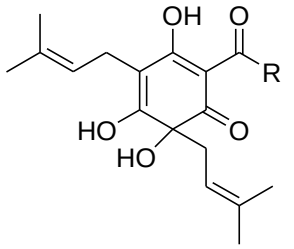
The recent literature concerns the use of humulones and other hop acids in a pharmaceutical context. Microbiological assays began on the application of humulones as antibiotics in the 1970's.[4] These simple antibiotic assays opened the door for further investigations into the use of humulones and other hop acids in the pharmaceutical arena. The toxicity of humulone was tested on beagle dogs and it was concluded unequivocally that hop acids are safe chemicals for use in biological systems.[5] Very recently, hops have reemerged in the literature because of the antioxidative properties of humulone and other hop acids in regard to their prospective application in cancer treatment.[6] A study done in 1995 by Tagashira et al. suggests that the highly oxidized humulone molecule, which will be presented in due course, is a potent antioxidant and radical scavenger.[7]

The most recent research involves the use of humulones and other hop acids as tumor suppressing agents. Research has shown that humulones, along with other hop acids, may act to improve treatment of certain cancers by Vitamin D, a carcinostatic process with major pros and cons. Leukemia cells lose their ability to proliferate in the presence of Vitamin D. Yet, the effective dose of Vitamin D needed to illicit tumor control leads to massive bone loss. Without a decrease in the potency of tumor control, bone loss was effectively limited when Vitamin D was administered in a mixture with humulones.[8] At the molecular level, a mechanism has been proposed in which humulone successfully stops cell division in leukemia cells by abolishing the cancerous cells' ability to transcribe the DNA of cyclooxygenase-2, an enzyme that is essential for cell division and metastasis.[9] Furthermore, the bone rebuilding properties of humulone were the central focus of a patent filed by Tobe and Kazuyuki concerning the utilization of humulones in the treatment of osteoporosis.[10]

Hop Chemistry

The major bittering components of the hop plant are found in the oxygenated fraction of the female hop cone extract, which is made up of a mixture of α-acids, or humulones, and β-acids, known as lupulones which will be covered sparingly in this report.[1,11,12] The chemicals of the utmost importance in the flavoring of beer are the aforementioned α-acids, known as α-humulones, found naturally in the hop plant in several homologues with humulone, adhumulone, and cohumulone being the most prevalent (Figure 1).[2,11,13] The literature suggests humulones and other hop acids used in experimentation are isolated directly from the hop cone efficiently by means of supercritical fluid extraction, making the hop acids readily available.[13,14]

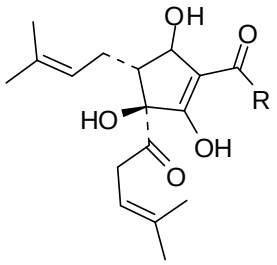
Figure 1. α-Humulone. R denotes side chain of $–CH_2CH(CH_3)_2$ (humulone); $-CH(CH_3)CH_2CH_3$ (adhumulone); or $–CH(CH_3)_2$ (cohumulone).



1

In their natural form, α-humulones **1** have little to no bittering capabilities. It is only upon the boiling of the wort mixture, a step in the brewing process that involves the application of heat to a fermenting mixture of sugar, yeast, and hops, that bitterness becomes present in beer. The emergence of bitter flavor is the result of the isomerization of α-humulone **1** to iso-α-humulone **2** (Figure 2).[14,15]

Figure 2. Iso-α-humulone. R denotes side chain of $–CH_2CH(CH_3)_2$ (isohumulone); $-CH(CH_3)CH_2CH_3$ (isoadhumulone); or $–CH(CH_3)_2$ (isocohumulone).



2

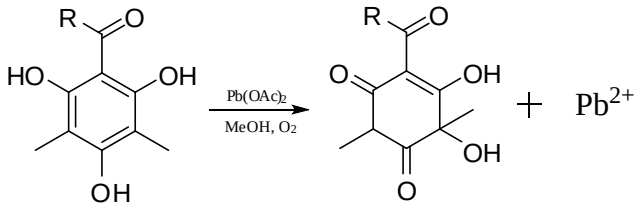
The yield in the brewing process for the isomerized product is not quantitative, with humulone homologue, cohumulone **1,** shown to be the most efficient at isomerizing into its respective iso-α-acid **2**.[14]

Historic Synthesis of α-Humulone

The literature seems to indicate that the last attempted full syntheses of α-humulone were carried out in the 1950's. By the end of the decade, it is assumed that the economic viability of synthesizing hop acids decreased. We presume that this trend was a result of increased use of analytical techniques that improved the efficiency of isolation (eg. supercritical fluid extraction) by allowing hop acids to be directly extracted from the hop cone. Yet, pertaining to the abovementioned economics study by van Braun, an increase in global food prices has led full-circle to the chemical synthesis of α-humulone once again becoming economically practical.[3]

The synthesis of an analogue of α-humulone for use as an antibiotic and tuberculostatic agent was attempted by Campbell and Coppinger in 1951. Methylacylphloroglucinol **3** was oxidized with lead acetate in open air to give α-humulone analogue acyl-3,5-dimethyl-2,3-dihydroxy-4,6-diketocyclohexene-1 **4** and lead salt precipitate (Scheme 1).[16]

Scheme 1. R = $-CH_2CH(CH_3)_2$

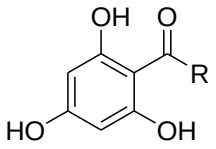


3 **4**

Wolfgang Reidl attempted the full synthesis of α-humulone in 1957 with a two-percent yield.[17] We will use Reidl's synthesis as the groundwork for our proposed method. Reidl employed a variety of electrophilic aromatic substitution methods, such as

Freidel-Crafts alkylation and the Hoesch method to produce, in a high yield, his starting material, phlorisovalerphenone **5** (Figure 3).[18]

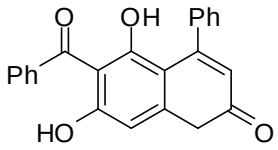
Figure 3. R= $–CH_2CH(CH_3)_2$



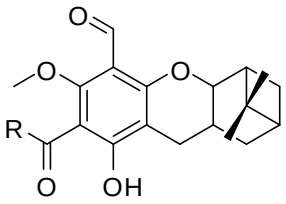
5

Interestingly, phlorisovalerphenone **5** has been a targeted intermediate in the synthetic pathway of 6-acyl-4-aryl/alkyl-5,7-dihydroxycoumarins, an anti-inflamitory agent, as well as two families of anti-malarial agents known as euglobals and rubustadials, illustrating the molecule's versatility in synthesis (Figure 4).[19-21]

Figure 4.

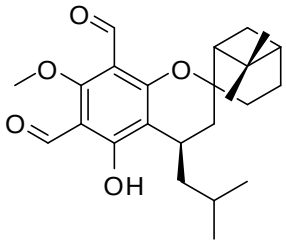


6-Acyl-4-aryl-5,7-dihydroxycoumarin



Euglobal

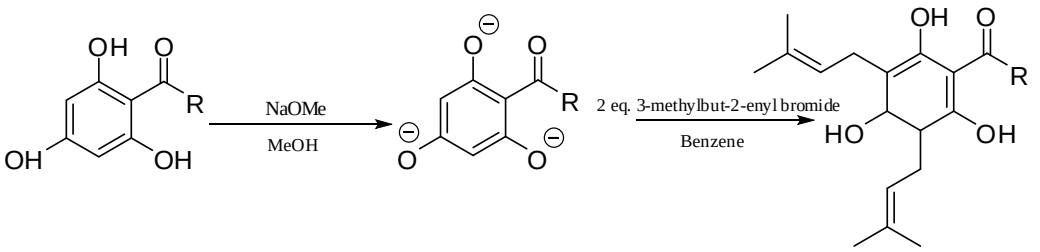
R= $-COCH_2CH(CH_3)_2$



Robustadial

Deprotonation of Phlorisovalerphenone **5** was carried out in a suspension of sodium methoxide. Azeotropic distillation was utilized by Reidl to replace methanol with benzene as the solvent. This was followed by alkylation with two equivalents of 3-methylbut-2-enyl bromide at 0^oC to produce 4-desoxyhumulone **6** which Reidl did not isolate upon initial reaction (Scheme 2).[17]

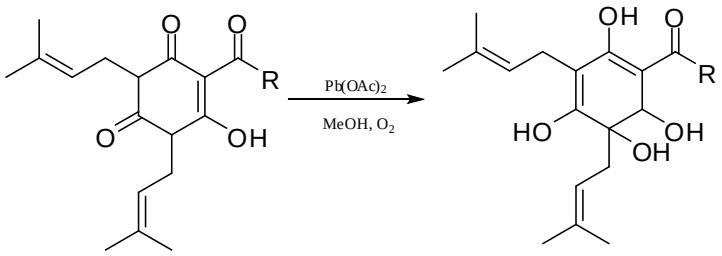
Scheme 2. R = $–CH_2CH(CH_3)_2$



5 **6**

Upon the removal of sodium bromide salt, Reidl was able to isolate 4-desoxyhumulone **6**. The residue was dried, taken up in a methanolic solution of lead acetate to induce oxidation, and the mixture was shaken in open air to produce an enantiomeric solution of α-humulone **1** (Scheme 3).[17]

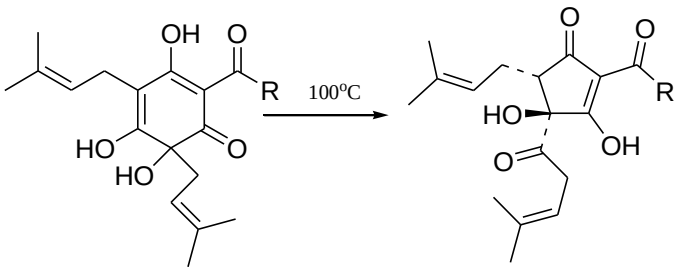
Scheme 3. R = $–CH_2CH(CH_3)_2$



7 **1**

The isomerization of α-humulone has been studied extensively for its usefulness in the flavoring of beer.[12-15] To produce the distinct bitter flavor, heat is used to induce isomerization of α-humulone **1** to the active flavoring agent iso-α-humulone **2** (Scheme 4).[15]

Scheme 4. R = $–CH_2CH(CH_3)_2$



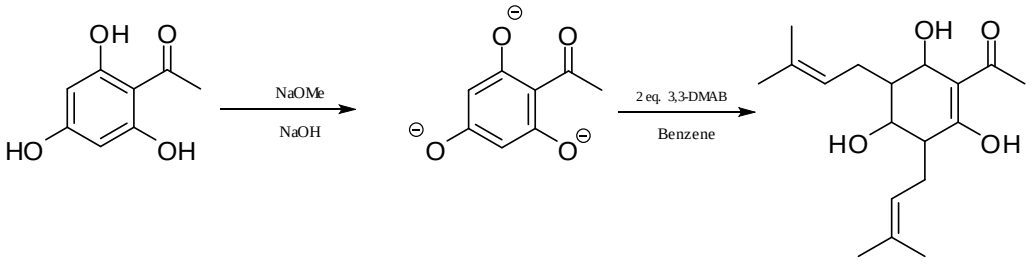
1 **2**

Proposed Chemistry

We are seeking to develop a contemporary method for the synthesis of α-humulone using 2,4,6-Tetrahydroxyacetophenone **8** (Figure 1, R= $-CH_3$) as the starting material. An α-humulone homologue with a methyl R-group **10** has yet to be synthesized or extracted. Although not based on precedence, the sheer number natural homologues of α-humulone that exist with only a variable R-group **1** lead us to believe an α-humulone homologue with a methyl R-group **10** will have the same chemical properties and retain the same bittering ability as the major natural α-humulone homologues (Figure 1).

The starting material, 2,4,6-Trihydroxyacetophenone **8**, will be deprotonated using sodium methoxide in the presence of molecular sieves to remove the potential for water to possibly quench the negative charge. Azeotropic distillation will then be carried out to exchange the methanolic solvent with benzene. Introduction of a non-polar solvent will lead to the precipitation of the tri-anionic ring. Under rigorous stirring, the tri-anionic ring system will be allylated with two equivalents 3,3-dimethylallyl bromide **12** (Figure 6) producing a 4-desoxyhumulone homologue **9** which will enter back into solution as sodium bromide salt precipitates out (Scheme 5).

Scheme 5.



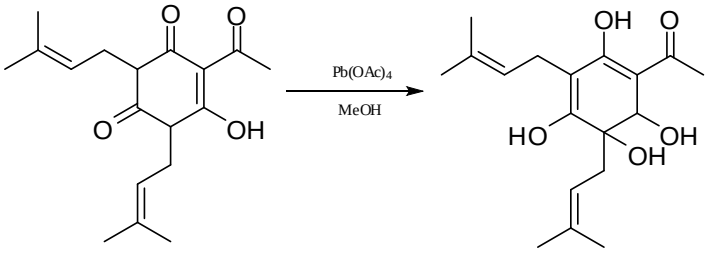
8 **9**

Filtration will be used to remove sodium bromide salt, and filtrate will be oxidized with lead tetraacetate to produce a new α-humulone homologue **10** (Scheme 6).

Alterations to Reidl's Oxidation Process

The literature seems to indicate that the conversion from phlorisovalerphenone **8** to 4-desoxyhumulone **9** is a fairly conventional process. If we were to base the remainder of the process on the synthesis proposed by Reidl[17] and Stevens[18], the next step would be to use a lead based oxidizing reagent to oxidize 4-desoxyhumulone **9** to our targeted humulone homologue **10** (Scheme 6).

Scheme 6.

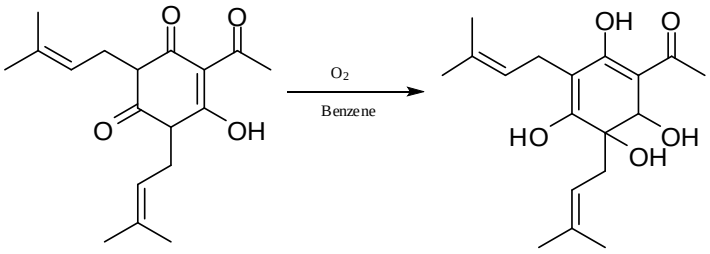


9 **10**

If that was the case, our method would follow Schemes 5 and 6 which are heavily based on the previous work done by Reidl[17] and Stevens[18] with minor changes (eg. Molecular sieves utilized in the deprotonation step and the use of lead tetraacetate in Scheme 6)

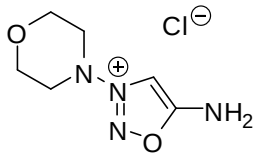
made to improve the efficiency of Reidl's strategy and his yield of two percent. Yet, there remain multiple strategies of oxidation that can be employed without producing the byproduct of reduced lead. One of these strategies is to bubble oxygen through a benzoic mixture of 4-desoxyhumulone **9** (Scheme 7) in a similar fashion to how bubbling oxygen has been used in medicinal chemistry to induce the release of nitric oxide from sydnonimines (Figure 5).[22]

Scheme 7.



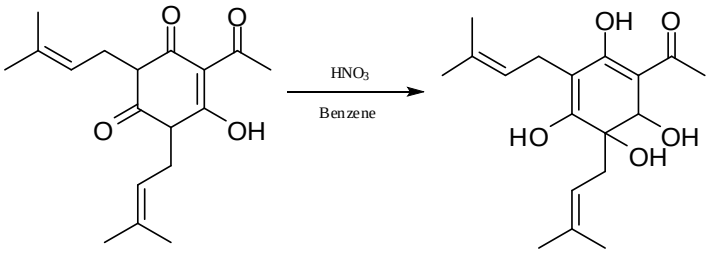
9 **10**

Figure 5.



Implicit in our search for oxidizing agents is the imperativeness to find a method that works well in unsaturated systems. Another method that may be useful is oxidation by nitric acid (Scheme 8).

Scheme 8.



9 **10**

The rationale behind this method is the preference of using nitric acid in the oxidation process of the side walls of carbon nanotubes, which are tubes made up of five and six membered arene rings that fold themselves so as to be as unreactive as possible.[23] This led us to presume that if the molecule could survive nitric acid treatment, then the ring could be transformed into its most oxidized state.

It is important to notice that our final α-humulone product **10** is very similar to the natural homologues of α-humulone **1** found in hops extract considering that our proposed homologue **10** retains all of the same functional and diene properties of natural α-humulone derivatives **1**. That being said, our target is not only an attractive compound itself for use towards a more cost efficient bittering of beer, but it also has the capability to be used to construct much larger and more elaborate molecules.

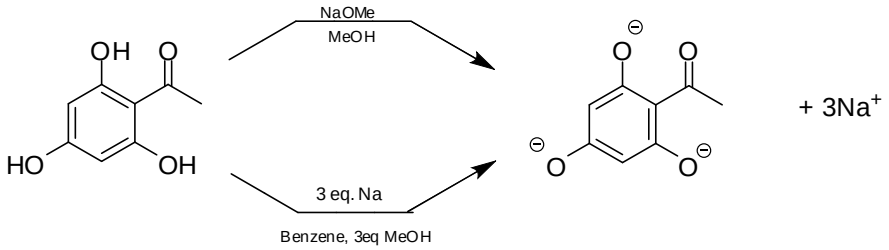
Results and Discussion

For the sake of clarity I will present my results and discussion in sections dealing with the major steps in the synthesis: deprotonation, allylation, oxidation and purification, and isomeration.

Deprotonation of 2,4,6-Trihydroxyacetophenone

Two different strategies were employed in the deprotonation process of 2,4,6-Trihydroxyacetophenone **8** (Scheme 9).

Scheme 9.



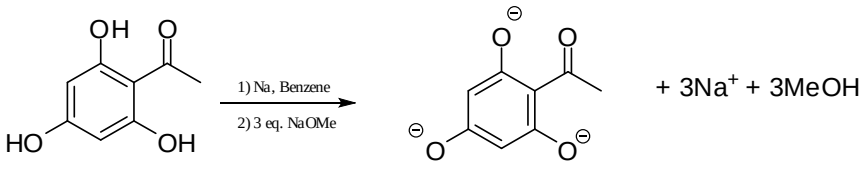
8 **11**

The top pathway is based on work by both Reidle[17] and Stevens[18]. Three equivalents of solid sodium were dissolved in methanol and added to solid 2,4,6-Trihydroxyacetophenone **8** along with 4 Å molecular sieves to remove water which could possibly quench the desired hydroxide anions, and the solution was allowed to stir in open air. The methanolic solvent denied us the ability to monitor the process of the deprotonation via TLC, yet a color change from pale green to dark green was seen after approximately an hour of stirring. Aziotropic distillation was then carried out at 58^{o} C[24] to replace the methanolic solvent with benzene, causing the tri-anionic salt **11** to precipitate out of solution increasing the ease at which it could be separated from the

solvent. The benzene was then removed from the system producing a dry, dark-green solid at a 70% yield.

In an attempt to generate the tri-anionic salt **11**, we devised the bottom pathway in Scheme 9. In this deprotonation strategy, 2,4,6-trihydroxyacetophenone **8** was dissolved in solution with three equivalents of sodium and taken up into a solution of benzene and the suspension was allowed to stir for three days. While it should be noted that a small portion of the elemental sodium was dissolved by the benzene, the addition of three equivalents of methanol was necessary to carry the deprotonation to finality. Again, after approximately an hour, a color change in the solution from a pale yellow to dark green was observed. Although this process was not reflected in any of the previous literature, we believe that the three equivalents of methanol would efficiently carry out the desired proton exchange while leaving behind an organic environment in which the following allylation step (Scheme 5) could be performed without interference from sodium methoxide. The rationale being that, once in solution, the methanol would dissolve the remaining sodium, proceed in the proton exchange process with the yet-to-be-deprotonated 2,4,6-Trihydroxyacetophenone **8**, producing the tri-anionic salt **11** and methanol (Scheme 10). Furthermore, inserting our deprotonation step for Reidl's[17] in the synthetic pathway allowed for a one pot synthesis of α-humulone.

Scheme 10.



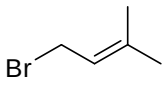
8 **11**

The ^{1}H-NMR data of the two deprotonation processes did not coincide, yet, as will be presented, this was rectified by ^{1}H-NMR data of products further along in the process. We assume that, whereas the top path allowed for the isolation of solid tri-anionic salt **11**, the process presented in Scheme 10 was in a dilute solution of mostly benzene, which was not efficient at dissolving the tri-anionic salt **11**. So, extracting a large enough quantity of the tri-anionic salt **11** to produce a useful spectrum proved to be difficult. Furthermore, correlated to the ^{1}H-NMR spectrum of the tri-anionic **11** salt of the bottom process, we are able to assume that the strategy did not produce any appreciable amount of organic byproduct which would have been present in the benzene fraction.

Allyation of the Tri-anionic Salt of 2,4,6-Trihydroxyacetophenone

The allylation process of the tri-anionic salt **11** of both of the abovementioned deprotonation processes was carried out in a similar fashion. At 0^{o} C and under rigorous stirring to control the extremely exothermic process[17], two equivalents of 3,3-dimethylallyl bromide **12** (Figure 6) were added drop-wise to the solution (Scheme 11).

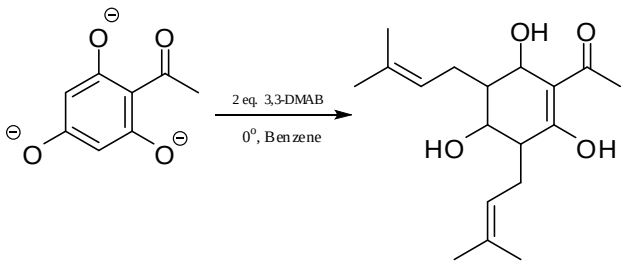
Figure 6.



3,3-DMAB

12

Scheme 11.



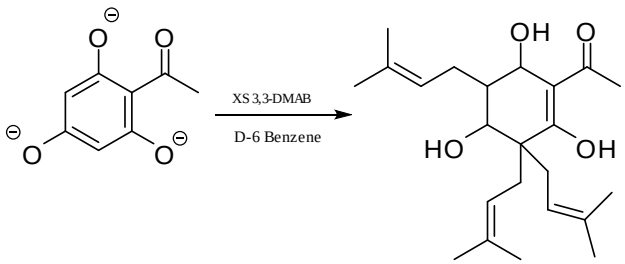
11 **9**

Upon aqueous work up with methanol, 4-desoxyhumulone **9** was produced.

To ensure the reaction of all of the 3,3-Dimethylallyl bromide **12**, the reaction was monitored using TLC. Every fifteen minutes 3,3-Dimethylally bromide **12** was spotted against the mixture in the pot. The 3,3-Dimethylallyl bromide **12** spot in the allylation of the tri-anion **11** salts produced in both of the deprotonation process above disappeared at around two-and-a-half hours.

^{1}H-NMR data further supported the ability of 3,3-Dimethylallyl bromide **12** to react with the tri-anionic salt. Excess 3,3-Dimethylallyl bromide **12** was added to an NMR tube with the tri-anionic salt **11** and deuterated benzene. We assume the reatction progressed to produce a tri-allylated product, which is an example of the above mentioned β-acid known as β-lupulone[25] (Scheme 12), which is similar in structure to our di-allylated target **9**.

Scheme 12.



11 β-lupulone

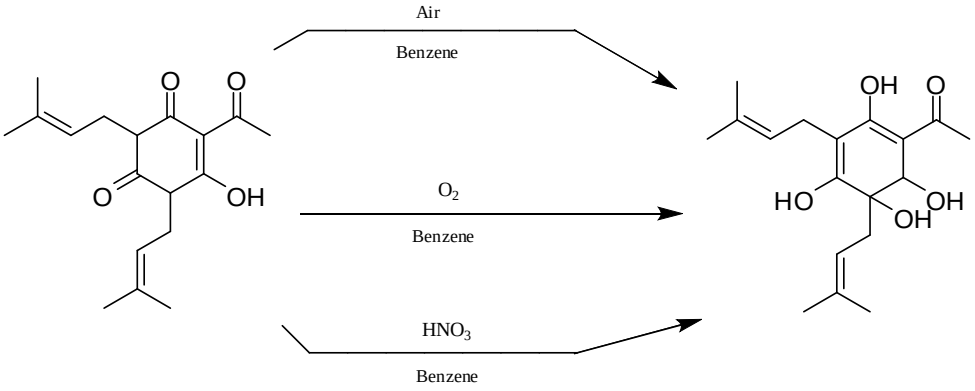
Although the excess of 3,3-dimethylallyl bromide caused its peaks to remain prevalent in the ^{1}H-NMR spectrum, peak shifts and the emergence of new peaks were observed leading us to assume that, in the presence of benzene, two equivalents of 3,3-dimethylallyl bromide **12** and our tri-anionic salt **11** are capable of reacting.

The process of isolating 4-desoxyhumulone **9** proved to be worth investigating more closely. In previous studies, we noticed a solid precipitate out of solution during the allylation process and, based on work by Stevens[18], we assumed the solid to be sodium bromide salt. In our earlier work, gravity filtration was employed to separate the liquid from the solid, with the solid being discarded. Thus, in our previous work, only the liquid was subjected to oxidation (Scheme 6). This method proved to be exceedingly unsuccessful. To resolve this problem, the ^{1}H-NMR data of both the liquid and the solid fractions was examined. The ^{1}H-NMR of the solid fraction showed emergence of peaks while nothing appeared in the spectrum of the liquid fraction. Under these circumstances we decided to progress treating the allylation and subsequent oxidation as a one-pot process removing neither the liquid nor the solid fraction.

Oxidation of 4-Desoxyhumulone and Purification of Crude

Once we concluded that the post-allylation product should be treated directly, we progressed to examining several different methods of oxidation in an attempt to identify the most efficient strategy (Scheme 13).

Scheme 13.



9 **10**

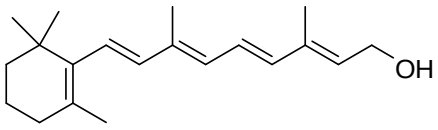
Only oxidation by pure oxygen was employed on the allylated product of both of the deprotonation pathways presented in Scheme 9. In both instances, pure oxygen was bubbled through the solution until dry resulting in a pale green solid. It was not until this point in the synthetic process that we were able to compare the effectiveness of our proposed deprotonation process to that of the process purported by Reidle.[17] Upon comparison of the ^{1}H-NMR spectrum of the final crude produced by the respective deprotonation processes proposed in Scheme 9, we see that the two processes produce similar final crude.

The oxidation of 4-desoxyhumulone **9** using nitric acid (Scheme 13) was examined on 4-desoxyhumulone **9** produced from the sodium methoxide in methanol step presented in Scheme 9. We took a very experimental approach in this process believing that, if the structure could remain intact under nitric acid treatment, then oxidation by

nitric acid would be useful in the preparation of α-humulone **10**. Upon observing the ^{1}H-NMR spectrum of the product of nitric acid oxidation, we observed peaks in regions very similar to the peaks seen in the ^{1}H-NMR spectrum of the crude produced by oxidation with oxygen, leading us to believe that both processes lead to the formation of what we presume is a similar product.

The third process we investigated involved bubbling air through a mixture of 4-desoxyhumulone **9**, which, again, was produced from the sodium methoxide in methanol deprotonation step in Scheme 9. Our rationale behind incorporating this oxidation process into those mentioned previously in the introduction is drawn from a study by Ihira et al. in which the photolytic resistance of the highly unsaturated molecule retinol (Vitamin A) (Figure7) was observed by objecting the molecule to oxidation by air, amongst other techniques.[26]

Figure 7.



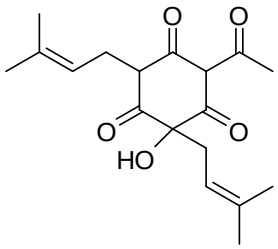
Retinol

After original attempts to obtain an ^{1}H-NMR spectrum in D4-Methanol and Chloroform-D proved to be unsuccessful, we were able to obtain an ^{1}H-NMR spectrum in deuterated DMSO. Although additional peaks in the spectrum indicative of organic byproduct dissolved by the DMSO were observed, the major peaks in the spectrum still coincide with the ^{1}H-NMR spectra from the other post-oxidation crude products. This leads us to believe that oxidation by air, pure oxygen, or nitric oxide, when enacted on 4-desoxyhumulone **9**, are all possible ways of producing a similar crude product.

A melting point of each crude product of oxidation was taken. The products formed by all methods in Scheme 13 of oxidation behaved similarly. Within the range of 61-67^{o} C, there was partial melting of the crude seen in the capillary tube. Comparing our melting point to the literature, which reports a melting point of 62^{o} C for α-humulone **10**[10], we concluded that there is a fair possibility that α-humulone **10** is present in our crude product. What the wide range of the melt confirms is that, much like what can be gleaned from the ^{1}H-NMR of the crude produced from oxidation by air, there are organic byproducts in the product. Still, a larger concern of ours at this point in the synthesis is the ionic salt (presumably NaBr from the allylation step) was still present in the capillary tube upon melting of the organic product. Accordingly, we sought to devise a method for separating the organic portion of the crude product from the ionic salt.

To extract the organic phase of the crude, the product was added to a vial with acetone and refrigerated overnight. The liquid portion of the mixture in the vial was then extracted and dried *in vacuo* in a round-bottom flask. To further ensure that only the organic phase was extracted, the dried product in the flask was taken up in a small amount of deuterated chloroform and added directly to an NMR tube. Data from ^{1}H-NMR of the acetone washed crudes produced by their respective process showed that, in all cases, there was a disappearance of peaks in a similar region (~3.4 ppm). Although this region is indicative of protons in the vicinity of hydroxyl groups, we assume that a tri-ketyl tautomer predominates in the solution (Figure 8).

Figure 8.



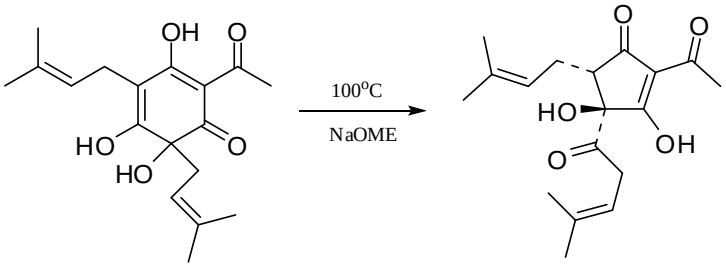
Tri-ketyl tautomer of **10**

At this point, all that we have done is proven further that all of the crude products are of the same or similar chemical composition. The abovementioned melting point agreement allows us to assume that we have produced α-humulone **10** to a certain extent. A key part in identifying if we have produced α-humulone **10** to an appreciable degree was to apply heat and see if the isomerization purported by Hay and Homiski[15] is able to be carried out with our product.

Isomerization of α-Humulone to Iso-α-Humulone

In the brewing process, the isomerization of α-humulone **10** to iso-α-humulone **13** is induced by a process that involves heating. This is reflected in the isomerization process purported by Hay and Homiski (Scheme 14).[15]

Scheme 14.



10 **13**

As in the purification process, an organic fraction was produced from the crude by separation in acetone. The fraction was dried and sodium methoxide in methanol were added under 100^{o} C[15] heat and rigorous stirring. The methanol was allowed to boil off several times, upon which more sodium methoxide in methanol was added to the pot. The final addition of base and solvent was allowed to boil off and the product was dried *in vacuo*. In a similar fashion to how the extracted α-humulone **10** was removed in its respective process, the dry product was treated with a small amount of deuterated chloroform to dissolve any organic in the process and the clear extract was added directly to an NMR tube. The data obtained from the ^{1}H-NMR shows that a chemical change certainly took place with far upfield peaks becoming clearer and a set of peaks emerging in the ~3.4 range indicative of protons in the vicinity of hydroxyl groups.

Conclusion

Throughout this study, we have investigated several methods concerned with the transformation of 2,4,6-Trihydroxyacetophenone **8** to our target α-humulone **10**, which may exists as several tautomers (Figure 9).

Figure 9.

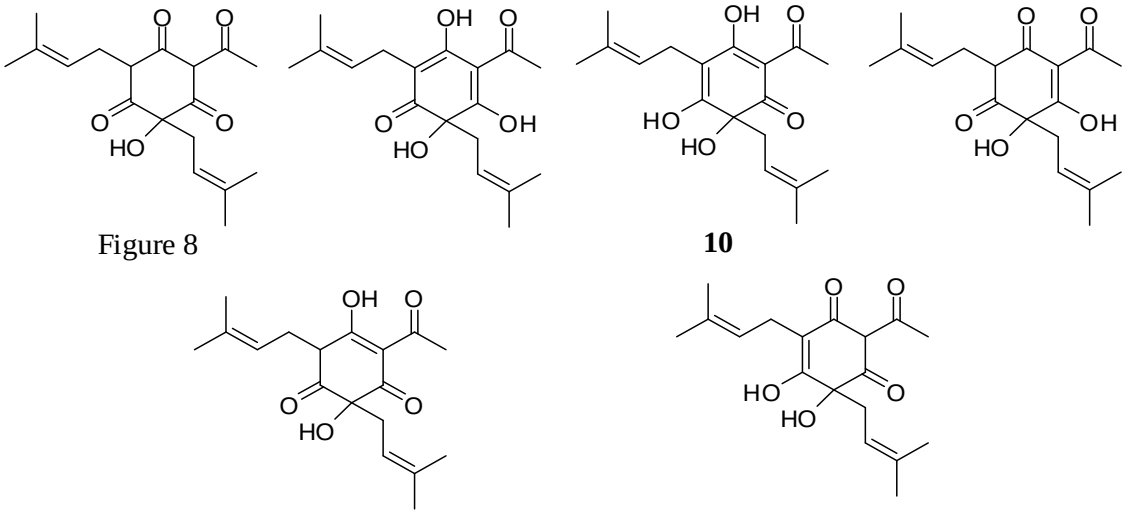
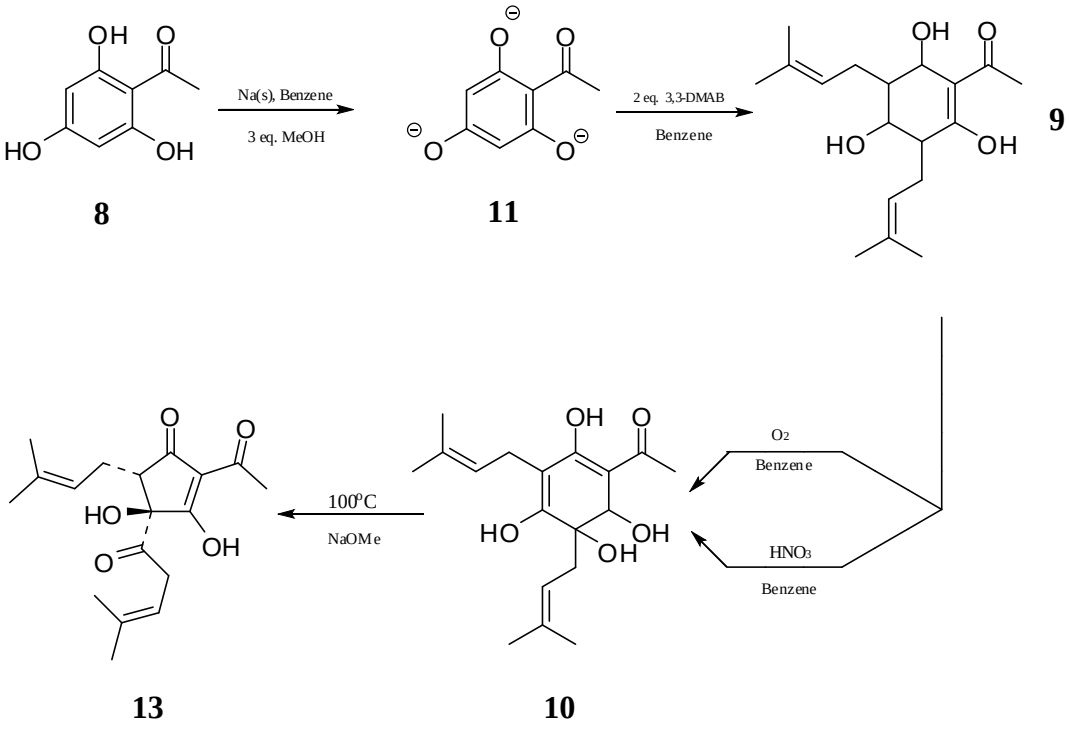


Figure 8 **10**

We have incorporated experimental procedures presented by Reidle[17] and Stevens[18] along with other techniques utilized in more recent syntheses as well as a technique devised by us. The isomerization, then, was a simple extrapolation using a similar method to Hay and Homiski[15], which allowed us to transform our product into the active flavoring agent iso-α-humulone **13** in a facile manner. In our attempts to explore multiple processes of carrying out certain steps in the synthetic pathway, we believe that we have devised a new, useful procedure in the synthesis of iso-α-humulone **13** (Scheme 15).

Scheme 15.



We have chosen the deprotonation step that involves the addition of three equivalents of methanol to sodium in benzene because it allows for the first three steps of Scheme 15 to be done in one pot. The ^{1}H-NMR data for oxidation by pure oxygen and nitric acid seemed to indicate that both were equally efficient at producing a similar

crude. As mentioned earlier, the final step is based off Hay and Homiski's isomerization process.[15]

There are several ways in which this study can be expanded. One obvious way is to focus on isolating pure α-humulone **10** in a higher yield rather than simply producing the molecule in a crude in an unknown, but presumably, low yield. We believe that this can be accomplished most effectively by improving the yield and purity of 4-desoxyhumulone **9**. Also, further studies could be performed that look deeper into ways to improve the isomerization process. The methodology proposed in this report can be expanded upon to improve the yield of the synthetic target, α-humulone **10**, and its subsequent isomerization to the active flavoring agent iso-α-humulone **13**.

Experimental

Solvents and Chemicals: Sodium methodixe was produced by dissolving solid sodium in methanol dried with activated 4 Å molecular sieves. All other solvents were used without further purification. All chemicals and deuterated solvents used in reactions were purchased from Aldrich and used without further purification.

Thin layer chromatography (TLC) plates used were Watman K6F silica gel 60 Å. Plates were run in a solution of 3:1 distilled hexane to ethyl acetate.

Equipment: The Joel JNM-ECP 400 FT-NMR spectrometer provided ^{1}H-NMR (400 MHz) and ^{12}C-NMR (100MHz) spectra in deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), deuterated water (D_2O), deuterated benzene (C_6D_6), and deuterated dimethyl sulfoxide (C_2D_6OS). Chemical shifts are reported in parts-per-million (ppm, δ).

Tri-sodium salt of 2,4,6-Trihydroxyacetophenone: To a dry round bottom flask, 2 μmol (.372g) 2,4,6-trihydroxyacetophenone **8** and 10mL of 0.6M sodium methoxide in methanol were added and stirred for 90 minutes at room temperature. Aziotropic distillation with benzene was carried out at 58^{o} C^{24}. The tri-anionic salt was dried *in vacuo* yielding 0.29g (70% yield) of the trisodium salt **11** of 2,4,6-Trihydroxyacetophenone **8**. ^{1}H-NMR (400 MHz, CD_3OD) δ 3.34 (s, 3H).

4-Desoxyhumulone: To a dry round bottom flask, 0.23g (1μmol) of the tri-sodium salt of 2,4,6-trihydroxyacetophenone **11** was added and taken up in benzene. The solution was placed on ice and 0.29g (2μmol) 3,3-dimethylallyl bromide **12** was added drop-wise under vigorous stirring for two and a half hours producing 4-desoxyhumulone **9** after aqueous work-up with methanol. The reaction vessel was capped and stored in the refrigerator. ^{1}H-NMR (400MHz, C_6D_6) δ 8.81 (s, 4H), δ 7.20 (t,1H).

4-Desoxyhumulone (one pot): To a dry round bottom flask, 2μmol (0.372g) 2,4,6-trihydroxyacetophenone **8**, 6 μmol (0.15g) of sodium, and 15mL of benzene were added. The solution was stirred for three days and 6μmol (0.242mL) of dry methanol was added and allowed to stir for three hours. The solution was placed on ice and, to the mixture 4μmol (0.6g) of 3,3-dimethylallyl bromide **12** were added drop-wise and stirred vigorously.

α-Humulone (crude): A solution of 4-Desoxyhumulone **9** in benzene was fitted with a septum punctured with a 16 gauge needle for ventilation and stirred vigorously. Air was bubbled into the solution through a plastic syringe fitted with a septum penetrated needle until a very small amount of solvent remained. The solid was dried *in vacuo* and 0.13g of α-humulone **10** crude was recovered in a 61% yield. ^{1}H-NMR (400MHz, CD_3OD) δ 7.37 (s, 1H), δ 5.19 (d, 1H), δ 3.50 (m, 8H), δ 2.52 (m, 6H), δ 2.45 (m, 4H), δ 1.60 (m, 5H)

α-Humulone (crude): A solution of 4-Desoxyhumulone **9** in benzene was fitted with a septum punctured with a 16 gauge needle for ventilation and stirred rigorously. Pure oxygen was bubbled into the solution through a plastic syringe fitted with a septum penetrated needle until a very small amount of solvent remained. The solid was dried *in vacuo* and 0.11g of α-humulone **10** crude was recovered in a 50% yield. ^{1}H-NMR (400MHz, CD_3OD) δ 7.33 (s, 1H), δ 4.87 (s, 8H), δ 3.35 (m, 6H), δ 1.91 (m, 1H), δ 1.60 (m, 4H).

α-Humulone (crude): To a solution of 4-Desoxyhumulone in benzene,1mL of concentrated nitric acid was added drop-wise under rigorous stirring. The solution was allowed to stir for 30 minutes. The solid was dried *in vacuo* and 0.14g of α-humulone **10**

crude was recovered in a 63% yield. ^{1}H-NMR (400MHz, CD_3OD) δ 7.32 (s, 1H), δ 4.85 (s, 8H), 3.30 (t, 5H), δ 1.98-1.56 (comp, 4H).

α-Humulone: Crude α-humulone **10** was collected into a vial and washed with acetone. The mixture was shaken vigorously and allowed to refrigerate overnight. The liquid fraction was then removed from the solution and added directly to a dry round bottom flask *in vacuo*. The dry oil was then diluted in a small amount of deuterated chloroform and added directly to an NMR tube for analysis. 1H-NMR (400MHz, $CDCl_3$) δ 7.26 (s, 3H), δ 2.53 (m, 1H), δ 1.61 (m, 8H).

Iso-α-humulone: Crude α-humulone **10** was collected into a vial and washed with acetone. The mixture was shaken vigorously and allowed to refrigerate overnight. The liquid fraction was then removed from the solution and added directly to a dry round bottom flask *in vacuo*. Sodium methoxide at a concentration of 1M was added to the system at 100^{o}C and allowed to boil off. After several additions of sodium methoxide the solution was dried *in vacuo*. The oil was then dissolved in a small amount of deuterated chloroform and added directly to an NMR tube for analysis. ^{1}H-NMR (400MHz, $CDCl_3$) δ 4.83 (m, 8H), δ 3.48 (m, 1H), δ 1.25 (d, 2H), δ 0.85 (t, 1H).

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Spectra is available from department upon request.