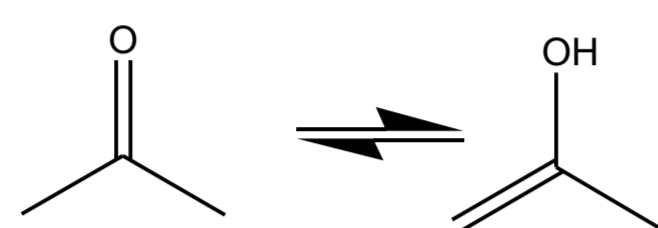


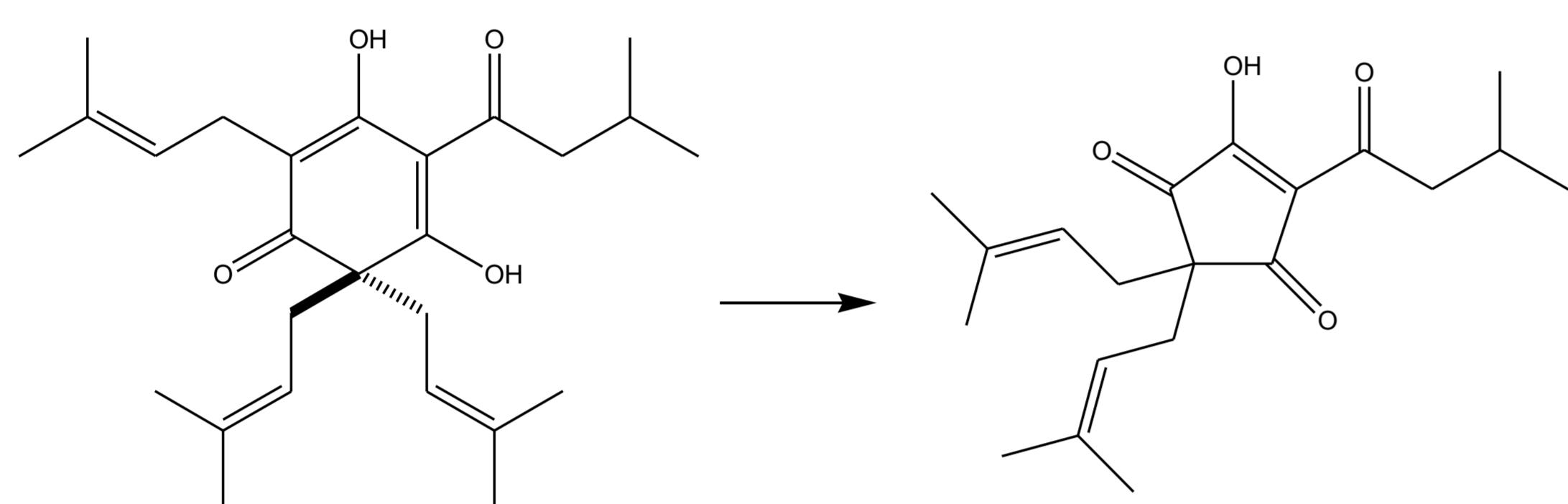
## Introduction

Hops have drawn chemists to the brewing industry since at least the 19<sup>th</sup> century. The chemistry of the hop- and beer-derived bitter acids has proved to be contrary. This is due to their existence in a potentially wide array of keto-enol tautomers:



As distinct chemical entities that are in dynamic equilibrium, tautomeric structures blur the properties of "single compounds". In the case of the hop- and beer bitter acids, there are potentially many keto-enol tautomeric variants.

In this study hulupones,  $\beta$ -acid oxidation products with appreciable bitterness, were studied to evaluate the scope and limitations of common computational chemistry methods to derive insight into hop chemistry, such as  $\alpha$ -acid isomerization, photodegradation of iso- $\alpha$ -acids and ligand binding studies with bitterness receptors.



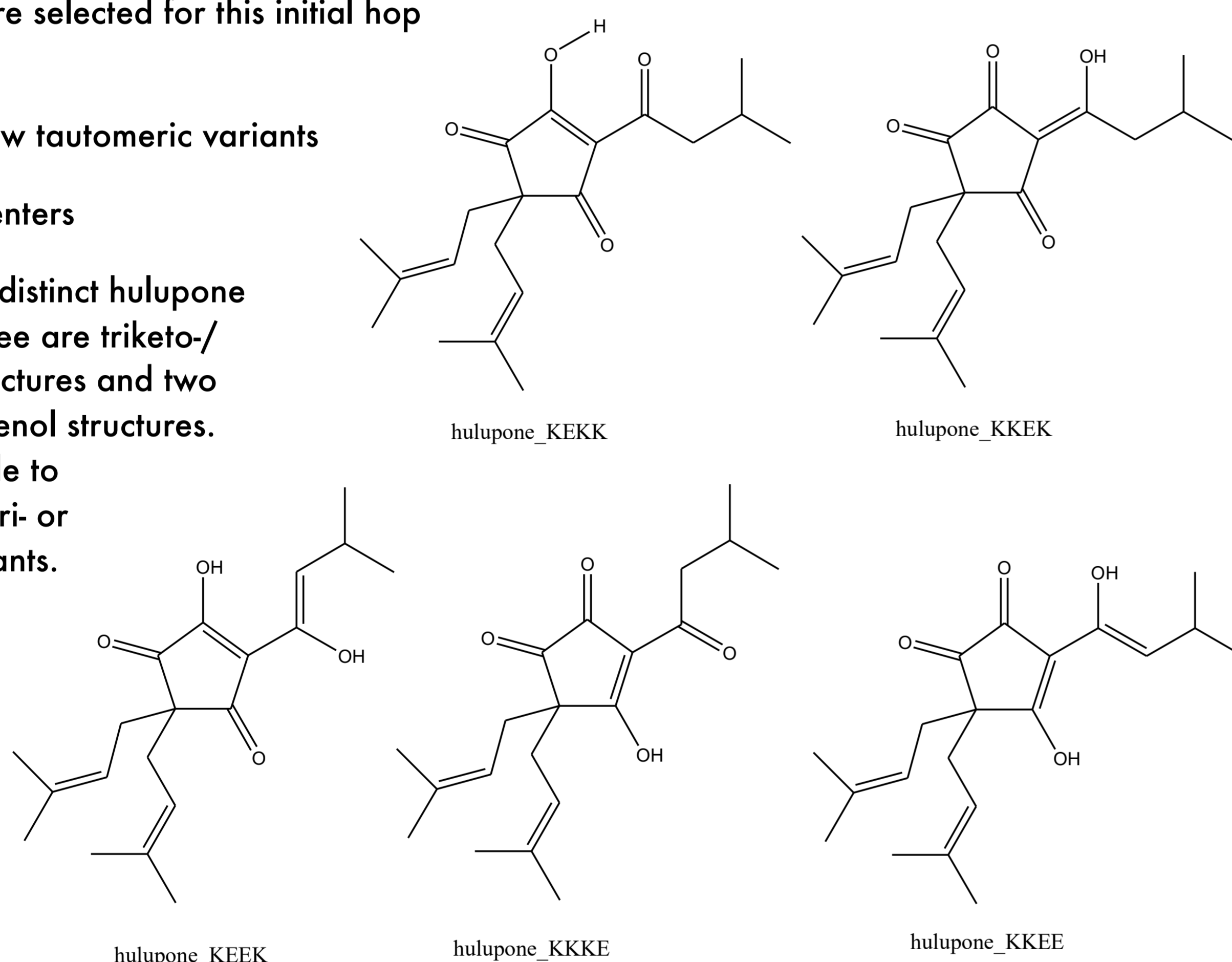
## Compounds studied

Hulupones were selected for this initial hop acid study:

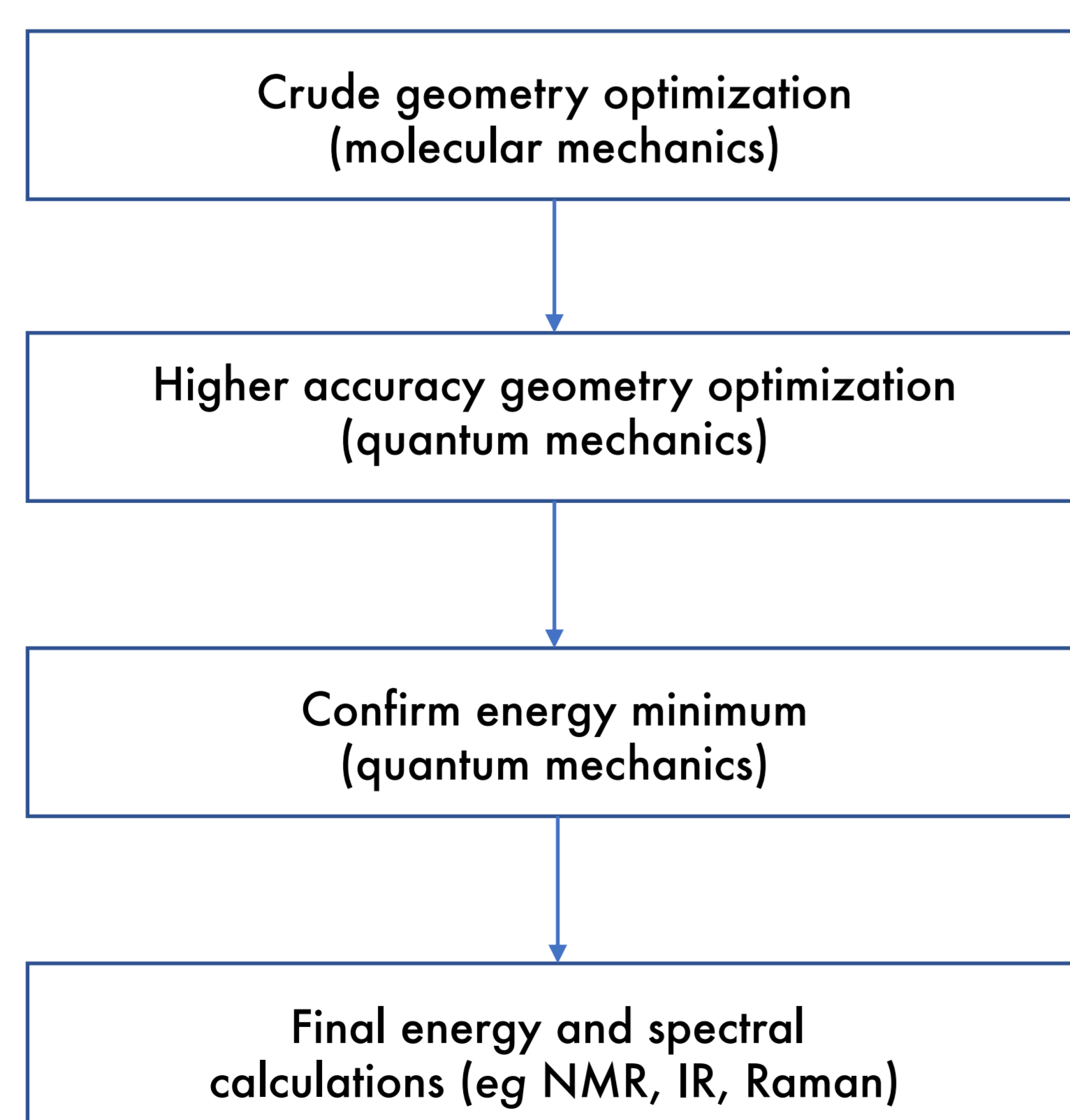
- Relatively few tautomeric variants
- No chiral centers

There are five distinct hulupone tautomers. Three are tri-keto-/mono-enol structures and two are di-keto-/di-enol structures.

It is not possible to construct any tri- or tetra-enol variants.



## Approach



(Each structure crudely optimized using the MOPAC algorithm included with Chem3D Ultra (v. 8.0, CambridgeSoft, Cambridge, MA), and then crudely geometry optimized. Energies, NMR and IR spectra calculated using Gaussian16W (64-bit, v. C.01; Gaussian Inc., Pittsburgh, PA). Three model chemistries, B3LYP, APFD and M062X were used in combination with the 6-31G(d) basis set. Single point energies were determined with the same chemistries and the 6-311++G(2d,p) basis set. All calculations were performed assuming a dielectric constant of unity (ie vacuum phase)).

## Results

Calculations using three hybrid functionals are as below. The Hartree is a common unit for expressing total electronic energies of atomic and molecular systems. 1 Hartree = 2625.5 kJ/mol

Structure code	Enthalpy of formation (Hartrees)	Gibbs free energy of formation (Hartrees)	Relative enthalpy of formation (kJ/mol)	Relative Gibbs free energy of formation (kJ/mol)
KEKK	-1079.728974	-1079.817436	0.0	0.0
KKEK	-1079.723398	-1079.810997	14.6	16.9
KKEE	-1079.700673	-1079.789213	74.3	74.1
KKKE	-1079.727888	-1079.816428	2.9	2.6
KEEK	-1079.713482	-1079.801324	40.7	42.3

Energies calculated using B3LYP/6-311++G(2d,p)//B3LYP/6-31G(d)

Structure code	Enthalpy of formation (Hartrees)	Gibbs free energy of formation (Hartrees)	Relative enthalpy of formation (kJ/mol)	Relative Gibbs free energy of formation (kJ/mol)
KEKK	-1078.859978	-1078.947006	0.0	0.0
KKEK	-1078.856716	-1078.942334	8.6	12.3
KKEE	-1078.831208	-1078.918537	75.5	74.7
KKKE	-1078.858928	-1078.946201	2.8	2.1
KEEK	-1078.843943	-1078.931417	42.1	40.9

Energies calculated using APFD/6-311++G(2d,p)//APFD/6-31G(d)

Structure code	Enthalpy of formation (Hartrees)	Gibbs free energy of formation (Hartrees)	Relative enthalpy of formation (kJ/mol)	Relative Gibbs free energy of formation (kJ/mol)
KEKK	-1079.257030	-1079.343049	0.0	0.0
KKEK	-1079.252089	-1079.337010	13.0	15.9
KKEE	-1079.231457	-1079.316014	67.1	71.0
KKKE	-1079.254924	-1079.341573	5.5	3.9
KEEK	-1079.241339	-1079.327806	41.2	40.0

Structure code	Relative proportion of tautomers (B3LYP; %)	Relative proportion of tautomers (APFD; %)	Relative proportion of tautomers (M062X; %)
KEKK	74.0	69.7	81.6
KKEK	0.08	0.5	1.3
KKEE	< 0.000001	< 0.000001	< 0.000001
KKKE	25.9	29.8	17.1
KEEK	< 0.000001	< 0.000001	< 0.000001

Calculated Boltzmann distributions based on Gibbs free energy calculations ( $\Delta G = -RT \ln K$ )

## Discussion and future scope

It is common practice to perform calculations with different theoretical models, as all have some deficiencies which may or may not be relevant to the systems being studied. Here three so-called hybrid functionals were used, which are combinations of parameterization and density functional theory.

For each of B3LYP, APFD and M062X it is clear that the three tri-keto-/mono-enol structures were the lowest in energy. Indeed each chemistry ranks the five tautomers in the same way, ie:



Increasing energy/decreasing abundance

The lowest energy structure, KEKK, corresponds to the <sup>1</sup>H NMR assignments of Tynan (1989) but this study suggests that KKKE should in principle also be measurable by NMR even if co-mixed with KEKK, although persual of both structures suggests that differences in their NMR spectra may be slight. This was tentatively confirmed by calculated <sup>1</sup>H and <sup>13</sup>C NMR spectra.

Further work is ongoing:

- Recalculate molecular parameters in simulated solvent (eg ethanol and water) environments
- Extend studies to  $\alpha$ -,  $\beta$ -, iso- $\alpha$ -, allo-iso- $\alpha$ - and anti-iso- $\alpha$ -acids
- Apply alternative model chemistries.

## Reference

Tynan, TJ, Synthesis and characterization of hop-derived compounds, and their application in quantitative high performance liquid chromatography, MSc thesis, 1989.