

1 Theoretical Study on Isomerization of α -Acids:

2 A DFT Calculation

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ABSTRACT:

The α -acids contained in hops are one of the ingredients of beer. The isomerization of α -acids produces iso- α -acids, the main source of bitterness in beer. In this study, the isomerization mechanism of the α -acid, cohumulone, was elucidated by using density functional theory in conjunction with the polarizable continuum model or 3D-RISM integral equation theory of liquids. The calculated reaction diagram is consistent with experimental results; the activation free energy difference between the *cis* and *trans* isomers is in good agreement with the experimental estimate. The activation energy difference results from solvation energy. Additionally, a calculation of NMR chemical shifts showed that the proton position of isocohumulone is different from that proposed previously. The effect of Mg^{2+} cation on the isomerization was also investigated. Both the activation and reaction free energy are stabilized by the presence of Mg^{2+} , which is consistent with experimental results. Water solvation reduces the activation free energy.

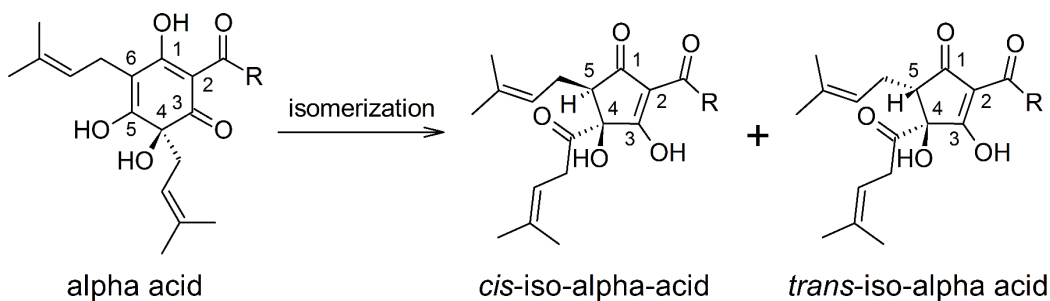
KEYWORDS: α -acid; iso- α -acid; isomerization; cohumulone; metal cation effect; solvent effect

1. Introduction

Hops (*Humulus lupulus* L.) have been cultivated around the world, such as in Europe, North America, Australia, and Asia (Delyser & Kasper, 1994). Among the many uses of hops, its biological activities, such as its anti-inflammatory and antioxidant properties, have been

exploited for medical purposes (Zanolli & Zavatti, 2008). Hops are a well-known raw material for beer and are one of the four essential ingredients of beer: barley malt, brewing water, yeast, and hops (Denis De Keukeleire, 2000). Hops impart a characteristic aroma and taste to beer, especially bitterness (Schönberger & Kostecky, 2011).

The important chemical compounds in hop flowers are α -acids. Unique side chains are found in some types of α -acids, such as humulone, cohumulone, and adhumulone (Scheme 1). α -acids do not have a bitter taste. Heating α -acids results in thermal isomerization via an acyloin-type ring contraction. The isomerization products are iso- α -acids, the main bitter-tasting components of beer. These iso- α -acids have *cis/trans* isomers (Scheme 1) (Jaskula-Goiris et al., 2010).



Scheme 1. Isomerization of α -acids to *cis/trans*-iso- α -acids: cohumulone, humulone, and adhumulone correspond to R= isopropyl, 2-methylpropyl, and 1-methylethyl, respectively

Many experimental studies have been performed to elucidate the isomerization mechanism of α -acids (Askew, 1964; Jaskula et al., 2008; Malowicki & Shellhammer, 2005;

Ocvirk & Košir, 2020; Verzele & van Boven, 1971). For example, the isomerization rate was measured in an aqueous buffer at pH 5.2 and 90–130°C (Malowicki & Shellhammer, 2005). The isomerization rate was found to be first order in the α -acid concentration, and the activation energy for the overall *cis* and *trans* isomerization was found to be 23.6 kcal/mol. Jaskula et al. (Jaskula et al., 2008) subsequently measured the *cis* and *trans* isomerization rates and proposed the difference in reaction profiles between the *cis* and *trans* isomers. The activation energy for the isomerization of *cis*-iso- α -acids is several kcal/mol higher than that of *trans*-iso- α -acids. There is a 3.4-kcal/mol difference in the activation energies of *cis* and *trans* cohumulone. By contrast, *cis*-iso- α -acids have a lower reaction energy than *trans*-iso- α -acids. The aforementioned studies determined the kinetics and thermodynamics of isomerization. However, the molecular mechanism for isomerization has not been elucidated.

Many experimental studies on the structural analysis of iso- α -acids have also been carried out (D. De Keukeleire & Verzele, 1971; Hoek et al., 2001; Khatib et al., 2007; Urban et al., 2013; Verzele & van Boven, 1971). The absolute configuration of *cis*-isohumulone was first determined as (4*R*, 5*S*) by Horeau's method in 1971 (D. De Keukeleire & Verzele, 1971; Verzele & van Boven, 1971). This structure has been used for over 40 years, including to assign ^{13}C and ^1H NMR chemical shifts (Hoek et al., 2001; Khatib et al., 2007). In 2013, the absolute configuration of *cis*-iso- α -acids was redetermined as (4*S*, 5*R*) by X-ray crystallography (Urban et al., 2013). These measurements were used to identify almost the entire molecular configuration. However, it is still difficult to accurately determine the positions of hydroxyl

protons using these measurements. An alternative molecular configuration, 1-OH-PRD (shown below), to the traditional configuration, 3-OH-PRD, is possible.

Isomerization yields are affected by various factors, such as the pH and metal cations (Jaskula et al., 2010; Steenackers et al., 2015). Several metal cations have long been known to affect isomerization (Lance et al., 1975). In particular, the acceleration effect of Mg^{2+} on isomerization has been well studied (Köller, 1969). Isomerization with the addition of magnesium oxide, MgO , at ambient temperature produces high yields with no side products. Increasing the quantity of MgO added has been shown to increase the yield over fewer days of reaction (Kostrzewa et al., 2016). This effect of Mg^{2+} on isomerization could be attributed to complex formation between an α -acid and a Mg^{2+} cation, because α -acids have a 1,4-diketone structure. This O-Mg-O structure is also found in the RNA folding, where Mg^{2+} cation is more suitable than other cations such as Na^+ and Ca^{2+} for its size and charge density (Petrov et al., 2011). However, Wietstock et al. recently carried out UV-VIS spectroscopy on α -acid and metal cation mixtures and proposed no complexation between α -acids and Mg^{2+} (Wietstock et al., 2016). The spectrum of equimolar mixtures of α -acids and Fe^{2+} was found to differ from that of α -acids alone, indicating complexation between α -acids and Fe^{2+} , whereas equimolar mixtures of α -acids and Mg^{2+} exhibited a similar spectrum to that of α -acids alone. Thus, the mechanism for the effect of Mg^{2+} on isomerization remains controversial.

The purposes of this study are to theoretically examine the isomerization mechanism of cohumulone ($\text{R}=\text{isopropyl}$), the hydroxyl proton position for isocohumulone, and the effect of Mg^{2+} addition on isomerization. There are some theoretical studies on the chemical reaction

of ingredients of foods and beverages (D. Milenković et al., 2018; D. A. Milenković et al., 2020; Tošović et al., 2017). However, to the best of our knowledge, no theoretical studies on hop α -acid isomerization have been performed to date. First, the transition state structure and the activation free energy are analyzed to elucidate the isomerization mechanism. Next, the position of the hydroxyl proton is determined by comparing calculated and experimentally measured NMR chemical shifts. Finally, the activation free energy of isomerization with Mg^{2+} is decomposed into several components to identify the main effect of the metal cation on the stability of isomerization.

2. Material and methods

We used density functional theory (DFT) in conjunction with the polarizable continuum model (PCM) or three-dimensional reference interaction site model (3D-RISM) integral equation theory (Beglov & Roux, 1997; Kovalenko & Hirata, 1998) to investigate the isomerization mechanism of cohumulone, the NMR spectra of cohumulone and isocohumulone, and the absorption spectra of cohumulone and complexes between cohumulone and metal cations. The combination of quantum chemical method and 3D-RISM theory is referred to as 3D-RISM-SCF method (Sato et al., 2000; Yoshida et al., 2011; Yoshida & Hirata, 2006), which enables us to obtain molecular solvation structure around a solute. The geometries of the reactants, intermediates, transition states, and products in solution were optimized at the B3LYP-GD3(BJ)/6-31+G(d,p) level with the SMD solvent model (Marenich et al., 2009). Notably, the dispersion correction, such as the D3 version of Grimme's dispersion

113 with Becke-Johnson damping, GD3(BJ) (Grimme et al., 2011), is needed to reproduce the
114 experimental reaction profile. To match the experimental conditions, the reaction profile and
115 absorption spectra were calculated in aqueous solution, whereas the NMR chemical shifts were
116 calculated in chloroform solution. Frequency calculations were used to obtain thermal
117 corrections to the Gibbs free energies at 25°C (Ochterski & Ph, 2000). Truhlar's quasiharmonic
118 approximation was adopted for the free energy calculations to correct for the spurious
119 overestimation of vibrational entropies introduced by treating low-frequency vibrational
120 modes as harmonic oscillators. This uses the same usual harmonic oscillator approximation in
121 the calculations of the vibrational partition functions, except that all of the vibrational
122 frequencies that are lower than 100 cm⁻¹ are set equal to 100 cm⁻¹ (Lam & Houk, 2014; Ribeiro
123 et al., 2011). The same geometries optimized at SMD-B3LYP-GD3(BJ)/6-31+G(d,p) level were
124 used for 3D-RISM-SCF calculations. The Kovalenko-Hirata closure is employed to solve the
125 3D-RISM calculation. The Lennard-Jones parameters for solutes were taken from the general
126 AMBER force field (GAFF) (Wang et al., 2004). The simple point charge (SPC) (Berendsen et
127 al., 1981) model with modified hydrogen parameters ($\sigma = 1.0$ Å and $\epsilon = 0.056$ kcal/mol) was
128 used for water solvent. The grid points in the 3D-RISM-SCF calculations were $128 \times 128 \times 128$
129 with a spacing of 0.5 Å. The gauge-independent atomic orbital (GIAO) (Ditchfield, 1974)
130 method and time-dependent DFT (TDDFT) were employed to calculate the NMR and
131 absorption spectra, respectively. NMR spectra are calculated by using the SMD model because
132 the solvent effect of non-polar chloroform is considered to be small. All the calculations were

performed using Gaussian 16 (Frisch et al., 2016), and a modified version of GAMESS (Schmidt et al., 1993) where the 3D-RISM-SCF method has been implemented (Yoshida & Hirata, 2006).

3. Results and Discussion

3-1. Isomerization reaction profile

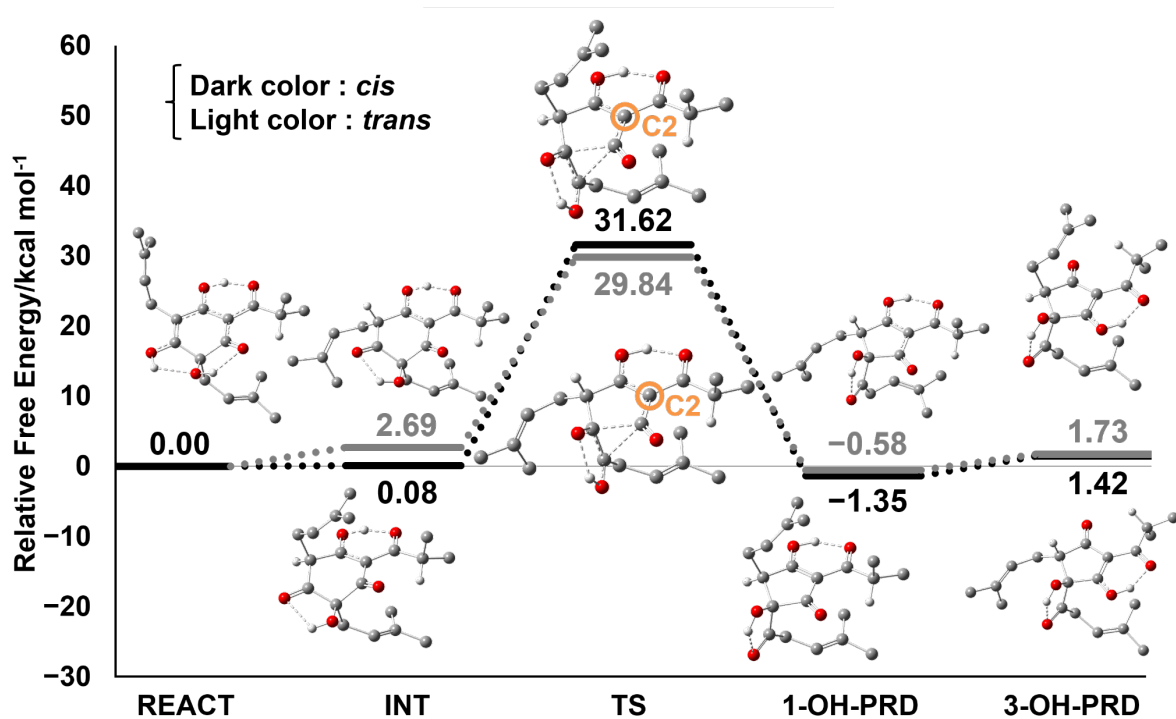


Figure 1. Cohumulone isomerization reaction profile

We first analyzed the mechanism of cohumulone isomerization. Following previous studies, (D. De Keukeleire & Verzele, 1971; Steenackers et al., 2015) the reaction path in Scheme S1 (Supplementary Materials) was assumed. In the first step, keto-enol tautomerization generates the *cis* and *trans* intermediates, INT_{*cis*} and INT_{*trans*}, from the reactant

cohumulone (REACT). Next, INT_{cis} and INT_{trans} pass through two transition states, TS_{cis} and TS_{trans}, and are converted into two products that both have a hydroxy group at the C1 position, 1-OH-PRD_{cis/trans}. Finally, the equilibrium between 1-OH-PRD_{cis/trans} and 3-OH-PRD_{cis/trans} with a hydroxy group at the C3 position is investigated. Note that 3-OH-PRD_{cis/trans} are traditionally regarded as the structures of *cis/trans* isocohumulone (Urban et al., 2013). It is also noted that no direct involvement of water in the reaction was assumed because intramolecular ring contraction and proton transfer are found to proceed at the TSs in a concerted manner.

The reaction profile calculated with the 3D-RISM-SCF method is shown in Figure 1. Both INT_{cis} and INT_{trans} are unstable compared with REACT by ~3 kcal/mol. The activation free energy of TS_{cis} (31.62 kcal/mol) is higher than that of TS_{trans} (29.84 kcal/mol), whereas the *cis* products, 1-OH-PRD_{cis} and 3-OH-PRD_{cis}, have lower reaction free energies (−1.35 and 1.42 kcal/mol, respectively) than the *trans* products, 1-OH-PRD_{trans} and 3-OH-PRD_{trans} (−0.58 and 1.73 kcal/mol, respectively). These calculated results are consistent with experimental results (Jaskula et al., 2008). The calculated activation free energy difference of 1.78 kcal/mol between the *cis* and *trans* products is in good agreement with the experimental value of 3.4 kcal/mol (Malowicki & Shellhammer, 2005). Furthermore, 1-OH-PRDs are found to be more stable than 3-OH-PRDs, which suggests that 1-OH-PRDs are more realistic candidates for the structure of isocohumulone (see discussion below).

3-2. Analysis of TS_{cis/trans}

The difference in the activation free energies of the *cis* and *trans* products was analyzed.

We decomposed the free energy difference between the TS_{cis} and TS_{trans}, $\Delta G = 1.78$ kcal/mol, into three contributions, the structural energy difference in the gas phase ΔE_{gas} , the solvation free energy difference $\Delta\mu_{\text{sol}}$, and thermal free energy difference $\Delta G_{\text{thermal}}$:

$$\Delta G = \Delta E_{\text{gas}} + \Delta\mu_{\text{sol}} + \Delta G_{\text{thermal}} \quad (1)$$

where $\Delta E_{\text{gas}} = E_{\text{gas}}(\text{TS}_{\text{cis}}) - E_{\text{gas}}(\text{TS}_{\text{trans}})$, and $\Delta\mu_{\text{sol}}$, and $\Delta G_{\text{thermal}}$ are defined analogously. ΔE_{gas} is calculated to be -4.71 kcal/mol, indicating that TS_{cis} has lower structural energy than TS_{trans}. On the other hand, TS_{trans} has lower solvation free energy than TS_{cis}, $\Delta\mu_{\text{sol}} = 6.33$ kcal/mol. $\Delta G_{\text{thermal}}$ is found to be as small as 0.16 kcal/mol. These results demonstrate that solvation plays a major role in the stabilization of TS_{trans}. Notably, the sophisticated 3D-RISM-SCF method was needed for the proper description of solvation. The SMD solvation model failed to reproduce the experimental result; the free energy of TS_{trans} (28.43 kcal/mol) calculated with the SMD model is similar to that of TS_{cis} (28.40 kcal/mol).

Further analysis decomposing the solvation free energy into each atom contribution showed that the main reason for the difference is the C2 atom, the center of the β -triketo system. TS_{cis} has low structural energy due to the interaction between the C2 atom and side chain whereas TS_{trans} has low solvation free energy due to the strong solvation of the C2 atom. The importance of the stabilization of the β -triketo system for the isomerization was suggested in the previous study (Jaskula-Goiris et al., 2010).

3-3. Proton position

Experimental studies have reported 3-OH-PRD as the isomerization end product (Urban et al., 2013). However, the calculated reaction profile showed that 1-OH-PRD is more stable than 3-OH-PRD (Figure 1). We analyzed the proton position in more detail by calculating the ^{13}C and ^1H NMR chemical shifts of REACT, PRD_{cis} , and PRD_{trans} for comparison with experimental data (Hoek et al., 2001; Khatib et al., 2007). Although direct comparison is precluded by the unavailability of NMR data for hydroxyl protons, a useful comparison can be made for NMR data for ^{13}C and other protons. The atomic numbering of REACT, PRD_{cis} , and PRD_{trans} is shown in Figure S1.

First, the NMR spectra of REACT were used as a reference. The calculated and experimental NMR chemical shifts of REACT and the correlation between these results are shown in Table S1 and Figure 2. Reasonably good correlations are obtained for both ^{13}C and ^1H , with coefficients of determination, R^2 , of 0.9980 and 0.9947, respectively. This result validates our NMR calculation.

Figure 2 shows the correlations between the experimental and calculated NMR chemical shifts for 3-OH-PRDs (the proposed isocohumulone structure) and 1-OH-PRDs (the lower free energy). The calculated and experimental NMR chemical shifts of 3-OH-PRDs and 1-OH-PRDs are summarized in Tables S2 and S3. The R^2 of the aforementioned correlations for 1-OH-PRDs are larger than those for 3-OH-PRDs for both the ^{13}C and ^1H NMR chemical shifts of the *cis* and *trans* products. For example, the correlation for ^{13}C NMR of 1-OH- PRD_{cis}

has a R^2 of 0.9987, which is larger than the R^2 of 0.9944 for the corresponding 3-OH-PRD_{cis} correlation. These results show that better correlations between the calculated and experimental results were obtained for the stable 1-OH-PRDs than for 3-OH-PRDs in both the *cis* and *trans* configurations. Therefore, 1-OH-PRD is considered to be the actual α -acid isomerization product, not 3-OH-PRD.

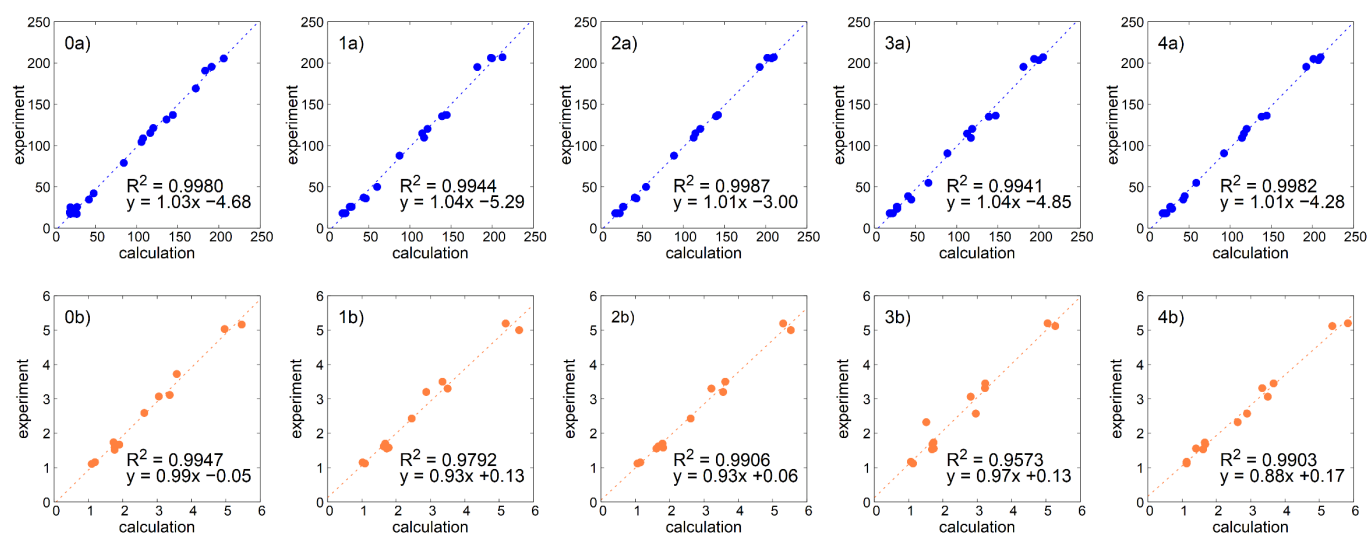


Figure 2. Correlations between experimental and calculated NMR chemical shifts for 0) cohumulone, 1) 3-OH-PRD_{cis}, 2) 1-OH-PRD_{cis}, 3) 3-OH-PRD_{trans}, and 4) 1-OH-PRD_{trans}: a) ^{13}C and b) ^1H

3-4. Reaction profile with Mg^{2+}

The effect of the magnesium cation on the isomerization reaction profile was investigated. The DFT calculation was used to determine the most stable structures of the Mg complexes of cohumulone and isocohumulone, which were used to propose the reaction

220 scheme with magnesium cation shown in Scheme S2. First, cohumulone becomes an anion by
221 protonating a water molecule and forms the Mg-REACT complex, which includes a 6-
222 membered ring structure with O-Mg-O coordination. Because Mg^{2+} cation is generally hexa-
223 coordinated, 4 water molecules were additionally coordinated to Mg^{2+} cation. Next, Mg-
224 REACT transforms into the Mg-INT intermediates, which pass through transition states, Mg-
225 TSs, and finally generate the products, Mg-PRDs.

226 The reaction profile of the isomerization of cohumulone with the Mg cation calculated
227 with the 3D-RISM-SCF method is shown in Figure 3b. The reaction profile of anionic
228 cohumulone is included in Figure 3a for comparison.

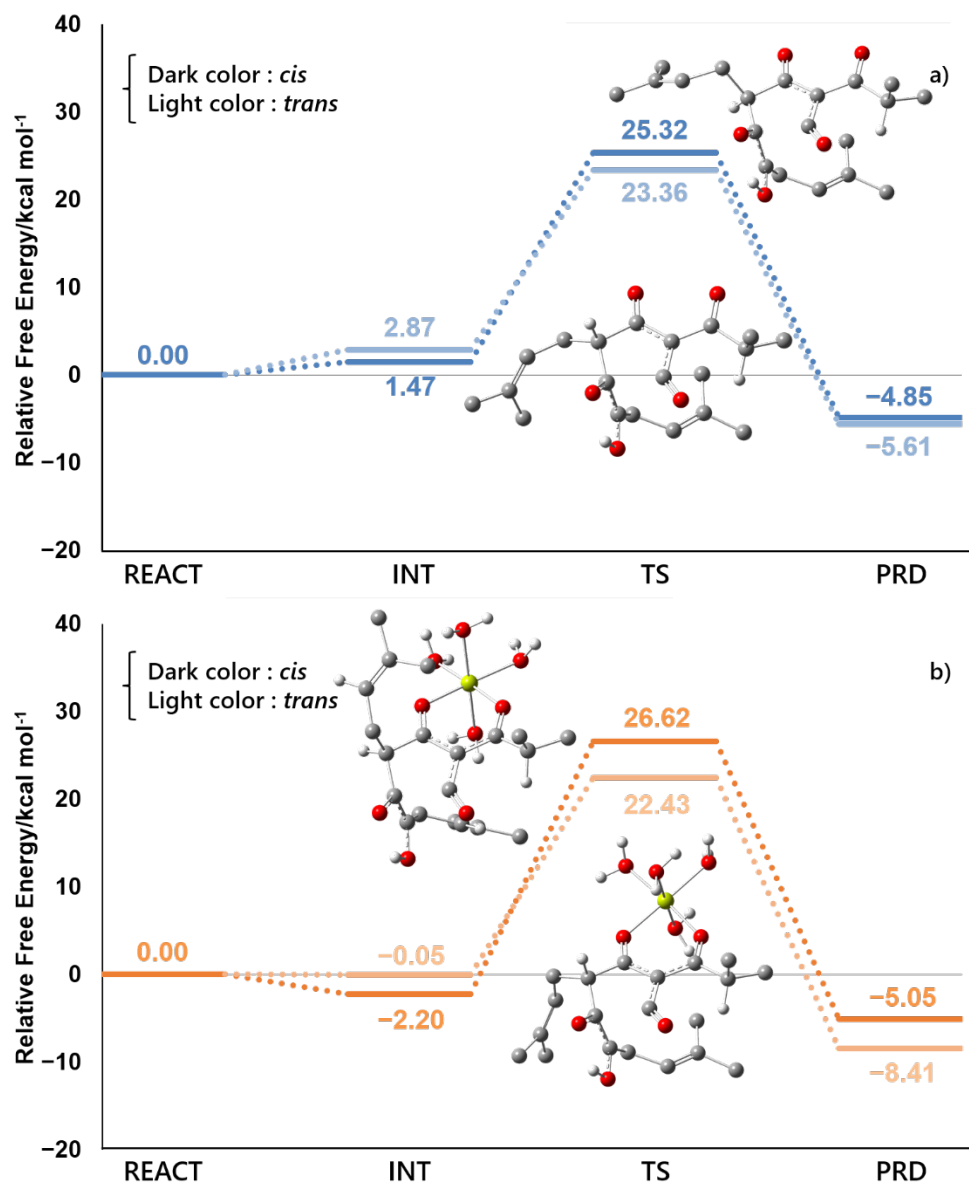


Figure 3. Isomerization reaction profiles for a) anionic cohumulone and b) Mg^{2+} complex.

Table 1 shows the activation free energy and the reaction free energy for the aforementioned isomerization reactions. The activation and reaction free energies of the Mg complex are approximately 5 kcal/mol lower than those of the neutral states. For example, the activation free energy of the Mg complex in the *trans* configuration (22.43 kcal/mol) is lower

than for anionic and neutral cohumulone (23.36 and 29.84 kcal/mol, respectively). This result is consistent with experimental results (Jaskula et al., 2010; Köller, 1969; Kostrzewa et al., 2016).

Table 1. Activation and reaction free energy differences (in kcal/mol)

	$\Delta G^\ddagger$		$\Delta_r G$	
	<i>trans</i>	<i>cis</i>	<i>trans</i>	<i>cis</i>
Mg ²⁺	22.43	26.62	−8.41	−5.05
anion	23.36	25.32	−5.61	−4.85
neutral	29.84	31.62	−0.58	−1.35

We determined the cause of the decrease in the activation free energy of the Mg complex by decomposing the activation free energy $\Delta G^\ddagger$ into three contributions, the structural energy in the gas phase ΔE_{gas} , the solvation free energy difference $\Delta\mu_{\text{sol}}$ and the thermal free energy difference $\Delta G_{\text{thermal}}$ between TS and REACT:

$$\Delta G^\ddagger = \Delta E_{\text{gas}} + \Delta\mu_{\text{sol}} + \Delta G_{\text{thermal}} \quad (2)$$

where $\Delta E_{\text{gas}} = E_{\text{gas}}(\text{TS}) - E_{\text{gas}}(\text{REACT})$, and $\Delta\mu_{\text{sol}}$ and $\Delta G_{\text{thermal}}$ are defined analogously. We evaluated the activation free energy differences between the Mg complexes and neutral cohumulone to compare the relative size of each contribution:

$$\Delta\Delta G^\ddagger = \Delta\Delta E_{\text{gas}} + \Delta\Delta\mu_{\text{sol}} + \Delta\Delta G_{\text{thermal}} \quad (3)$$

where $\Delta\Delta G^\ddagger = \Delta G^\ddagger(\text{Mg}^{2+}) - \Delta G^\ddagger(\text{neutral})$, and $\Delta\Delta E_{\text{gas}}$, $\Delta\Delta\mu_{\text{sol}}$, and $\Delta\Delta G_{\text{thermal}}$ are defined analogously. The calculated results for the *cis* and *trans* activation free energies are shown in

Table 2. The solvation energy difference contributes considerably to the decreases in the activation free energies of -13.61 and -27.47 kcal/mol for the *cis* and *trans* configurations, respectively. This result shows that one of the major factors that stabilize the TS of Mg complexes is the large difference in the solvation free energies between REACT and the TS of Mg complexes.

Table 2. Decomposition analysis of activation free energy differences (in kcal/mol)

Mg ²⁺ -neutral	$\Delta\Delta G^\ddagger$	$\Delta\Delta E_{\text{gas}}$	$\Delta\Delta\mu_{\text{sol}}$	$\Delta\Delta G_{\text{thermal}}$
<i>cis</i>	-5.00	9.39	-13.61	-0.79
<i>trans</i>	-7.41	20.35	-27.47	-0.30

Finally, the effect of complex formation on the absorption spectra was investigated by calculating UV-VIS absorption spectra at the TD-B3LYP-GD3(BJ)/6-31+G(d,p) level for the neutral and anionic states and the Mg²⁺, Fe²⁺, and Fe³⁺ complexes (Figure S2). As in the case of the Mg²⁺ complex, 4 water molecules were additionally coordinated to Fe²⁺ and Fe³⁺ complexes. In Fe²⁺ and Fe³⁺ complexes, only SMD results are shown because of the difficulty of calculating the higher excited states using 3D-RISM-SCF. It is noted that there is no qualitative difference between 3D-RISM-SCF and SMD results in the lower excited states. Considering the long-wavelength tail, the spectrum of the Mg²⁺ complex (Figure S2c) is similar to those of the neutral and anionic states (Figures S2a and S2b). In these cases, no peak is found at >400 nm, and the

longest-wavelength (lowest-energy) peak corresponding to the π - π^* excitation is located at approximately 350 nm. By contrast, the spectra of the Fe^{2+} and Fe^{3+} complexes (Figures S2d and S2e) are very different from that of REACT. The spectra of these complexes exhibit several peaks at >400 nm and long-wavelength tails. The complexes have an empty low-energy d -type molecular orbital that enables ligand-to-metal charge transfer (LMCT) transitions to proceed at a low excitation energy. These results are consistent with experimental results (Wietstock et al., 2016) and indicate that it is difficult to determine the complexation of Mg^{2+} cation with α -acids using UV-VIS spectra alone.

5. Conclusions

In summary, the mechanism for the isomerization of cohumulone, the hydroxyl proton position of isocohumulone, and the effect of Mg^{2+} addition on isomerization were theoretically investigated. The calculated reaction profile and the activation free energy difference between *cis* and *trans* configurations are consistent with experimental results. The activation free energy difference between the *cis* and *trans* configurations is attributed to the solvation energy. An analysis of the calculated reaction profile and the NMR spectra showed that 1-OH-PRD is the actual isomerization product, not 3-OH-PRD. The isomerization reaction profile for cohumulone with Mg^{2+} demonstrated that Mg^{2+} increases iso- α -acids yields and accelerates isomerization, which is in good agreement with experimental results. The solvent effect of water contributes considerably to the decrease in the activation free energy of the isomerization reaction with Mg^{2+} . Similar spectra were obtained for the Mg^{2+} complex and

cohumulone, showing that it is difficult to determine complexation using UV-VIS spectra alone.

Declaration of Competing Interests

The authors declare no competing financial interest.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Numbers JP16KT0165, JP17K05757, JP18H04657, JP20H04813, and JP20H05839. The computations were performed in part at the Research Center for Computational Science, Okazaki, Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org>.

References

Askew, H. O. (1964). Changes in Hop α Acids Concentrations on Heating in Aqueous Solutions and Unhopped Worts. *Journal of the Institute of Brewing*, 70(6), 503–513.
<https://doi.org/10.1002/j.2050-0416.1964.tb06356.x>

307 Beglov, D., & Roux, B. (1997). An integral equation to describe the solvation of polar
 308 molecules in liquid water. *Journal of Physical Chemistry B*, 101(39), 7821–7826.
 309 <https://doi.org/10.1021/jp971083h>

310 Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., & Hermans, J. (1981). Interaction
 311 Models for Water in Relation to Protein Hydration. In *Intermolecular Forces* (Vol. 3,
 312 Issue September, pp. 331–342). https://doi.org/10.1007/978-94-015-7658-1_21

313 De Keukeleire, D., & Verzele, M. (1971). The absolute configuration of the isohumulones and
 314 the humulinic acids. *Tetrahedron*, 27(20), 4939–4945. [https://doi.org/10.1016/S0040-](https://doi.org/10.1016/S0040-4020(01)98199-2)
 315 [4020\(01\)98199-2](https://doi.org/10.1016/S0040-4020(01)98199-2)

316 De Keukeleire, Denis. (2000). Fundamentals of beer and hop chemistry. *Química Nova*,
 317 23(1), 108–112. <https://doi.org/10.1590/S0100-40422000000100019>

318 Delyser, D. Y., & Kasper, W. J. (1994). Hopped Beer: The Case For Cultivation. *Economic*
 319 *Botany*, 48(2), 166–170. <https://doi.org/10.1007/BF02908210>

320 Ditchfield, R. (1974). Self-consistent perturbation theory of diamagnetism I. A gauge-
 321 invariant LCAO method for N.M.R. Chemical shifts. *Molecular Physics*, 27(4), 789–807.
 322 <https://doi.org/10.1080/00268977400100711>

323 Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R.,
 324 Scalmani, G., Barone, V., Petersson, G. A., Nakatsuji, H., Li, X., Caricato, M., Marenich,
 325 A. V., Bloino, J., Janesko, B. G., Gomperts, R., Mennucci, B., Hratchian, H. P., Ortiz, J.

326 V., Izmaylov, A. F., Sonnenberg, J. L., Williams-Young, D., Ding, F., Lipparini, F., Egidi,
 327 F., Goings, J., Peng, B., Petrone, A., Henderson, T., Ranasinghe, D., Zakrzewski, V. G.,
 328 Gao, J., Rega, N., Zheng, G., Liang, W., Hada, M., Ehara, M., Toyota, K., Fukuda, R.,
 329 Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T.,
 330 Throssell, K., Montgomery, J. A., Jr., Peralta, J. E., Ogliaro, F., Bearpark, M. J., Heyd, J.
 331 J., Brothers, E. N., Kudin, K. N., Staroverov, V. N., Keith, T. A., Kobayashi, R., Normand,
 332 J., Raghavachari, K., Rendell, A. P., Burant, J. C., Iyengar, S. S., Tomasi, J., Cossi, M.,
 333 Millam, J. M., Klene, M., Adamo, C., Cammi, R., Ochterski, J. W., Martin, R. L.,
 334 Morokuma, K., Farkas, O., Foresman, J. B., Fox, D. J. (2016). Gaussian 16, Revision A.03,
 335 Gaussian, Inc., Wallingford CT.

336 Grimme, S., Ehrlich, S., & Goerigk, L. (2011). Effect of the damping function in dispersion
 337 corrected density functional theory. *Journal of Computational Chemistry*, *32*(7), 1456–
 338 1465. <https://doi.org/10.1002/jcc.21759>

339 Hoek, A. C., Hermans-Lokkerbol, A. C. J., & Verpoorte, R. (2001). An improved NMR
 340 method for the quantification of α -acids in hops and hop products. *Phytochemical*
 341 *Analysis*, *12*(1), 53–57. [https://doi.org/10.1002/1099-1565\(200101/02\)12:1<53::AID-PCA550>3.0.CO;2-E](https://doi.org/10.1002/1099-1565(200101/02)12:1<53::AID-PCA550>3.0.CO;2-E)
 342

343 Jaskula-Goiris, B., Aerts, G., & De Cooman, L. (2010). Hop α -acids isomerisation and
 344 utilisation: An experimental review. *Cerevisia*, *35*(3), 57–70.
 345 <https://doi.org/10.1016/j.cervis.2010.09.004>

346 Jaskula, B., Aerts, G., & De Cooman, L. (2010). Potential impact of medium characteristics on
 347 the isomerisation of hop α -acids in wort and buffer model systems. *Food Chemistry*,
 348 123(4), 1219–1226. <https://doi.org/10.1016/j.foodchem.2010.05.090>

349 Jaskula, B., Kafarski, P., Aerts, G., & De Cooman, L. (2008). A kinetic study on the
 350 isomerization of hop α -acids. *Journal of Agricultural and Food Chemistry*, 56(15), 6408–
 351 6415. <https://doi.org/10.1021/jf8004965>

352 Khatib, A., Wilson, E. G., Kim, H. K., Supardi, M., Choi, Y. H., & Verpoorte, R. (2007). NMR
 353 assignment of iso- α -acids from isomerised extracts of *Humulus lupulus* L. cones.
 354 *Phytochemical Analysis*, 18(5), 371–377. <https://doi.org/10.1002/pca.991>

355 Köller, H. (1969). Magnesium Ion Catalysed Isomerization of Humulone: A New Route to
 356 Pure Isohumulones. *Journal of the Institute of Brewing*, 75(2), 175–179.
 357 <https://doi.org/10.1002/j.2050-0416.1969.tb03197.x>

358 Kostrzewa, D., Dobrzyńska-Inger, A., Rój, E., Grzęda, K., & Kozłowski, K. (2016).
 359 Isomerization of hop extract α -acids. *Journal of the Institute of Brewing*, 122(3), 493–
 360 499. <https://doi.org/10.1002/jib.349>

361 Kovalenko, A., & Hirata, F. (1998). Three-dimensional density profiles of water in contact
 362 with a solute of arbitrary shape: A RISM approach. *Chemical Physics Letters*, 290(1–3),
 363 237–244. [https://doi.org/10.1016/S0009-2614\(98\)00471-0](https://doi.org/10.1016/S0009-2614(98)00471-0)

364 Lam, Y. H., & Houk, K. N. (2014). How cinchona alkaloid-derived primary amines control
 365 asymmetric electrophilic fluorination of cyclic ketones. *Journal of the American*
 366 *Chemical Society*, 136(27), 9556–9559. <https://doi.org/10.1021/ja504714m>
 367 Lance, D. G., White, A. W., Hildebrand, R. P., & Clarke, B. J. (1975). the Effect of Heat on
 368 Metal Humulate and Isohumulate Salts. *Journal of the Institute of Brewing*, 81(5), 364–
 369 367. <https://doi.org/10.1002/j.2050-0416.1975.tb06406.x>
 370 Malowicki, M. G., & Shellhammer, T. H. (2005). Isomerization and degradation kinetics of
 371 hop (*Humulus lupulus*) acids in a model wort-boiling system. *Journal of Agricultural*
 372 *and Food Chemistry*, 53(11), 4434–4439. <https://doi.org/10.1021/jf0481296>
 373 Marenich, A. V., Cramer, C. J., & Truhlar, D. G. (2009). Universal solvation model based on
 374 solute electron density and on a continuum model of the solvent defined by the bulk
 375 dielectric constant and atomic surface tensions. *Journal of Physical Chemistry B*,
 376 113(18), 6378–6396. <https://doi.org/10.1021/jp810292n>
 377 Milenković, D. A., Dimić, D. S., Avdović, E. H., Amić, A. D., Dimitrić Marković, J. M., &
 378 Marković, Z. S. (2020). Advanced oxidation process of coumarins by hydroxyl radical:
 379 Towards the new mechanism leading to less toxic products. *Chemical Engineering*
 380 *Journal*, 395(April), 124971. <https://doi.org/10.1016/j.cej.2020.124971>
 381 Milenković, D., Đorović, J., Petrović, V., Avdović, E., & Marković, Z. (2018). Hydrogen atom
 382 transfer versus proton coupled electron transfer mechanism of gallic acid with different

383 peroxy radicals. *Reaction Kinetics, Mechanisms and Catalysis*, 123(1), 215–230.
 384 <https://doi.org/10.1007/s11144-017-1286-8>
 385 Ochterski, J. W., & Ph, D. (2000). Thermochemistry in Gaussian. *Gaussian Inc Pittsburgh*
 386 *PA*, 264(1), 1–19. <https://doi.org/10.1016/j.ijms.2007.04.005>
 387 Ocvirk, M., & Košir, I. J. (2020). Dynamics of isomerization of hop alpha-acids and transition
 388 of hop essential oil components in beer. *Acta Chimica Slovenica*, 67(3), 720–728.
 389 <https://doi.org/10.17344/acsi.2020.5394>
 390 Petrov, A. S., Bowman, J. C., Harvey, S. C., & Williams, L. D. (2011). Bidentate RNA-
 391 magnesium clamps: On the origin of the special role of magnesium in RNA folding.
 392 *RNA*, 17(2), 291–297. <https://doi.org/10.1261/rna.2390311>
 393 Ribeiro, R. F., Marenich, A. V., Cramer, C. J., & Truhlar, D. G. (2011). Use of solution-phase
 394 vibrational frequencies in continuum models for the free energy of solvation. *Journal of*
 395 *Physical Chemistry B*, 115(49), 14556–14562. <https://doi.org/10.1021/jp205508z>
 396 Sato, H., Kovalenko, A., & Hirata, F. (2000). Self-consistent field, ab initio molecular orbital
 397 and three-dimensional reference interaction site model study for solvation effect on
 398 carbon monoxide in aqueous solution. *The Journal of Chemical Physics*, 112(21), 9463–
 399 9468. <https://doi.org/10.1063/1.481564>
 400 Schmidt, M. W., Baldridge, K. K., Boatz, J. A., Elbert, S. T., Gordon, M. S., Jensen, J. H.,
 401 Koseki, S., Matsunaga, N., Nguyen, K. A., Su, S., Windus, T. L., Dupuis, M., &
 402 Montgomery, J. A. (1993). General atomic and molecular electronic structure system.

403 *Journal of Computational Chemistry*, 14(11), 1347–1363.
404 <https://doi.org/10.1002/jcc.540141112>

405 Schönberger, C., & Kostecky, T. (2011). 125th anniversary review: The role of hops in
406 brewing. *Journal of the Institute of Brewing*, 117(3), 259–267.
407 <https://doi.org/10.1002/j.2050-0416.2011.tb00471.x>

408 Steenackers, B., De Cooman, L., & De Vos, D. (2015). Chemical transformations of
409 characteristic hop secondary metabolites in relation to beer properties and the brewing
410 process: A review. *Food Chemistry*, 172, 742–756.
411 <https://doi.org/10.1016/j.foodchem.2014.09.139>

412 Tošović, J., Marković, S., Dimitrić Marković, J. M., Mojović, M., & Milenković, D. (2017).
413 Antioxidative mechanisms in chlorogenic acid. *Food Chemistry*, 237, 390–398.
414 <https://doi.org/10.1016/j.foodchem.2017.05.080>

415 Urban, J., Dahlberg, C. J., Carroll, B. J., & Kaminsky, W. (2013). Absolute configuration of
416 beer's bitter compounds. *Angewandte Chemie - International Edition*, 52(5), 1553–1555.
417 <https://doi.org/10.1002/anie.201208450>

418 Verzele, M., & van Boven, M. (1971). The Isomerization Mechanism of Humulone. *Bulletin*
419 *Des Sociétés Chimiques Belges*, 80(5–6), 677–682.
420 <https://doi.org/10.1002/bscb.19710800538>

- Wang, J., Wolf, R. M., Caldwell, J. W., Kollman, P. A., & Case, D. A. (2004). Development and testing of a general Amber force field. *Journal of Computational Chemistry*, 25(9), 1157–1174. <https://doi.org/10.1002/jcc.20035>
- Wietstock, P. C., Kunz, T., Pereira, F., & Methner, F. J. (2016). Metal chelation behavior of hop acids in buffered model systems. *BrewingScience*, 69(9–10), 56–63.
- Yoshida, N., & Hirata, F. (2006). A new method to determine electrostatic potential around a macromolecule in solution from molecular wave functions. *Journal of Computational Chemistry*, 27(4), 453–462. <https://doi.org/10.1002/jcc.20356>
- Yoshida, N., Kiyota, Y., & Hirata, F. (2011). The electronic-structure theory of a large-molecular system in solution: Application to the intercalation of proflavine with solvated DNA. *Journal of Molecular Liquids*, 159(1), 83–92. <https://doi.org/10.1016/j.molliq.2010.04.019>
- Zanoli, P., & Zavatti, M. (2008). Pharmacognostic and pharmacological profile of *Humulus lupulus* L. *Journal of Ethnopharmacology*, 116(3), 383–396. <https://doi.org/10.1016/j.jep.2008.01.011>

Graphical abstract

