

1 Theoretical Study on Isomerization of α -Acids:

2 A DFT Calculation

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15 **ABSTRACT:**

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

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

30

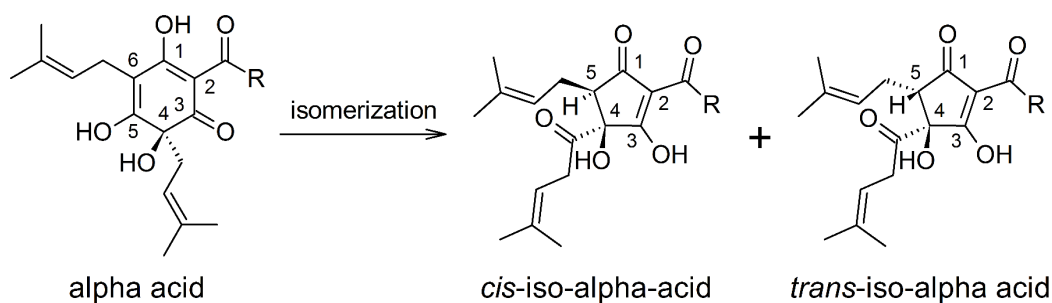
31 **1. Introduction**

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

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

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

45



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

49

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

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

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

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

74 Isomerization yields are affected by various factors, such as the pH and metal cations
75 (Jaskula et al., 2010; Steenackers et al., 2015). Several metal cations have long been known to
76 affect isomerization (Lance et al., 1975). In particular, the acceleration effect of Mg^{2+} on
77 isomerization has been well studied (Köller, 1969). Isomerization with the addition of
78 magnesium oxide, MgO, at ambient temperature produces high yields with no side products.
79 Increasing the quantity of MgO added has been shown to increase the yield over fewer days
80 of reaction (Kostrzewa et al., 2016). This effect of Mg^{2+} on isomerization could be attributed to
81 complex formation between an α -acid and a Mg^{2+} cation, because α -acids have a 1,4-diketone
82 structure. This O-Mg-O structure is also found in the RNA folding, where Mg^{2+} cation is more
83 suitable than other cations such as Na^+ and Ca^{2+} for its size and charge density (Petrov et al.,
84 2011). However, Wietstock et al. recently carried out UV-VIS spectroscopy on α -acid and
85 metal cation mixtures and proposed no complexation between α -acids and Mg^{2+} (Wietstock et
86 al., 2016). The spectrum of equimolar mixtures of α -acids and Fe^{2+} was found to differ from
87 that of α -acids alone, indicating complexation between α -acids and Fe^{2+} , whereas equimolar
88 mixtures of α -acids and Mg^{2+} exhibited a similar spectrum to that of α -acids alone. Thus, the
89 mechanism for the effect of Mg^{2+} on isomerization remains controversial.

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

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

101 2. Material and methods

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

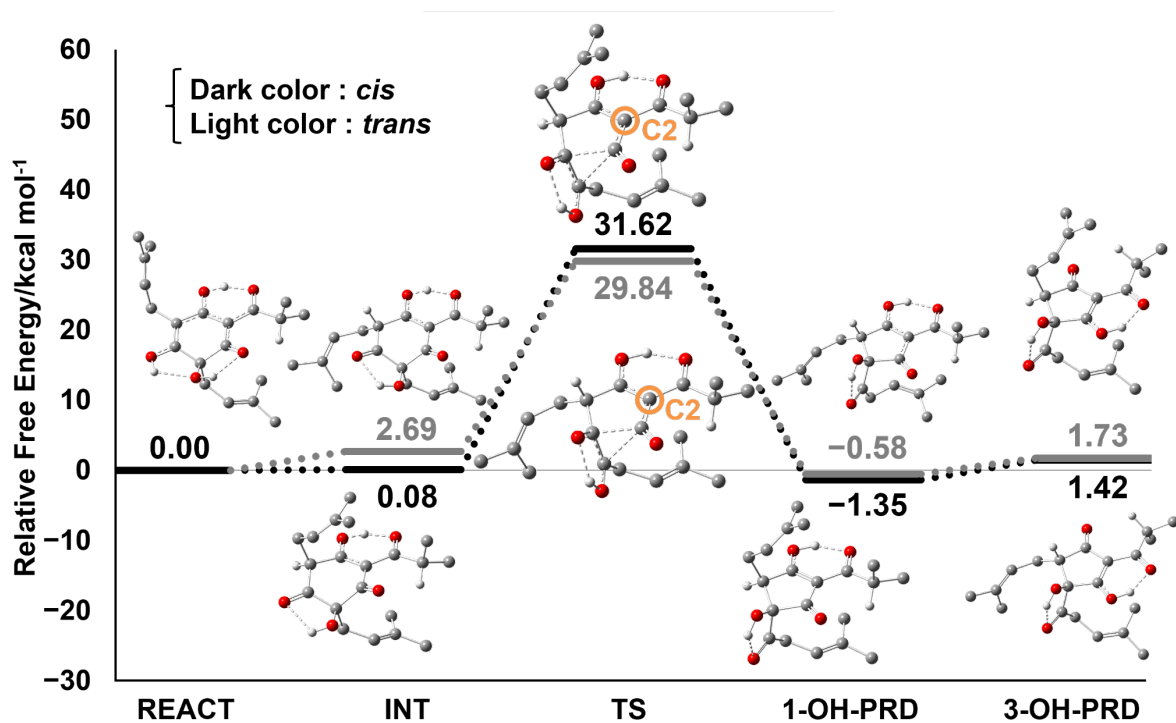
113 with Becke-Johnson damping, GD3(BJ) (Grimme et al., 2011), is needed to reproduce the
114 experimental reaction profile. To match the experimental conditions, the reaction profile and
115 absorption spectra were calculated in aqueous solution, whereas the NMR chemical shifts were
116 calculated in 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 ($\sigma = 1.0 \text{ \AA}$ and $\epsilon = 0.056 \text{ kcal/mol}$) 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

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

135 3. Results and Discussion

136 3-1. Isomerization reaction profile

137



138

139 **Figure 1.** Cohumulone isomerization reaction profile

140

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

145 cohumulone (REACT). Next, INT_{cis} and INT_{trans} pass through two transition states, TS_{cis} and
146 TS_{trans}, and are converted into two products that both have a hydroxy group at the C1 position,
147 1-OH-PRD_{cis/trans}. Finally, the equilibrium between 1-OH-PRD_{cis/trans} and 3-OH-PRD_{cis/trans} with
148 a hydroxy group at the C3 position is investigated. Note that 3-OH-PRD_{cis/trans} are traditionally
149 regarded as the structures of *cis/trans* isocohumulone (Urban et al., 2013). It is also noted that
150 no direct involvement of water in the reaction was assumed because intramolecular ring
151 contraction and proton transfer are found to proceed at the TSs in a concerted manner.

152 The reaction profile calculated with the 3D-RISM-SCF method is shown in Figure 1.
153 Both INT_{cis} and INT_{trans} are unstable compared with REACT by ~3 kcal/mol. The activation free
154 energy of TS_{cis} (31.62 kcal/mol) is higher than that of TS_{trans} (29.84 kcal/mol), whereas the *cis*
155 products, 1-OH-PRD_{cis} and 3-OH-PRD_{cis}, have lower reaction free energies (-1.35 and 1.42
156 kcal/mol, respectively) than the *trans* products, 1-OH-PRD_{trans} and 3-OH-PRD_{trans} (-0.58 and
157 1.73 kcal/mol, respectively). These calculated results are consistent with experimental results
158 (Jaskula et al., 2008). The calculated activation free energy difference of 1.78 kcal/mol between
159 the *cis* and *trans* products is in good agreement with the experimental value of 3.4 kcal/mol
160 (Malowicki & Shellhammer, 2005). Furthermore, 1-OH-PRDs are found to be more stable than
161 3-OH-PRDs, which suggests that 1-OH-PRDs are more realistic candidates for the structure
162 of isocohumulone (see discussion below).

163

164 **3-2. Analysis of TS_{cis/trans}**

165 The difference in the activation free energies of the *cis* and *trans* products was analyzed.
166 We decomposed the free energy difference between the TS_{cis} and TS_{trans}, $\Delta G = 1.78$ kcal/mol,
167 into three contributions, the structural energy difference in the gas phase ΔE_{gas} , the solvation
168 free energy difference $\Delta\mu_{\text{sol}}$, and thermal free energy difference $\Delta G_{\text{thermal}}$:

169
$$\Delta G = \Delta E_{\text{gas}} + \Delta\mu_{\text{sol}} + \Delta G_{\text{thermal}} \quad (1)$$

170 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}
171 is calculated to be -4.71 kcal/mol, indicating that TS_{cis} has lower structural energy than TS_{trans}.
172 On the other hand, TS_{trans} has lower solvation free energy than TS_{cis}, $\Delta\mu_{\text{sol}} = 6.33$ kcal/mol.
173 $\Delta G_{\text{thermal}}$ is found to be as small as 0.16 kcal/mol. These results demonstrate that solvation plays
174 a major role in the stabilization of TS_{trans}. Notably, the sophisticated 3D-RISM-SCF method was
175 needed for the proper description of solvation. The SMD solvation model failed to reproduce
176 the experimental result; the free energy of TS_{trans} (28.43 kcal/mol) calculated with the SMD
177 model is similar to that of TS_{cis} (28.40 kcal/mol).

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

184

185 3-3. Proton position

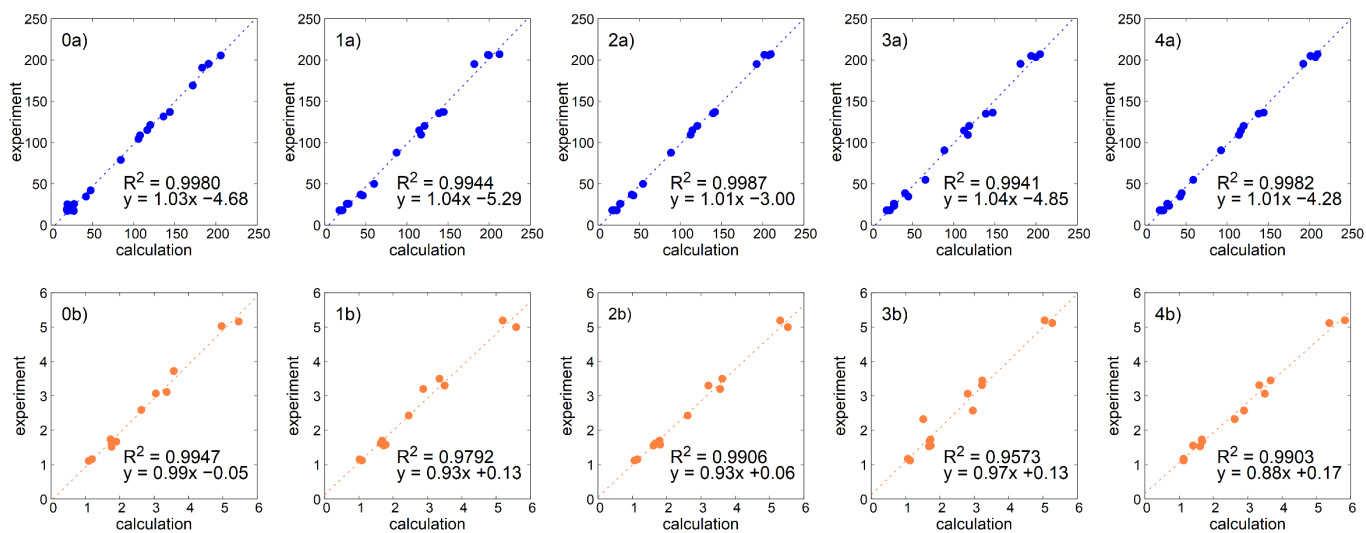
186 Experimental studies have reported 3-OH-PRD as the isomerization end product
187 (Urban et al., 2013). However, the calculated reaction profile showed that 1-OH-PRD is more
188 stable than 3-OH-PRD (Figure 1). We analyzed the proton position in more detail by
189 calculating the ^{13}C and ^1H NMR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

194 First, the NMR spectra of REACT were used as a reference. The calculated and
195 experimental NMR chemical shifts of REACT and the correlation between these results are
196 shown in Table S1 and Figure 2. Reasonably good correlations are obtained for both ^{13}C and
197 ^1H , with coefficients of determination, R^2 , of 0.9980 and 0.9947, respectively. This result
198 validates our NMR calculation.

199 Figure 2 shows the correlations between the experimental and calculated NMR
200 chemical shifts for 3-OH-PRDs (the proposed isocohumulone structure) and 1-OH-PRDs (the
201 lower free energy). The calculated and experimental NMR chemical shifts of 3-OH-PRDs and
202 1-OH-PRDs are summarized in Tables S2 and S3. The R^2 of the aforementioned correlations
203 for 1-OH-PRDs are larger than those for 3-OH-PRDs for both the ^{13}C and ^1H NMR chemical
204 shifts of the *cis* and *trans* products. For example, the correlation for ^{13}C NMR of 1-OH- PRD_{cis}

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

210



211
 212 **Figure 2.** Correlations between experimental and calculated NMR chemical shifts for 0)

213 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

214 and b) ^1H

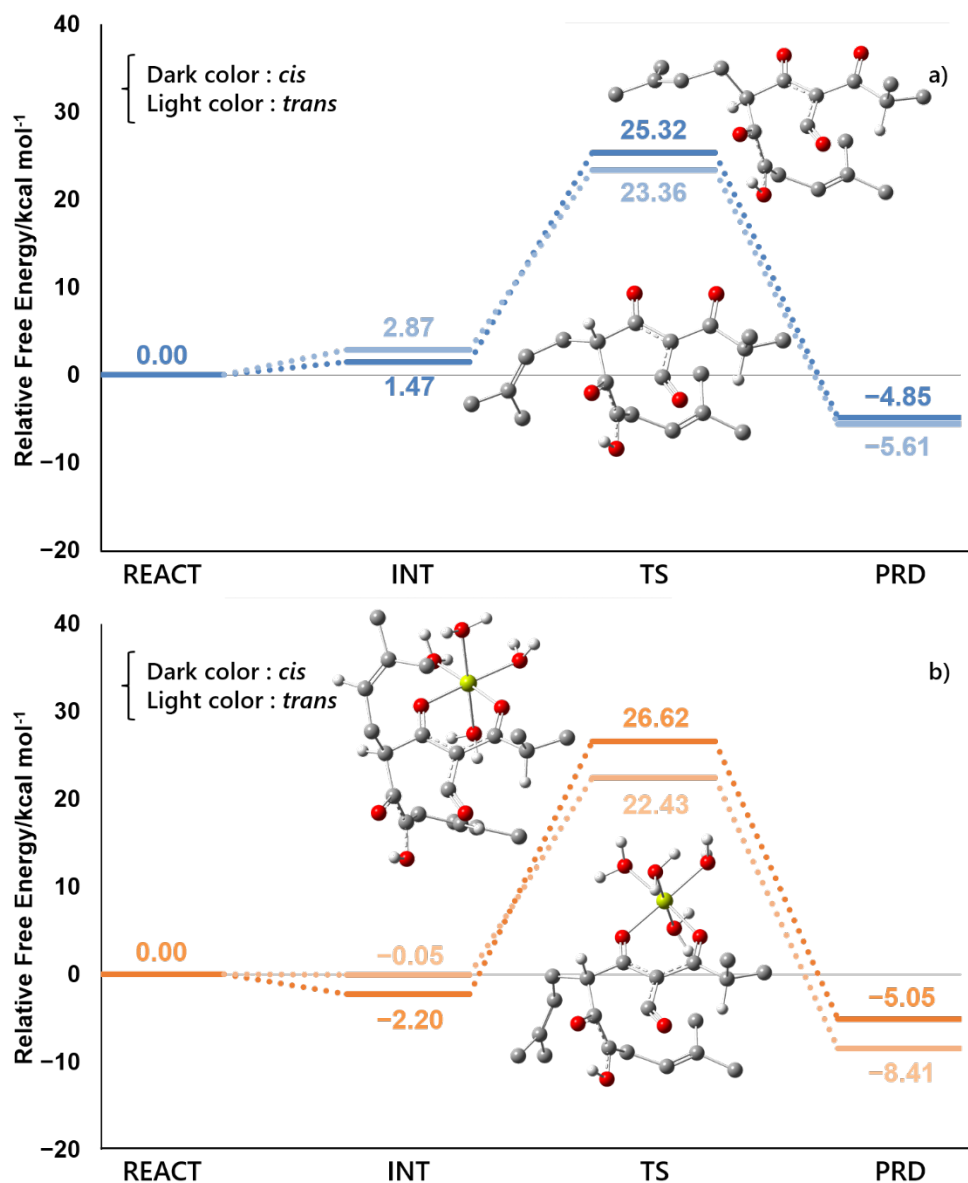
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216 3-4. Reaction profile with Mg^{2+}

217 The effect of the magnesium cation on the isomerization reaction profile was
 218 investigated. The DFT calculation was used to determine the most stable structures of the Mg
 219 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.



229

230 **Figure 3.** Isomerization reaction profiles for a) anionic cohumulone and b) Mg²⁺ complex.

231

232 Table 1 shows the activation free energy and the reaction free energy for the

233 aforementioned isomerization reactions. The activation and reaction free energies of the Mg

234 complex are approximately 5 kcal/mol lower than those of the neutral states. For example, the

235 activation free energy of the Mg complex in the *trans* configuration (22.43 kcal/mol) is lower

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

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

	ΔG^\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

239

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

$$244 \quad \Delta G^\ddagger = \Delta E_{\text{gas}} + \Delta\mu_{\text{sol}} + \Delta G_{\text{thermal}} \quad (2)$$

245 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
 246 evaluated the activation free energy differences between the Mg complexes and neutral
 247 cohumulone to compare the relative size of each contribution:

$$248 \quad \Delta\Delta G^\ddagger = \Delta\Delta E_{\text{gas}} + \Delta\Delta\mu_{\text{sol}} + \Delta\Delta G_{\text{thermal}} \quad (3)$$

249 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
 250 analogously. The calculated results for the *cis* and *trans* activation free energies are shown in

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

256

257 **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

258

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

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

276 5. Conclusions

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

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

290

291 **Declaration of Competing Interests**

292 The authors declare no competing financial interest.

293

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298

299 **Appendix A. Supplementary data**

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

302

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437 **Graphical abstract**

