DFT study of the radical scavenging activity of isoxanthohumol, humulones (α-acids) and iso-α-acids from beer

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Research Article

Keywords: Structure-antioxidant activity relationship, Density functional theory, Prenylated flavonoids, Bond dissociation enthalpy, Hop

Posted Date: February 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-231625/v1

Version of Record: A version of this preprint was published at Structural Chemistry on April 21st, 2021.
See the published version at https://doi.org/10.1007/s11224-021-01780-4.

DFT study of the radical scavenging activity of isoxanthohumol, humulones (α-acids) and iso-α-acids from beer

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Abstract

Humulones and iso-humulones are potent natural antioxidants found in beer. In this study, density functional theory (DFT) method was applied for elucidating the structure-antioxidant activity relationship and molecular mechanism of antioxidant activity of eight bioactive humulones previously identified in different beer samples: isoxanthohumol, (*R*)- and (*S*)-adhumulone, *cis*- and *trans*-iso-adhumulone, *cis*- and *trans*-iso-n-humulone, and desdimetyhyl-octahydro-iso-cohumulone. The calculated bond dissociation enthalpies (BDEs) suggest that desdimethyl-octahydro-iso-cohumulone was the most potent compound with BDEs 5.1 and 23.9 kJ/mol lower compared to the values for resveratrol in gas phase and water, respectively. The enolic –OH is the most reactive site for hydrogen atom transfer (HAT). The presence of β-keto group with respect to enolic –OH diminishes the HAT potency via the formation of a strong intramolecular hydrogen bond. Another common antioxidant mechanism, single electron transfer followed by proton transfer (SET-PT), is only feasible for isoxanthohumol. The results of this study indicate a strong correlation between the increased antioxidant activity of beer products and the higher content of reduced iso-α-acids.

Keywords: Structure-antioxidant activity relationship, Density functional theory, Prenylated flavonoids, Bond dissociation enthalpy, Hop

1. Introduction

Beer is the second most-consumed low alcoholic beverage in Europe, accounting for 37% of the total EU alcohol consumption [1]. Beer contains a wide range of compounds such as proteins, carbohydrates, B vitamins (niacin, riboflavin, folate, cobalamin, and pyridoxine), amino acids, phenolic compounds as well as minerals (mainly potassium and magnesium). The pharmaceutical properties of beer mainly depend on the chemical composition of hops and malt, as well as parameters involved in brewing *e.g.* the variety of barley and hops, temperature and pH during mashing, sparing, boiling, as well as yeast fermentation. Although the harmful effects associated with the increased alcohol intake are well-known, the effects of moderate consumption of beer (one glass for woman and two for a man per day) require further study. These effects mainly vary for diverse alcoholic beverages due to their heterogeneous content of non-alcoholic components. Amongst those, polyphenolic compounds from beer are potent radical scavengers and could stabilize beer products [2] and potentially be responsible for various beneficial effects in moderate consumption. About 70-80% of beer polyphenols originate from malt, and the remaining fraction belongs to bitter acids from hops. Hops, one of the main ingredients, contains about 14.4% of polyphenols such as phenolic acids, prenylated chalcones, flavonoids, catechins and proanthocyanidins [3]. Bitter acids are prenylated polyketides divided into two classes: α-acids (humulones) and β-acids (lupulones). During the wort boiling, tasteless humulones (α-acids) isomerize into bitter-taste iso-humulones (iso-α-acids). Iso-α-acids are significantly more abundant in beers compared to non-isomerized humulones [1], and its content highly influences the flavor [4] and foam stability of beer [5]. Beer is one of the most important dietary sources of prenylflavonoids. Isoxanthohumol and 6-prenylnaringenin were recognized as major

prenylflavonoids in various beer samples, accompanied by minor amounts of 8-prenylnaringenin [6]. However, xanthohumol is generally a minor prenylflavonoid in beer due to the thermal isomerization into isoxanthohumol during the brewing process.

Various biological activities of humulones and their isomers have been confirmed. For example, unisomerized bitter acids such as adhumulone, cohumulone and n-humulone selectively inhibit enzyme aldo-keto-reductase AKR1B10, an enzyme upregulated in various cancer types [7]. Xanthohumol degraded BCR-ABL oncoprotein, a significant factor for the development of chronic myelogenous leukemia [8]. Additionally, beer ingredients have significant effects on skin health [9]. Isoxanthohumol from hops can be activated into the potent phytoestrogen in the human intestine [10].

Antioxidant activity of hops components has been confirmed using various *in vitro* assays [11–13]. The radical scavenging activity of natural polyphenols is determined by the number and position of phenolic –OH groups and their tendency to donate hydrogen atom and quench free radicals. However, a quantitative study on the pharmacophoric features and the mechanism of antioxidant activity of potent hops ingredients is not reported in the literature. The antioxidant activity of isoxanthohumol and humulones might originate from the inactivation of highly reactive free radicals ($R^{\cdot}$) through several mechanisms. In the hydrogen atom transfer (HAT), the antioxidant compound (HuOH) transfers hydrogen atom ($H^{\cdot}$) to $R^{\cdot}$ via homolytic cleavage of O–H bond:

$$HuOH + R \longrightarrow HuO^{\cdot} + RH \qquad (1)$$

This reaction generates another free radical species ($HuO^{\cdot}$), stabilized via electronic and resonance effects and therefore less reactive than $R^{\cdot}$.

Single electron transfer (SET) mechanism inactivates the free radicals ($R^{\cdot}$) through electron transfer from the antioxidant (HuOH) to $R^{\cdot}$:

$$HuOH + R^{\cdot} \longrightarrow HuOH^{\cdot+} + R^{-} \qquad (2)$$

The anion R^{-} is a stable, closed-shell structure, while radical cation $HuOH^{\cdot+}$ is more stabilized compared to reactive $R^{\cdot}$ species. This step is usually followed by proton-transfer (PT) from the radical cation.

Sequential proton-loss electron-transfer (SPLET) is a three-step process, where initial proton dissociation from a molecule (Equation 3) is followed by the electron transfer, forming a radical species (Equation 4). The last step is the protonation of anion:

$$HuOH \longrightarrow HuO^{-} + H^{+} \qquad (3)$$

$$HuO^{-} + R^{\cdot} \longrightarrow HuO^{\cdot} + R^{-} \qquad (4)$$

$$R^{-} + H^{+} \longrightarrow RH \qquad (5)$$

The net result of HAT, SET and SPLET is the same, *i.e.* transfer of H atom (which can be represented as $H^{+} + e^{-}$) from antioxidant to the free radical. All these processes can be quantitatively examined using quantum chemical calculations, where DFT methodology proved to be particularly useful for an accurate prediction of thermodynamic data at a moderate computational cost. This methodology proved to be a useful tool for studying the antioxidant properties of many polyphenols and the design of novel antioxidants [14–18].

In a previous study, Ristivojevic et. al. [19] identified several hops phenyl flavonoids (isoxanthohumol, (*R*)- and (*S*)-adhumulone, *cis*- and *trans*-iso-adhumulone, *cis*- and *trans*-iso-n-humulone, and desdimetyhyl-octahydro-iso-cohumulone) as potent antioxidant compounds from

beer extracts. The main aim of the present study was to investigate pharmacophoric features and antioxidant mechanisms of these hops phenyl flavonoids using DFT calculations. The HAT and SET mechanisms were predicted in the gas phase and water. All results were systematically compared to those of resveratrol, a highly potent phenolic antioxidant. The stability of corresponding radical species was studied and visualized using spin density distribution maps. The strength of intramolecular hydrogen bonds was calculated for several isomers to aid the rationalization of structure-antioxidant relationships. The most important structural features are highlighted and structural modifications leading to more potent humulone antioxidants are suggested. The obtained results could be used in the brewing practice to increase the content of bioactives in beer and develop novel super beers enriched with antioxidant prenylated chalcones.

2. Methods

Initial 3D structures of compounds were generated using Vega ZZ 3.2.0 [20] and MMFF94 force field [21] and afterward submitted to conformational analysis in AMMP program [22] implemented in Vega ZZ. The lowest-energy conformer was located through the systematic search by rotating all single bonds by 30º and setting the dielectric constant of the environment to 78.4 (water). All other parameters were at default settings. Several possible isomers of desdimethyl-octahydro-iso-cohumulone were modeled using MMFF94 force field, and all-*R* isomer appeared as the lowest energy isomer. The geometry of the most stable conformer of each compound was further optimized at the DFT level of theory using B3LYP functional and 6-311g++(2d,2p) basis set. Starting from the optimized ground state, the geometries of corresponding radicals, radical

cations and anions were optimized at the same level of theory. For radicals and radical cations, the unrestricted calculations were performed to assure the correct treatment of species with unpaired electrons. The non-specific solvent effects were simulated using the IEF-PCM model of water [23]. Frequency calculations were performed after each optimization to confirm that geometries correspond to the minimum (absence of imaginary vibrational frequencies) and to obtain thermodynamic data. All DFT calculations were performed in Gaussian 16, version B.01 [24]. For the analysis of intramolecular non-covalent interactions (NCI) [25], bond critical points (BCPs) were calculated in Multiwfn 3.7 [26] using Quantum Theory of Atoms in Molecules (QTAIM) [27]. The NCI were visualized in VMD [28].

3. Results and discussion

The structures of potent beer antioxidants are shown in Fig. 1. To study the structure-antioxidant activity relationship of these compounds, DFT descriptors of two dominant antioxidant mechanisms, HAT and SET-PT, were computed and discussed herein.

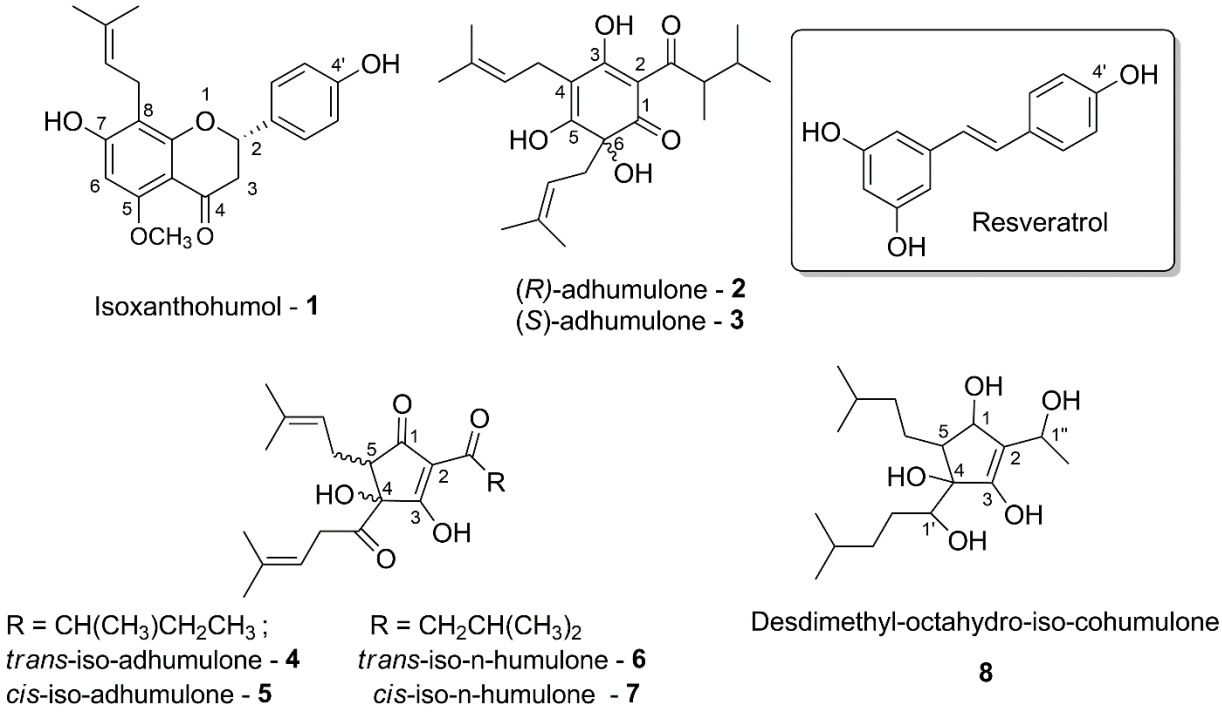

Fig. 1 Structure of isoxanthohumol (**1**), humulones (**2**-**8**) and resveratrol. The position of each –OH group evaluated as HAT site is labeled.

3.1. Hydrogen atom transfer (HAT) mechanism

Bond dissociation enthalpy (BDE) is a descriptor of HAT mechanism of antioxidants. It can be computed as:

$$\mathrm{BDE} = H(\mathrm{H}^{\cdot}) + H(\mathrm{HuO}^{\cdot}) - H(\mathrm{HuOH}), \qquad (6)$$

where $H(\mathrm{H}^{\cdot})$, $H(\mathrm{HuO}^{\cdot})$ and $H(\mathrm{HuOH})$ denote the enthalpies of hydrogen atom, radical and molecule, respectively. The lower BDE indicates higher antioxidant ability to transfer hydrogen atom and neutralize the harmful radical species.

The BDEs of isoxanthohumol and seven humulones/iso-humulones (compounds **1**-**8**, Fig. 1) were calculated. In order to screen for the most probable site where hydrogen atom abstraction occurs, BDE of each –OH bond of compounds **1**-**8** was computed and compared to those of phenol and resveratrol (Table 1).

Table 1 Bond dissociation enthalpies and ionization potentials (in kJ/mol) of compounds **1**-**8**, phenol and resveratrol computed on B3LYP/6-311g++(2d,2p) level in the gas phase and using PCM model of water. The position of –OH group is indicated in brackets, see Figure 1.

Comp.	BDE (water)[a]			IP (water)	BDE (gas)			IP (gas)
1	354.6 (7-)	344.8 (4'-)		566.1	346.6 (7-)	340.0 (4'-)		666.3
2	406.7 (3-)	331.2 (5-)	422.5 (6-)	595.6	398.7 (3-)	322.8 (5-)	416.6 (6-)	701.9
3	405.7 (3-)	331.1 (5-)	420.3 (6-)	594.7	398.4 (3-)	323.2 (5-)	413.0 (6-)	701.4
4	399.1 (3-)	352.4 (4-)		605.5	395.4 (3-)	336.1 (4-)		739.7
5	391.7 (3-)	400.6 (4-)		605.4	390.2 (3-)	392.3 (4-)		727.7
6	400.6 (3-)	352.9 (4-)		605.6	400.7 (3-)	342.4 (4-)		731.1
7	397.0 (3-)	404.0 (4-)		598.2	400.5 (3-)	396.6 (4-)		716.2
8	409.2 (1-)	313.1 (3-)	408.9 (4-)*	599.9	409.3 (1-)	296.5 (3-)	399.5 (4-)**	733.2
Phenol	345.5			601.7	368.2[b]			820.9[b]
Resveratrol	318.2 (4'-)			524.6	320.4 (4'-)			642.9

[a]The position of –OH group is indicated in brackets.
*BDEs for 1'- and 1''- OH groups were 411.5 and 406.6 kJ/mol, respectively.
**BDE for 1'- and 1''- OH groups were 393.6 and 396.6 kJ/mol, respectively.
[b]Experimentally determined values

The results depict an excellent HAT ability of desdimethyl-octahydro-iso-cohumulone (compound **8**), with the BDE 5.1 and 23.9 kJ/mol lower than the value for resveratrol in water and gas phase, respectively. The exceptionally low BDE for 3–OH bond originates from the conjugation of enolic 3–O$^{\cdot}$ via with C=C bond, providing the additional spin density (SD) delocalization compared to allylic –OH groups at positions 1 and 4. As can be seen from Fig. 2, only 29% of 3-O$^{\cdot}$ SD remains on this atom, compared to 63.9% for SD of 1-O$^{\cdot}$.

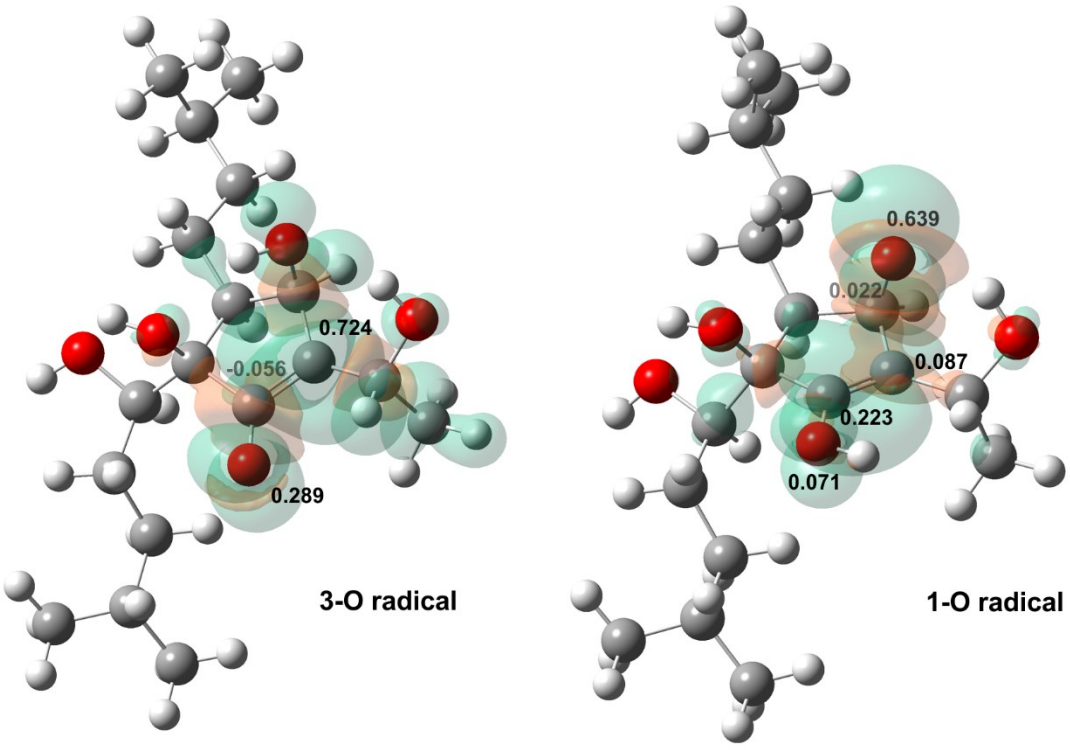

Fig. 2 Spin density distribution maps for 3-O (enolic) and 1-O (allylic) radicals of compound **8**. The spin density distribution is shown as a transparent surface, where labels indicate Mulliken spin density on corresponding atoms.

The *trans*-isomers of iso-adhumulone (compound **4**) and iso-n-humulone (compound **6**) have 48 kJ/mol lower BDEs of 4-OH group, indicating higher antioxidant activity of *trans*-isomers. Compared to *cis*-counterparts, 4-O radicals of **4** and **6** are additionally stabilized through the intramolecular addition of O radical to prenyl group attached to C_4-atom, forming the 5-membered ring (Fig. 3). This results in the transfer of the entire SD from 4-O radical to the prenyl group.

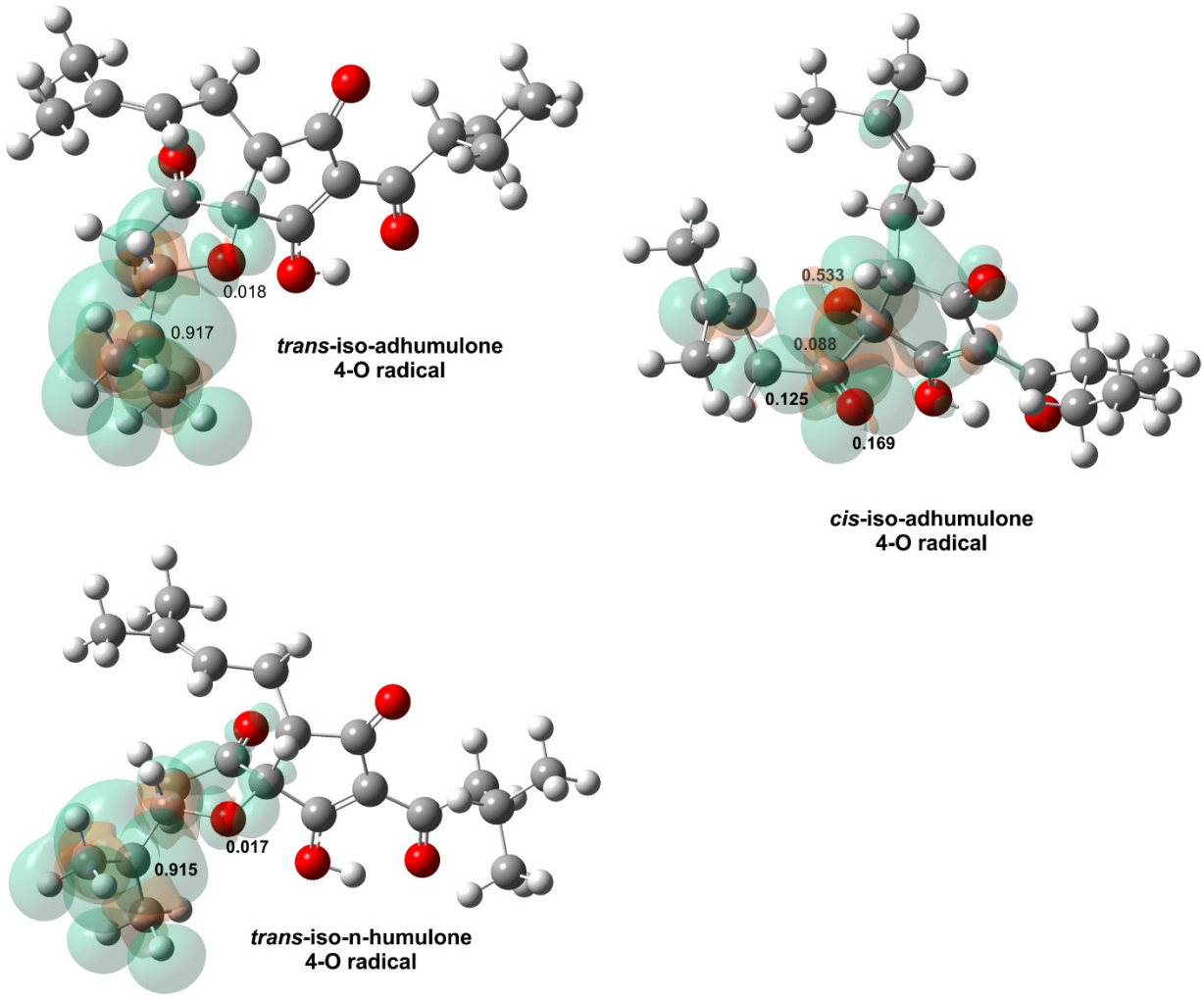

Fig. 3 Spin density distribution maps for 4-O radical of *trans*-and *cis*-iso-adhumulone (**4** and **5**) and *trans*-iso-n-humulone (**6**). The Mulliken SD values on the corresponding atom are labeled.

Negligible differences have been found between BDEs of (*R*)- and (*S*)-isomers of adhumulone (**2** and **3**). The most susceptible OH group is the enolic 5-OH group, which confirms the importance of enolic –OH moiety for the antioxidant activity of humulones. On the other hand, the enolic 3–OH group is significantly less reactive than 5–OH as BDE is nearly 80 kJ/mol higher. The possible explanation lies in the presence of carbonyl group in the β-position to enolic –OH, which interacts

with –OH group via intramolecular hydrogen bonding (IHB) forming a pseudo-6-membered ring. This interaction might stabilize the ground state of a molecule and increase the BDE according to Eq. 6. This trend is also observed in compounds **4**-**7**, where enolic 3–OH have large BDEs (~400 kJ/mol) due to IHB with β-keto groups. Therefore, enolic –OH groups that lack the β-keto group are the most important pharmacophoric features for the antioxidant activity of humulones.

According to literature, *trans*-iso-α-acids are more sensitive to the presence of reactive oxygen species (ROS) compared to *cis*-isomers, while reduced forms of iso-α-acids are remarkably stable [29]. Therefore, compound **8** as a fully reduced isomer is the most promising natural antioxidant from hops due to exceptionally low BDE and excellent stability.

3.2. Single-electron transfer (SET)

Ionization potential (IP) reflects the ability of a molecule to donate one electron to a free radical, creating a closed-shell species with lower reactivity. This is another mechanism of how antioxidants exert their activity, as anion formed upon electron transfer from antioxidant to the free radical generally stabilizes the harmful, reactive radical species. Upon the electron transfer, the antioxidant molecule turns into radical-cation, where stabilization through the resonance and electronic effects decreases the IP and increases the feasibility of the SET process.

IP can be calculated from the following equation:

$$IP = H(HuO^{\cdot+}) + H(e^{-}) - H(HuOH), \quad (7)$$

where $H(HuO^{\cdot+})$ is the enthalpy of radical-cation and $H(e^{-})$ is the enthalpy of an electron [30]. The IPs of all studied compounds, computed in the gas phase and water, are listed in Table 1.

Wright et al. reported that the dominant antioxidant mechanism of a compound can be elucidated through the comparison between the compound's IP and BDE with the corresponding values for phenol in the gas phase (ΔIP and ΔBDE) [31]. They suggested that two mechanisms, HAT and SET, occur simultaneously but with different rates. For the antioxidants having $\Delta IP \geq -150$ kJ/mol and ΔBDE around –40 kJ/mol, the main working mechanism is HAT, while SET is a principal antioxidant mechanism when $\Delta IP \leq -188$ kJ/mol.

The low ΔIP values for humulones **2**-**8** indicate that SET is not a favorable mechanism of their radical scavenging activity. The SET mechanism is the most probable for isoxanthohumol (**1**), with an IP value higher than resveratrol by 23.4 kJ/mol. On the other hand, low BDE values of compounds **2**, **3**, and particularly 3–OH group of compound **8** along with relatively high ΔIP suggest that the main radical scavenging mechanism of humulones is HAT.

The results obtained using the water model show that the solvation has a more pronounced influence on IP compared to BDE. This is expected, as polar solvents better stabilize radical cations formed upon ionization (Eq. 7) compared to the products of homolytical bond cleavage (Equation 6). Compound **8** appears to be more potent toward HAT compared to resveratrol, while SET is more feasible for resveratrol than for **1**-**8**.

The A ring of isoxanthohumol stabilizes the radical cation formed in the first step of SET mechanism, although to a lesser extent than resveratrol (Fig. 4). Compounds **2**-**8** do not possess this structural feature, which is reflected in higher IP values and lower probability for SET process.

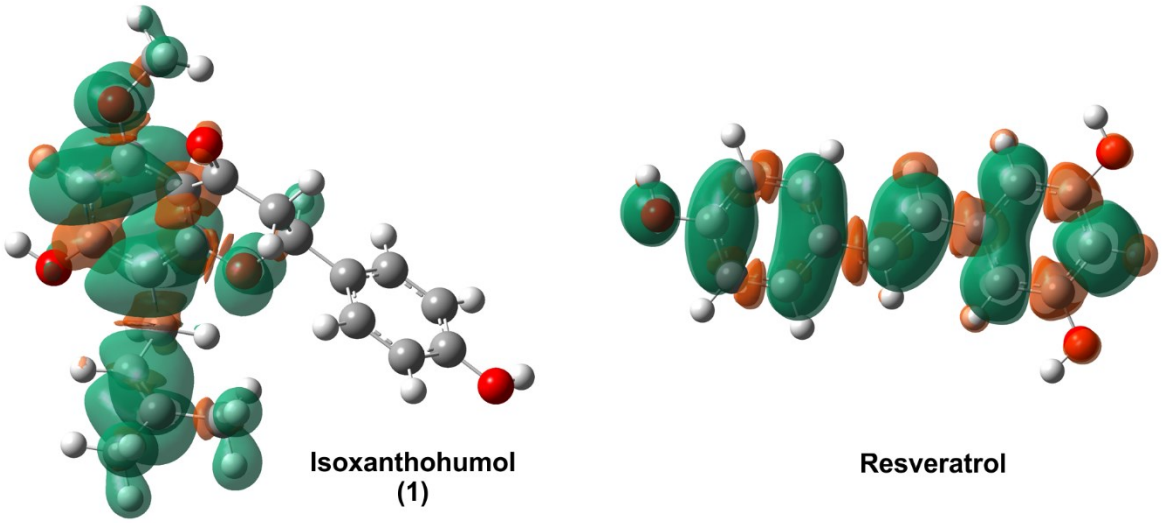

Fig. 4 Spin density distribution of radical cations of **1** and resveratrol.

3.3. The analysis of non-covalent intramolecular interactions (NCI)

The NCI analysis [25] of compounds **1**, **4** and **8** was performed to quantify the strength of IHB and give more details on the structure-antioxidant activity relationship of humulones. NCI analysis provided several parameters correlated with the hydrogen bond energy such as electron density (ρ), Laplacian of electron density ($\nabla^2\rho$) and potential energy density (V(r)). According to Espinosa et. al [32], the strength of hydrogen bonding (E_{HB}) can be approximated by 1/2 of V(r). The results of NCI analysis are given in Table 2.

Table 2 Electron density, Laplacian of electron density, potential energy density, H-bond binding energy, and donor-acceptor distance for bond critical points (CP) representing non-covalent interactions of compounds **1**,**4** and **8**.

Comp.	ρ, a.u.	$\nabla^2\rho$, a.u.	D-A distance, Å	V(r), a.u.	E_{HB}, kJ/mol
1 (CP 1)	0.01723	0.06256	N.A	-0.01163	15.3
1 (CP 2)	0.01246	0.05109	N.A	-0.00995	13.1
4 (CP 1)	0.05991	0.13170	1.612	-0.05580	73.3
8 (CP 1)	0.02234	0.09620	2.070	-0.01849	24.3
8 (CP 2)	0.01013	0.03543	2.413	-0.00685	9.0
8 (CP 3)	0.02452	0.08201	1.992	-0.01850	24.3

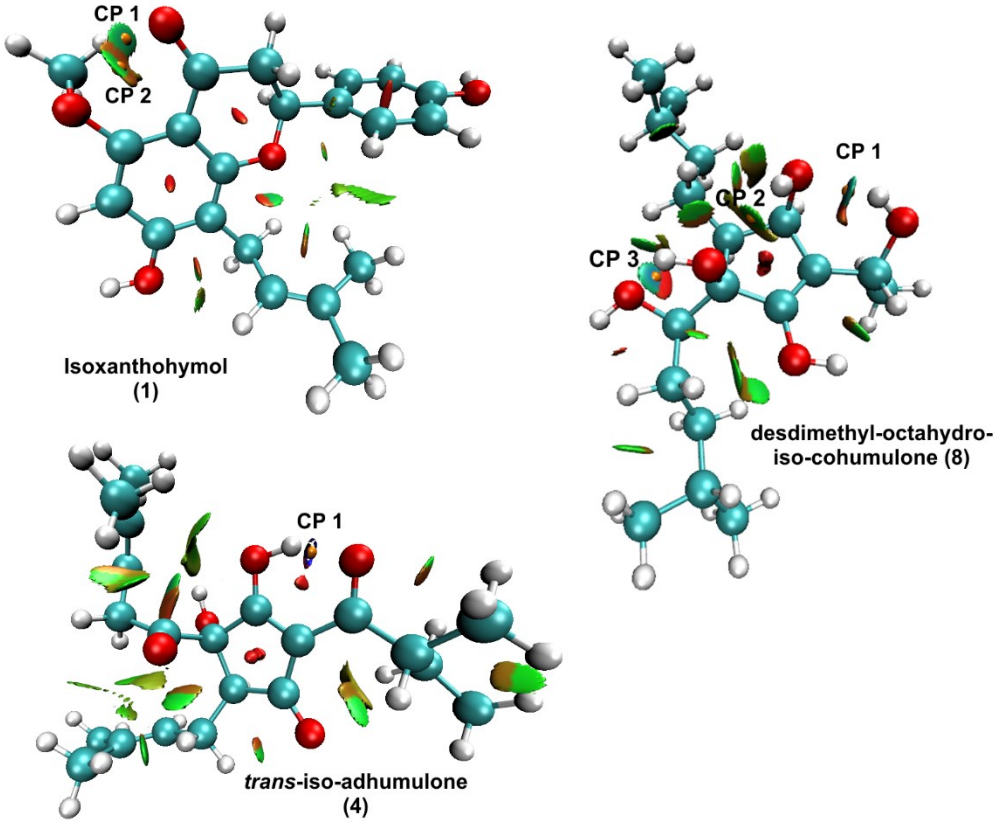

Fig. 5 The BCPs and NCI surfaces. The surface is color-coded according to the strength of intramolecular interactions: blue – highly attractive regions (hydrogen bonding), green – weak attractions (VdW), red – steric repulsions. Orange spheres represent bond critical points.

The results reveal exceptionally strong IHB for between enolic 3–OH group and side-chain keto group of compound **4** (73.3 kJ/mol). On the other hand, three CPs of compound **8** are located far from the enolic 3–OH group (Fig. 5), and the corresponding strength of intramolecular interaction is much lower compared to **4**. These findings confirmed the hypothesis that the presence of keto group in β-position to enolic –OH increases the BDE and decreases the antioxidant activity of compounds.

3.4. Suggested structural modifications for the improvement of humulone antioxidants

The summary of BDE calculations and NCI analysis suggests several structural motifs important for the antioxidant activity of humulones. The hydrogen atom transfer (HAT) is the most probable antioxidant mechanism of compounds **2**-**8**, while SET is only feasible for isoxanthohumol (**1**). The –OH group in the vinylic (enolic) position of cyclopentene ring represents a highly active site toward HAT. However, no β-keto groups next to this moiety should be present in the structure of humulone antioxidant, as strong IHB increases the BDE of vinylic –OH by ~80 kJ/mol (see Table 1, BDEs for 3–OH of **8** (β-keto group) and **4**-**7**). In the case of *cis*- and *trans*- isomers of iso-α-acids (compounds **4**-**7**), the most potent HAT site is the allylic –OH group (4–OH). The results suggest a higher antioxidant activity of *trans*-iso-α-acids. On the other hand, higher reactivity of *trans*-isomers favors the reaction with ROS in beer and confirms the instability of beers with a high content of *trans*-isomers [29].

The reduced form of iso-α-acids (compound **8**) appears to be a more potent antioxidant compared to iso-α-acids (**4**-**7**) and unisomerized α-acids (**2** and **3**). Therefore, reductive conditions during the

fermentation process may result in a higher content of reduced iso-α-acids and yield beer products with increased antioxidant activity.

The results of DFT calculations corroborate with our previous studies using DPPH-High-Performance Thin-Layer Chromatography (HPTLC) assay, where isoxanthohumol and desdimethyl-octahydro-iso-cohumulone were recognized as prenylflavonoids with the highest radical scavenging activity [19, 33].

4. Conclusion

In conclusion, the DFT study on structure-antioxidant relationship indicated a fully reduced iso-α-acid, desdimethyl-octahydro-iso-cohumulone, as a compound particularly prone to HAT mechanism, with BDEs 5.1 and 23.9 kJ/mol lower than the values for resveratrol in the gas phase and water, respectively. The enolic –OH is the most reactive site toward HAT. The presence of the β-keto group next to enolic –OH diminishes the HAT potency via the formation of a strong intramolecular hydrogen bond. The SET mechanism is most pronounced in isoxanthohumol. The results of this study strongly suggest the increased antioxidant activity of beer products with the higher content of reduced iso-α-acids. This study deepens the understanding of antioxidant activity of compounds in beer products and could contribute to unraveling potential beneficial effects of this popular beverage.

Declarations

Funding: This research was funded by the Ministry of Education, Science and Technological Development of Serbia, Contract No 451-03-68/2020-14/200168. I.C. acknowledges the National Institute of Chemistry in Ljubljana, Slovenia and Ažman Supercomputing Centre for providing software and computational resources for this work.

Conflict of interest: The authors declare no conflict of interest. M.Z. is founder and director of Nanopuzzle Medicines Design Ltd, that will derive no financial gain from this work.

Availability of data and material: Not applicable

Code availability: Not applicable

Authors' contributions: Conceptualization, P.R. and I.C.; methodology, M.B. and I.C.; software, I.C., M.B.; validation, M.Z. and D.M-O.; formal analysis, D.M-O.; investigation, M.B., P.R. and I.C.; resources, I.C. and M.Z.; data curation, M.V-T.; writing—original draft preparation, I.C. and M.B.; writing—P.R., D.M-O.; visualization, I.C.; supervision, M.Z.; project administration, M.V-T.; funding acquisition, M.B., D.M-O. and M.Z.

Ethics approval: Not applicable

Consent to participate: Not applicable

Consent for publication: All authors approved this submission.

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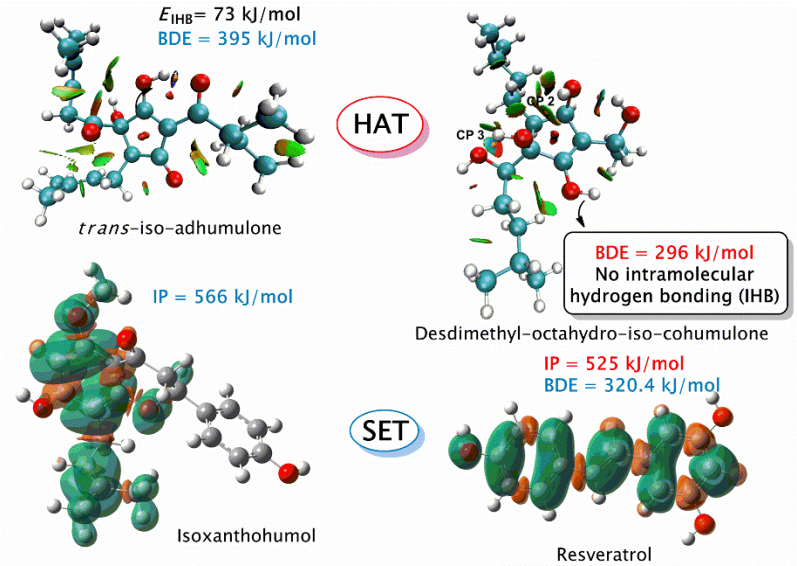
Graphic for Table of Content



Figures

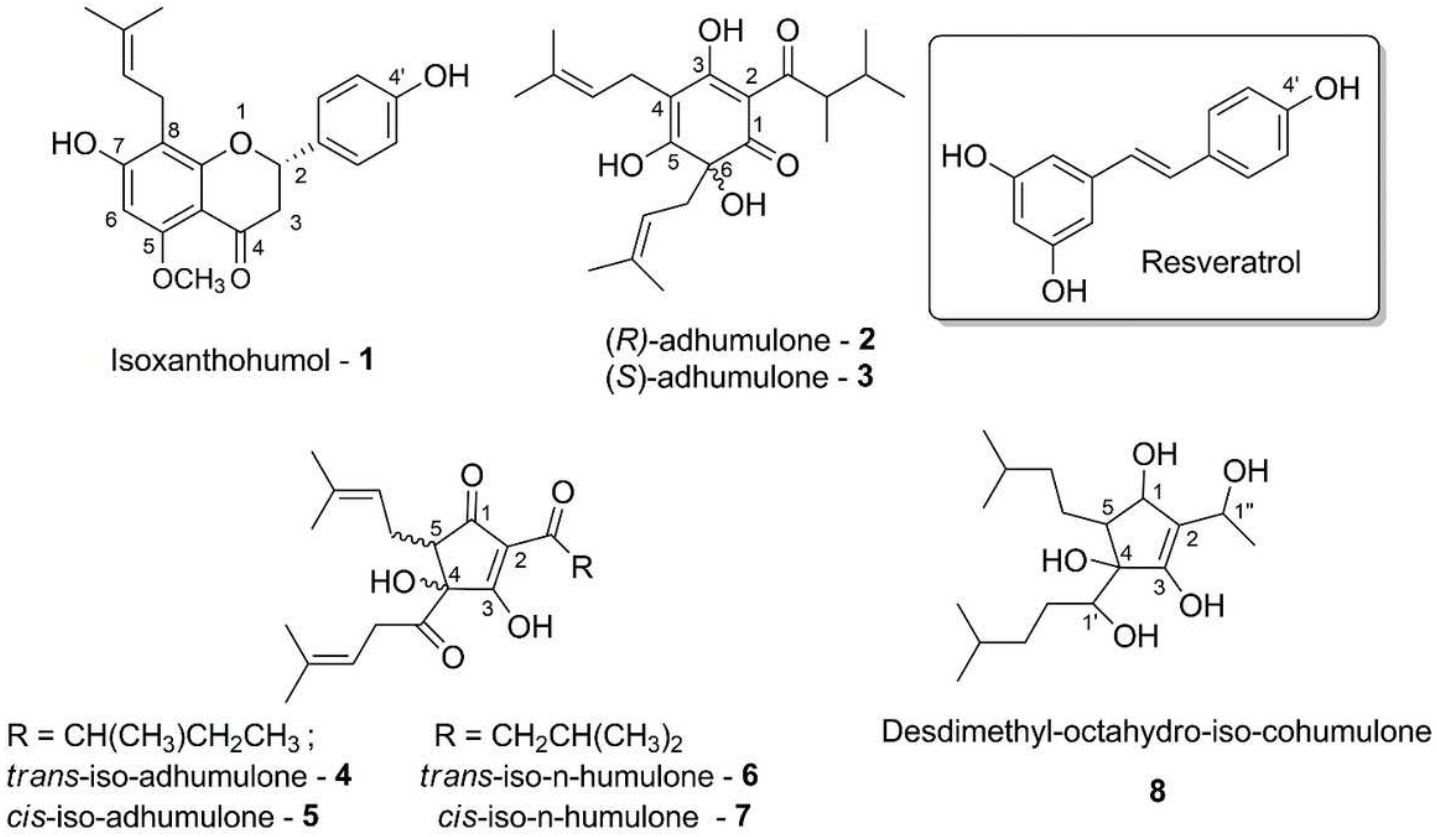


Figure 1

Structure of isoxanthohumol (1), humulones (2-8) and resveratrol. The position of each –OH group evaluated as HAT site is labeled.

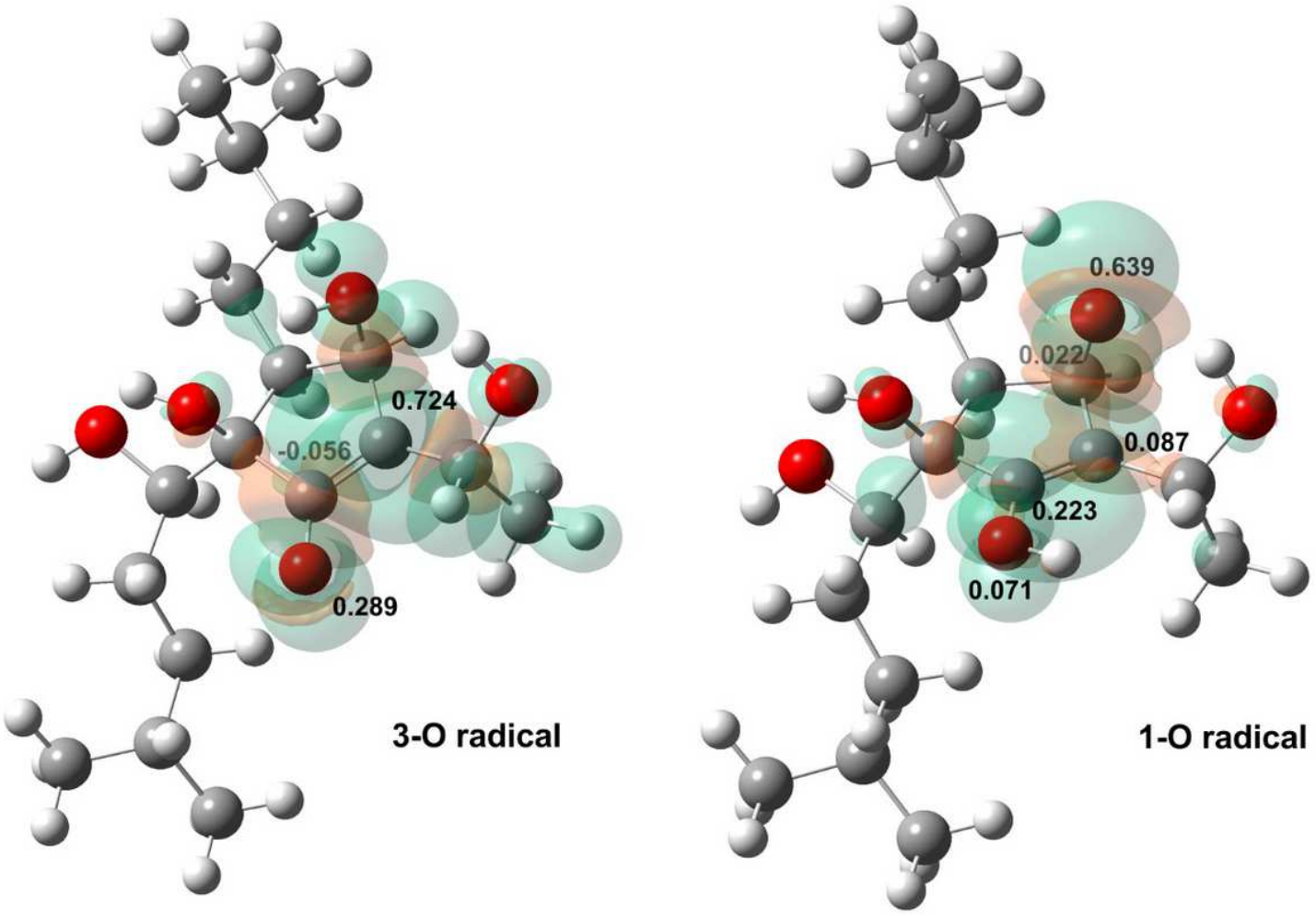


Figure 2

Spin density distribution maps for 3-O (enolic) and 1-O (allylic) radicals of compound 8. The spin density distribution is shown as a transparent surface, where labels indicate Mulliken spin density on corresponding atoms.

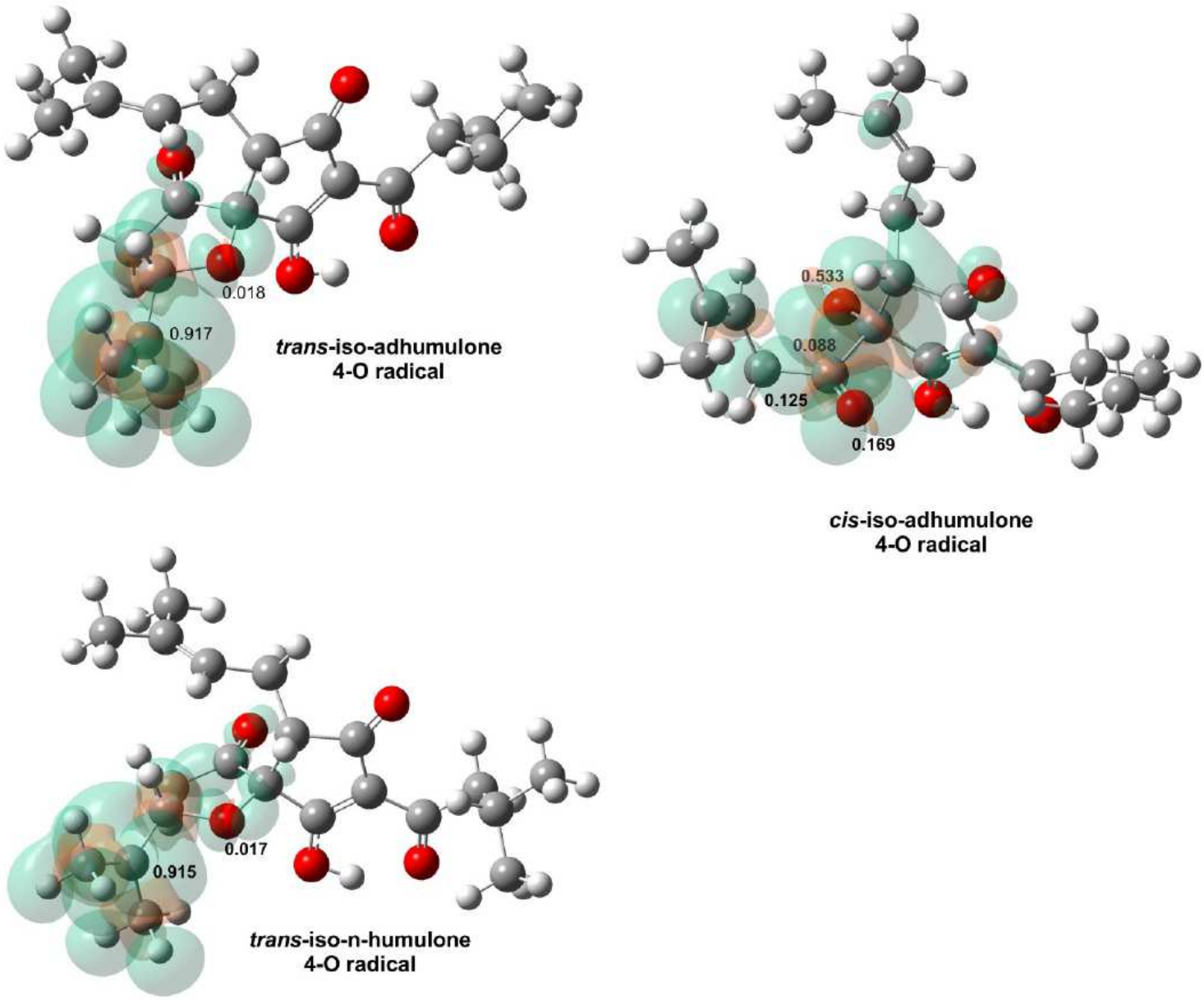


Figure 3

Spin density distribution maps for 4-O radical of trans-and cis-iso-adhumulone (4 and 5) and trans-iso-n-humulone (6). The Mulliken SD values on the corresponding atom are labeled.

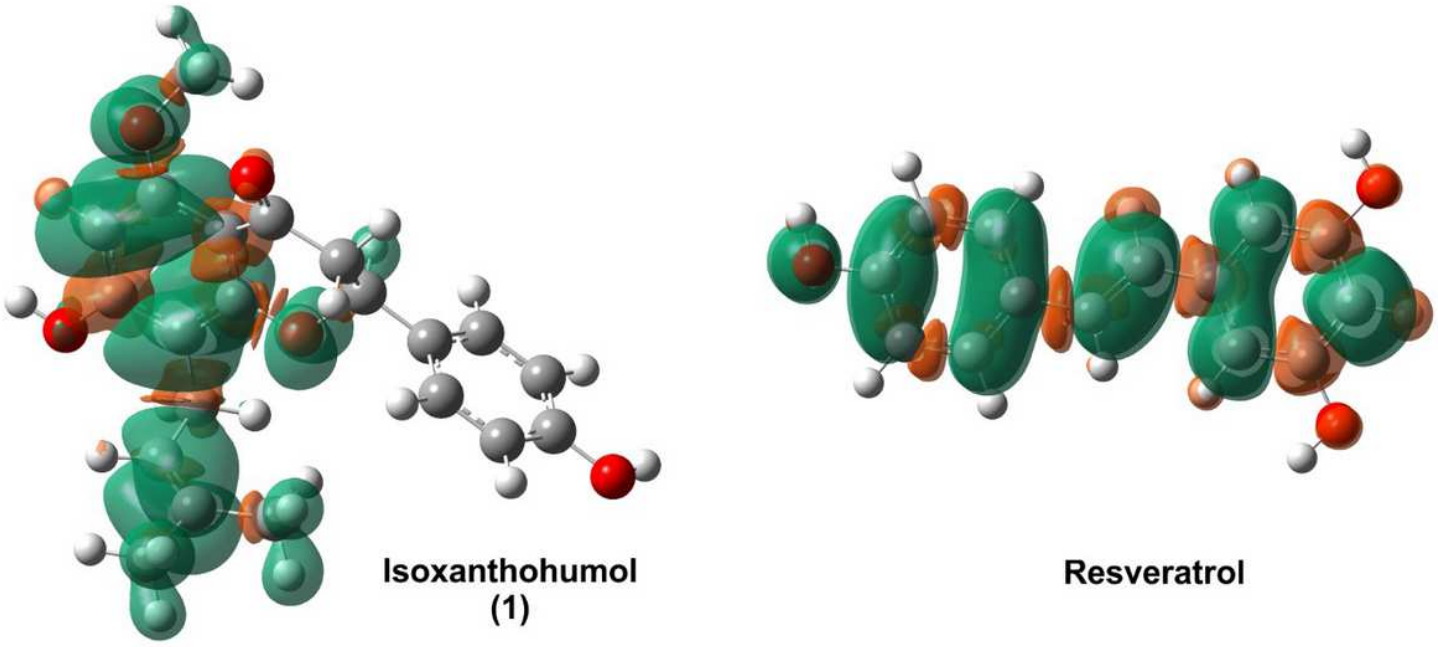


Figure 4

Spin density distribution of radical cations of 1 and resveratrol.

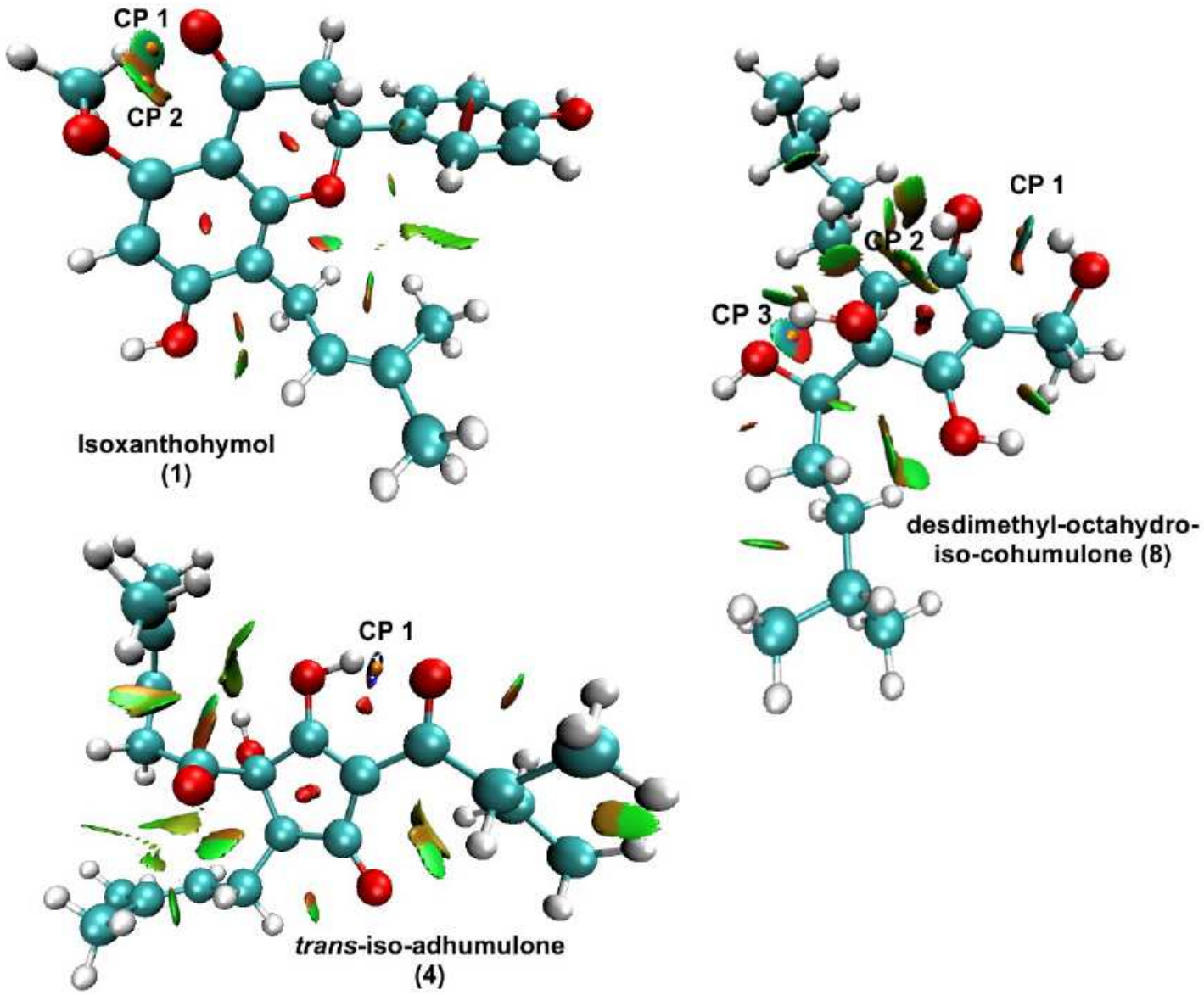


Figure 5

The BCPs and NCI surfaces. The surface is color-coded according to the strength of intramolecular interactions: blue – highly attractive regions (hydrogen bonding), green – weak attractions (VdW), red – steric repulsions. Orange spheres represent bond critical points.