7-O-Galloyltricetifavan: a promising natural radical scavenger

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7-O-Galloyltricetifavan (7OGT), a natural flavonoid, is isolated from the leaves of Pithecellobium clypearia. The compound exhibits a variety of biological activities. This study details the evaluation of the HOO• antiradical activity of 7OGT by quantum chemistry calculations. The HOO• trapping activity of 7OGT in the gas phase (reference state) was discovered to follow the formal hydrogen transfer mechanism with a rate constant of \( k = 4.58 \times 10^8 \text{ M}^{-1} \text{s}^{-1} \). In physiological environments, 7OGT is predicted to be an excellent HOO• radical scavenger with \( k_{\text{overall}} = 2.65 \times 10^8 \text{ M}^{-1} \text{s}^{-1} \) and \( 1.40 \times 10^4 \text{ M}^{-1} \text{s}^{-1} \) in water and pentyl ethanoate solvents, respectively. The HOO• antiradical activity of 7OGT in water at physiological pH is approximately 2000 times that of Trolox and substantially higher than that of other well-known natural antioxidants such as trans-resveratrol or ascorbic acid. Thus, 7OGT is an excellent natural antioxidant in polar environments.

1. Introduction

7-O-Galloyltricetifavan (7OGT; figure 1), a natural flavonoid, was first isolated from the leaves of Pithecellobium clypearia [1–3]. 7OGT is a flavan derivative that has antiviral properties against respiratory syncytial virus, influenza H1N1 virus, herpes simplex virus type 1 and coxsackie B3 virus as well as anti-inflammatory, anti-Alzheimer, anti-allergic and antioxidant properties [1–7]. Studies showed that 7OGT has potent xanthine oxidase inhibition with an IC\(_{50}\) = 25.5 µmol l\(^{-1}\) [3] and inhibits soluble epoxide hydrolase enzymatic activity with IC\(_{50}\) values 10.0 ± 0.4 µM [5]. Thus, 7OGT is indicated as a good natural antioxidant with the known neuroprotective activity that is believed to underpin the prevention of Alzheimer’s disease [7].

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Oxidative stress is now thought to play a role in several chronic diseases [8–10]. The ability of natural products to scavenge free radicals is an essential aspect of their anti-inflammatory, antibacterial and cancer-preventive properties, and it is the driving force behind the investigation of the antioxidant properties [11–13]. There is currently no information about the kinetics and mechanism of the $\text{HOO}^+$ + 7OGT in physiological conditions; however, computer calculations offer a convenient way to predict the antioxidant activity of organic compounds in physiological media [11,14,15]. Since 7OGT has exhibited a broad range of potent biological activities, this study aims to delve into the underpinning antiradical activity of 7OGT by a quantum chemical approach, using HOO$^+$ as a model radical.

### 2. Computational methods

The density functional theory-based quantum chemical calculations used here had been outlined in a range of former works for modelling antioxidant activities of various compounds [16–20]. In brief, the M06-2X/6-311++G(d,p)/M06-2X/6-31 + G(d) method was used to calculate thermodynamic parameters in the gas phase [21]. The kinetic calculations were performed at the M06-2X/6-311++G(d,p) level of theory, following the quantum mechanics-based test for overall free radical scavenging activity (QM-ORSA) protocol [22–24] with the SMD solvation model [25] for water and pentyl ethanoate solvents [11,14,17,19,26–35]. This protocol delivers results in reasonably good agreement with experimental data (\(k_{\text{calc}}/k_{\text{exp}}\) ratio = 1–2.9) [11,26,36], and therefore, it is commonly used to assess the radical scavenging activity of natural and synthetic compounds [14,15,20,37,38].

Figure 1. The structure of 7OGT.

Using the transition state (TS) theory at 298.15 K, 1 M standard state, the rate constant \((k)\) was computed as follows [31–34,39]:

\[
k = \sigma \kappa \frac{k_B T}{h} e^{-(\Delta G^*/RT)}
\]

where \(\sigma\) is the reaction symmetry number [29,30], \(\kappa\) contains the tunnelling corrections calculated using the Eckart barrier [35], \(k_B\) is the Boltzmann constant, \(h\) is the Planck constant and \(\Delta G^*\) is the Gibbs free energy of activation.

The reaction barriers of single electron transfer (SET) reactions in media were determined using the Marcus theory [40,41]. The equations used to calculate the Gibbs free energy change of reaction \(\Delta G^\lambda\) for the SET pathway are

\[
\Delta G^\lambda_{\text{SET}} = \frac{\lambda}{4} \left(1 + \frac{\Delta G^0_{\text{SET}}}{\lambda}\right)^2
\]

and

\[
\lambda \approx \Delta E_{\text{SET}} - \Delta G^0_{\text{SET}}
\]

where \(\Delta E_{\text{SET}}\) is the non-adiabatic energy difference among reactants and vertical products for SET, and \(\Delta G^0_{\text{SET}}\) is the standard Gibbs free energy change of the reaction [42,43].

A correction was applied to rate constants that were close to the diffusion limit [11]. The apparent rate constants \((k_{\text{app}})\) for an irreversible bimolecular diffusion-controlled reaction were computed using the Collins–Kimball theory in solvents at 298.15 K [44]; the steady-state Smoluchowski rate constant \((k_D)\)
was estimated using the literature [11,45],

$$k_{\text{app}} = \frac{k_{\text{TST}}k_D}{k_{\text{TST}} + k_D}$$  \hspace{1cm} (2.4)

and

$$k_D = 4\pi R_{AB}D_{AB}N_A$$  \hspace{1cm} (2.5)

$$D_{AB} = D_A + D_B$$ (\(D_{AB}\) is the mutual diffusion coefficient of the reactants \(A\) and \(B\)) [44,46], where \(D_A\) or \(D_B\) is determined using the Stokes–Einstein formulation (2.6) [47,48].

$$D_{A\text{ or } B} = \frac{k_BT}{6\pi\eta a_{A\text{ or } B}}.$$  \hspace{1cm} (2.6)

\(\eta\) is the viscosity of the solvents (i.e. \(\eta\) (pentyl ethanoate) = 8.62 × 10\(^{-4}\) Pa s, \(\eta\) (H\(_2\)O) = 8.91 × 10\(^{-4}\) Pa s) and \(a\) is the radius of the solute.

To avoid over-penalizing entropy losses in solution, the solvent cage effects were added using Okuno’s adjustments [49], which were modified with the free volume theory according to the Benson correction [11,50–52].

For species with numerous conformers, all of them were energy minimized, with the lowest electronic energy conformer being included in the study. The existence of only one single imaginary frequency was a defining feature of all transition stages. To verify that each TS is accurately related to the pre-complex and post-complex, intrinsic coordinate calculations were completed. The calculations were carried out using Gaussian 09 software [53].

### 3. Results and discussions

#### 3.1. The gas phase evaluation

Following the established protocol [19,54], the antioxidant activity of 7OGT was first evaluated according to the three main radical scavenging mechanisms: sequential electron transfer followed by proton transfer (SETPT), formal hydrogen transfer (FHT) and sequential proton loss followed by electron transfer (SPLET). In the two-step reactions such as the SETPT and SPLET pathways, the first step reaction (i.e. SET and proton loss (PL) for the SETPT and SPLET pathways, respectively) normally has the higher activation energy, with the exception of the proton dissociation of acidic moieties in water that is considered separately. Therefore, the thermochemical parameters i.e. proton affinity (PA), bond dissociation energy (BDE) and ionization energies (IE) that characterize the PL, FHT and SET reactions, respectively, were computed for all relevant bonds of 7OGT with the M06-2X/6-311++G(d, p)//M06-2X/6–31+G(d) method in the gas phase [21].

The results are presented in table 1. The results showed that the BDE values of the C–H range from 84.3 to 99.0 kcal mol\(^{-1}\), while the BDEs are 73.9–85.3 kcal mol\(^{-1}\) for O–H bonds. This suggests that the hydroxyl groups are the thermodynamically preferred sites of activity via the hydrogen transfer reaction. The lowest BDE values were presented at the O4’–H bond (73.9 kcal mol\(^{-1}\)) and the O13–H bond (77.1 kcal mol\(^{-1}\)). These sites are believed to play a key role in 7OGT’s radical scavenging activity via the FHT mechanism. According to this data, 7OGT has lower BDE(O–H) values than e.g. vanillic acid (85.2 kcal mol\(^{-1}\)), [16] puerarin (87.3 kcal mol\(^{-1}\)), [54] resveratrol (83.9 kcal mol\(^{-1}\)), [55] or viniferifuran (82.7 kcal mol\(^{-1}\)) [56]. This suggests that 7OGT could exhibit faster antiradical activity (following the FHT mechanism) than these natural antioxidants.

As shown in table 1, the lowest IE and PA values are significantly higher (about 2.42 and 4.60 times, respectively) than those of the BDE. Therefore, the antiradical activity of 7OGT is expected to favour the FHT mechanism in lipid media. The calculated Gibbs free energy changes (\(\Delta G^0\)) of the 7OGT + HOO\(^{\cdot}\) reaction via the main mechanisms: FHT, PL, which is the first step of SPLET, and SET as the first step of the SETPT suggest that the FHT reaction is spontaneous (\(\Delta G^0 < 0\)) for most of the sites, apart from the C3(4)H–bonds; however, the PL and SET reactions are not spontaneous (\(\Delta G^0 > 0\)) in any cases. The Marcus theory was also used to estimate the reaction barriers of the SET reaction in the gas phase [40,41]; however, this reaction was negative in the studied conditions (\(\Delta G_{\text{SET}}^0 = 155.6\) and \(\lambda = 21.2\) kcal mol\(^{-1}\)). Thus kinetics of HOO\(^{\cdot}\) + 7OGT reaction were computed following the FHT mechanism at the positions that yielded negative \(\Delta G^0\).
Kinetic studies of the HOO$^+$ + 7OGT reaction were performed following the QM-ORSA protocol with the M06-2X/6-311++G(d,p) method [11,27,28], and results are presented in table 2 and figure 2. The energy barriers for the 7OGT + HOO$^+$ reaction following the FHT pathway are within the range of $-2.0$ to $7.3$ kcal mol$^{-1}$ (table 2). The O4$^-'$H + HOO$^+$ reaction had the lowest barrier height with $\Delta H = -2.0$ kcal mol$^{-1}$. It can be affirmed that the HOO$^+$ scavenging activity of the O4$^-'$H bond is the highest among all of the studied bonds. The HOO$^+$ trapping activity of 7OGT is mainly due to the H-abstraction of the O4$^-'$H bond ($\Delta G^\neq = 7.4$ kcal mol$^{-1}$; $k_{Eck} = 4.58 \times 10^8$ M$^{-1}$ s$^{-1}$; $\Gamma = 100\%$). The activation Gibbs energies ($\Delta G^\neq$) range 7.4–16.6 kcal mol$^{-1}$, while the $\kappa$ values vary 19.3–53.3. Thus, the $\kappa$ values play an important role in the rate constants of the hydroperoxyl antiradical activity of the 7OGT. This result is consistent with previous studies on phenolic compounds [22,27]. The calculated results suggest that the HOO$^+$ trapping activity of 7OGT is defined by the FHT reaction at the O4$^-'$ position; therefore, this reaction will be further analysed in physiological media.

### 3.2. The radical scavenging activity of 7-O-Galloyltricetifavan in physiological environments

Previous research has shown that the antiradical activity of phenolic compounds in aqueous solutions is dominated by anion states [37,38]. The protonation states of 7OGT were investigated at physiological pH to discover potential radical scavenging mechanisms [16,38,57]. Based on the calculated data [58], $pK_{a1}$ and $pK_{a2}$ values were 6.87 and 8.29, respectively (figure 3). Therefore, in water at pH = 7.4, three states, including neutral (H$_2$A: 20.7%), anion (HA$: 70.2\%$) and dianion (A$^{2-}$: 9.1%) will be used for studying the radical scavenging activity, whereas the neutral state will be considered in the lipid medium (pentyl ethanoate solvent).

The calculated results in the vacuum suggest that the HOO$^+$ antiradical activity in non-polar media follows the hydrogen transfer mechanism at the O4$^-'$H bond. Thermodynamic evaluation in pentyl
Figure 2. The FHT TSs between the 7OGT and HOO$^-$ radical.

Figure 3. The deprotonation of 7OGT.
ethanoate and water (electronic supplementary material, table S2) did not differ at the most likely site of activity ($BDE = 74.6$ and $78.9$ kcal mol$^{-1}$ and $\Delta G^o = -10.7$ and $-9.6$ kcal mol$^{-1}$ in the lipid and water media, respectively) for the neutral state (H$_2$A), however, the PL and SET pathways of this state are not spontaneous in either media ($\Delta G^o > 0$). Previous studies showed that for a compound containing acidic moieties the SET reaction of the dissociated states should be also considered in the aqueous solution [16,18,21,37,54,57]. Thus, the kinetics of the radical scavenging activity of 7OGT against HOO$^*$ radical in physiological media were carried out following equations (3.1) and (3.2) below, and the results are presented in table 3.

Lipid medium,

$$k_{\text{overall}} = k_{\text{app}}(\text{FHT(O4}$' - H) - \text{neutral}).$$

(3.1)

Water at physiological pH,

$$k_{\text{overall}} = k_j (\text{FHT (O4}' - H) - \text{neutral}) + k_j (\text{SET - anion}) + k_j (\text{SET - dianion}).$$

(3.2)

According to the results (table 3), the HOO$^*$ + 7OGT reaction in the aqueous solution ($k_{\text{overall}} = 2.65 \times 10^8$ M$^{-1}$ s$^{-1}$) is approximately $10^4$ times faster than that ($k_{\text{overall}} = 1.40 \times 10^4$ M$^{-1}$ s$^{-1}$) in the lipid medium. The SET of dianion $\Lambda^-$ plays a principal role ($k_j = 2.64 \times 10^8$ M$^{-1}$ s$^{-1}$, $\Gamma = 99.6\%$) in the HOO$^*$ antiradical activity of 7OGT in the aqueous solution. Compared with typical antioxidants indicated that the HOO$^*$ scavenging activity of 7OGT is faster than those of Trolox (approx. 2000 times, $k = 1.30 \times 10^5$ M$^{-1}$ s$^{-1}$), [24] trans-resveratrol (approx. five times, $k = 5.62 \times 10^7$ M$^{-1}$ s$^{-1}$) [55], ascorbic acid (approx. two times, $k = 1.00 \times 10^9$ M$^{-1}$ s$^{-1}$) [11], ramalin (approx. 1692 times, $k = 1.56 \times 10^9$ M$^{-1}$ s$^{-1}$) [57], deoxynimbidiol (approx. 1.5 times, $k = 1.69 \times 10^8$ M$^{-1}$ s$^{-1}$) [24] and 8-hydroxyconiothyrinone B (approx. 4.5 times, $k = 5.80 \times 10^7$ M$^{-1}$ s$^{-1}$) [18]. Hence, 7OGT is one of the most excellent natural antioxidants in polar environments.

### 4. Conclusion

The hydroperoxyl antiradical activity of 7OGT was successfully evaluated by computational chemistry. The results showed that the antiradical activity of the 7OGT in non-polar media such as gas phase and pentyl ethanoate follows the FHT reaction ($k = 4.58 \times 10^8$ and $1.40 \times 10^8$ M$^{-1}$ s$^{-1}$, respectively). 7OGT also presented excellent antiradical activity ($k_{\text{overall}} = 2.65 \times 10^8$ M$^{-1}$ s$^{-1}$) in the aqueous solution. The HOO$^*$ radical scavenging of 7OGT is faster than that of typical antioxidants such as trans-resveratrol, Trolox, deoxynimbidiol, ramalin, 8-hydroxyconiothyrinone B and ascorbic acid. Thus, 7OGT is one of the most potent natural antioxidants identified thus far in polar environments.

Data accessibility. Data are available at the Dryad Digital Repository: https://doi.org/10.5061/dryad.jh9w0vtcq [59].

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All authors gave final approval for publication and agreed to be held accountable for the work performed therein. Conflict of interest declaration. We declare we have no competing interests.

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37. Galano A, Raúl Alvarez-Idaboy J. 2019 Computational strategies for predicting free radical scavengers’ protection against oxidative stress: where are we and what might follow? Int. J. Quantum Chem. 119, e25665. (doi:10.1002/qua.25665)

38. Boulebd H. 2021 Is cannabidiolic acid an overlooked natural antioxidant? Insights from quantum chemistry calculations. New J. Chem. 46, 162–168. (doi:10.1039/D1NJ04771J)

39. Evans MG, Polanyi M. 1935 Some applications of the transition state method to the calculation of reaction velocities, especially in solution. Trans. Faraday Soc. 31, 875–894. (doi:10.1039/tf9353100875)

40. Marcus RA. 1964 Chemical and electrochemical electron-transfer theory. Annu. Rev. Phys. Chem. 15, 155–196. (doi:10.1146/annurev.pc.15.100164.001103)

41. Marcus RA. 1993 Electron transfer reactions in chemistry: theory and experiment. Rev. Mod. Phys. 65, 599–619. (doi:10.1103/RevModPhys.65.599)

42. Nelsen SF, Blackstock SC, Kim Y. 1987 Estimation of inner shell Marcus terms for amino nitrogen compounds by molecular orbital calculations. J. Am. Chem. Soc. 109, 677–682. (doi:10.1021/ja00237a007)

43. Nelsen SF, Weaver MN, Luo Y, Piazdziewicz JI, Ausman UK, Jentzsch TL, O’Konek II. 2006 Estimation of electronic coupling for intermolecular electron transfer from cross-reaction data. J. Phys. Chem. A 110, 11 665–11 676. (doi:10.1021/jp064406v)

44. Collins FC, Kimmall GE. 1949 Diffusion-controlled reaction rates. J. Colloid Sci. 4, 425–437. (doi:10.1016/0095-8522(49)90023-9)

45. Von Smoluchowski M. 1917 Versuch einer Mathematischen Theorie der Koagulations Kinetic Kolloider Lousungen. Z. Phys. Chem. 92, 129–168.

46. Truhlar DG. 1985 Nearly encounter-controlled reactions: the equivalence of the steady-state and diffusional viewpoints. J. Chem. Educ. 62, 104. (doi:10.1021/ed062p104)

47. Einstein A. 1905 Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. Ann. Phys. 322, 549–560. (doi:10.1002/andp.19053220806)

48. Stokes GG. 1905 Mathematical and physical papers. Cambridge, UK: University Press.

49. Okuno Y. 1997 Theoretical investigation of the mechanism of the Baeyer-Villiger reaction in nonpolar solvents. Chem. Eur. J. 3, 212–218. (doi:10.1002/chem.19970030208)

50. Benson S. 1960 The foundations of chemical kinetics. New York, NY: McGraw-Hill.

51. Iuga C, Alvarez-Idaboy JR, Vivier-Bunge A. 2011 ROS initiated oxidation of dopamine under oxidative stress conditions in aqueous and lipidic environments. J. Phys. Chem. B 115, 12 234–12 246. (doi:10.1021/jp063474u)

52. Alvarez-Idaboy JR, Reyes L, Mora-Diez N. 2007 The mechanism of the Baeyer–Villiger rearrangement: quantum chemistry and TST study supported by experimental kinetic data. Org. Biomol. Chem. 5, 3682–3689. (doi:10.1039/b712068e)

53. Frisch M et al. 2009 Gaussian 09. Wallingford, CT: Gaussian Inc. 

54. Zhou H, Li X, Shang Y, Chen K. 2019 Radical scavenging activity of puerarin: a theoretical study. Antioxidants 8, 590. (doi:10.3390/antiox8200590)

55. Cordova-Gomez M, Galano A, Alvarez-Idaboy JR. 2013 Piceatannol, a better pentaoyl radical scavenger than resveratrol. JOC Adv. 3, 20 209–20 218. (doi:10.1021/acs.joc42923g)

56. Shang Y, Zhou H, Li X, Zhou J, Chen K. 2019 Theoretical studies on the antioxidant activity of viniferifuran. New J. Chem. 43, 15 736–15 742. (doi:10.1039/C9NJ02735A)

57. Vo QV, Tam N, Van Bay M, Mechler A. 2020 The radical scavenging activity of natural ramalin: a mechanistic and kinetic study. Chem. Phys. Lett. 739, 137004. (doi:10.1016/j.cplett.2019.137004)

58. Galano A et al. 2016 Empirically fitted parameters for calculating pK a values with small deviations from experiments using a simple computational strategy. J. Chem. Inf. Model 56, 1714–1724. (doi:10.1021/acs.jcim.6b00310)

59. Hieu LV, Van Thi TT, Hoa NT, Mechler A, Vo QV. 2022 Data from: 7-O-Galloyltricetifavan: a promising natural radical scavenger. Dryad Digital Repository. (doi:10.5061/dryad.jh9w0vtqj)