Enhanced radical-scavenging activity of naturally-oriented artepillin C derivatives†

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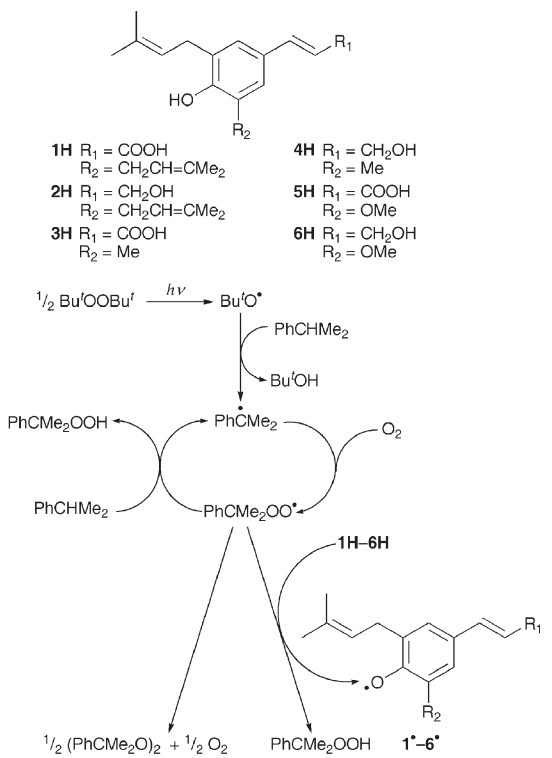
More than two-fold augmentation in the radical-scavenging activity of artepillin C could be achieved *via* altering the O–H bond dissociation enthalpy of artepillin C by means of structural modifications.

Reactive oxygen species (ROS) and other free radicals have been implicated as pathological mediators in many clinical disorders. The reactivity of the most frequently encountered active free radicals, such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), alkyl radical ($R^{\bullet}$), alkoxy radical ($RO^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), nitric oxide ($NO^{\bullet}$) and lipid peroxyl radical ($LOO^{\bullet}$), varies, but some may cause severe damage to biological molecules, especially to DNA, lipids and proteins. However, several attempts including the use of naturally occurring and chemically synthesized antioxidants have been made to find out the possible ways for scavenging of these free radicals[1] and synthetic attempts have also been made in the last two decades to develop more potential antioxidants.[2] The plant-derived phenolic compounds have attracted much attention due to their limited or zero toxicity in *in vivo* systems. Artepillin C [3-{4-hydroxy-3,5-bis(3-methyl-2-butenyl)phenyl}-2(*E*)-propenoic acid] (**1H**), a major component (>5%) of Brazilian propolis,[3] has been reported to show antioxidative activity[4] alongside other important biological activities.[5] Recently, we reported the free radical-scavenging activity of artepillin C and also discussed the possible scavenging mechanism.[6] Since O–H bond dissociation enthalpy (D_{HT}) is known to regulate the antioxidative potency in phenolic compounds,[7] synthetic approaches towards the lowering of D_{HT} by structural modifications of artepillin C (**1H**) may result in remarkable changes in its antioxidative activity. We report herein the synthesis of five naturally-oriented artepillin C derivatives (**2H–6H**) (Scheme 1) and their enhanced scavenging activity towards cumylperoxyl radical ($PhCMe_2OO^{\bullet}$). $PhCMe_2OO^{\bullet}$, which is less reactive than $RO^{\bullet}$, is known to follow the same pattern of relative reactivity with a variety of substrates.[8] The structure–activity relationship is also discussed based on the results obtained in this study, providing a valuable insight into the development of antioxidants stronger than the naturally occurring ones.

Synthesis of **2H–6H** was based on regioselective *C*-prenylation of corresponding *ortho*-substituted phenols according to our established procedure[9] (see ESI,† S1). This indicates that the artepillin C derivatives could efficiently scavenge $PhCMe_2OO^{\bullet}$. In the presence of the artepillin C derivatives the decay rate of $PhCMe_2OO^{\bullet}$ follows pseudo-first-order kinetics. The pseudo-first-order rate constant (k) exhibits first-order dependence with respect to the concentration of the artepillin C derivatives. From the slopes of the linear plots were determined the second-order rate constants (k_{obs}) for the reaction between artepillin C derivatives and $PhCMe_2OO^{\bullet}$ in EtCN at 203 K. The k_{obs} value for the



Scheme 1

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Table 1 Rate constants (k_{obs}) for scavenging of cumylperoxyl radical by **1H–6H** in EtCN at 203 K, energy difference values (D_{HT}) between phenoxyl radicals and phenols with reference to **1H**, and ionization potential (IP) values determined by density functional theory (DFT) calculations

Compound	k_{obs}/mol^{-1} dm^3 s^{-1}	D_{HT}/kcal mol^{-1}	IP/kcal mol^{-1}
1H	4.9×10^2	0	162.4
2H	5.7×10^2	−2.7	152.3
3H	1.5×10^2	0.5	164.9
4H	5.5×10^2	−2.1	154.3
5H	9.4×10^2	−4.2	162.3
6H	1.2×10^3	−6.0	153.2

$PhCMe_2OO^{\bullet}$ scavenging by **6H** (1.2×10^3 mol^{-1} dm^3 s^{-1}) is the largest among the examined artepillin C derivatives and is much larger than that for (+)-catechin (5.0×10^2 mol^{-1} dm^3 s^{-1}), which is one of the strongest antioxidants.[14,15] The rate constants of the $PhCMe_2OO^{\bullet}$-scavenging reactions by other derivatives have also been determined and are listed in Table 1. All the artepillin C derivatives, except **3H**, could afford significantly larger k_{obs} values than **1H** (4.9×10^2 mol^{-1} dm^3 s^{-1}). The k_{obs} value of **3H** was found to be smaller than that of **1H**, and this may be due to the absence of an electron-donating alkene group in **3H**. The k_{obs} value increases with an increasingly electron-rich environment in the molecule.

Direct measurements of the rate of the reaction between artepillin C derivatives and $PhCMe_2OO^{\bullet}$ were performed in propionitrile (EtCN) at 203 K by means of electron paramagnetic resonance (EPR). $PhCMe_2OO^{\bullet}$ is formed *via* a radical chain process as shown in Scheme 1.[10] The photoirradiation of Bu^tOOBu^t results in the homolytic cleavage of the O–O bond to produce $Bu^tO^{\bullet}$,[11] which abstracts a hydrogen atom from cumene to give cumyl radical, followed by the facile addition of molecular oxygen to cumyl radical. $PhCMe_2OO^{\bullet}$ can also abstract a hydrogen atom from cumene in the propagation step to yield cumene hydroperoxide, accompanied by regeneration of cumyl radical (Scheme 1).[12] In the termination step, $PhCMe_2OO^{\bullet}$ decays by a bimolecular reaction to yield the corresponding peroxide and molecular oxygen (Scheme 1).[13] In the presence of the artepillin C derivatives, the decay rate of $PhCMe_2OO^{\bullet}$ was much faster than that in their absence (Fig. 1).

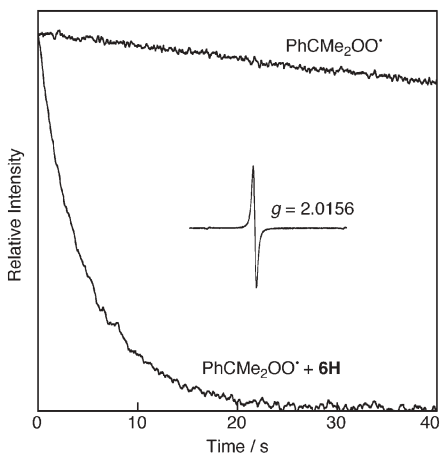

Fig. 1 Time course changes of the EPR signal intensity of $PhCMe_2OO^{\bullet}$ in the absence and presence of **6H** (1.3×10^{-4} mol dm^{-3}) in EtCN at 203 K. Inset: EPR spectrum of $PhCMe_2OO^{\bullet}$.

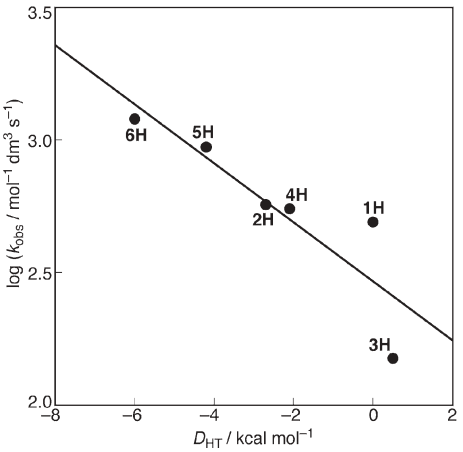

Fig. 2 Plot of log k_{obs} *vs.* calculated energy difference values (D_{HT}) between phenoxyl radicals and phenols with reference to **1H**.

It was also found that the k_{obs} values for the artepillin C derivatives were linearly correlated with the corresponding energy difference values, equal to D_{HT}, (Table 1) between the phenoxyl radicals (**1**$^{\bullet}$–**6**$^{\bullet}$) and the phenols determined by density functional theory (DFT) calculations[16] as shown in Fig. 2. On the other hand, as shown in Fig. 3, such a linear correlation cannot be observed between the k_{obs} values and the ionization potential (IP) calculated by DFT (Table 1). These results suggest that the $PhCMe_2OO^{\bullet}$-scavenging reaction by the artepillin C derivatives may proceed *via* a one-step hydrogen atom transfer rather than *via* an electron transfer oxidation of the artepillin C derivatives by $PhCMe_2OO^{\bullet}$.

Furthermore, enhancement in the antioxidative activity of the artepillin C derivatives can be explained by the fact that electron-donating (ED) groups reduce the D_{HT} and electron-withdrawing (EW) groups have the reverse effect.[17,18] By comparing the radical-scavenging efficiency of a series of artepillin C derivatives, we found the same trend and can conclude that the two structural factors are important for the antioxidative potency of the artepillin C derivatives, *i.e.*, the additional presence of ED substituents and the absence of EW groups. In fact, the k_{obs} value for **6H**, which is 2.4 times larger than that of **1H**, can be explained by the presence of two electron-donating groups, methoxy and hydroxypropenyl, in the molecule. Similarly, it can be explained why **3H** is least

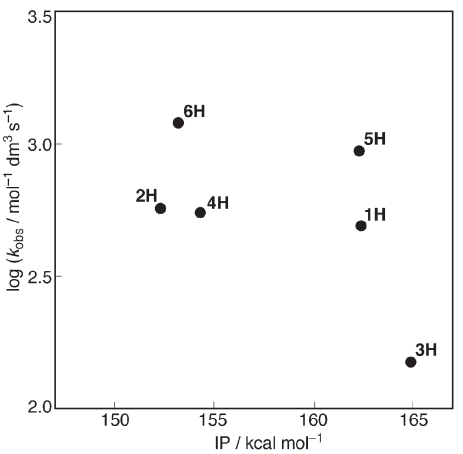

Fig. 3 Plot of log k_{obs} *vs.* calculated ionization potentials (IP) of **1H–6H**.

reactive followed by **1H**, **4H**, **2H**, **5H**, and **6H** among the examined artepillin C derivatives. By comparing the k_{obs} values for **1H–6H**, it is clear that the more electron-rich is the environment the compound has, the lower is its D_{HT} value, and the higher is its $PhCMe_2OO^{\bullet}$ scavenging activity.

In conclusion, structural modification of artepillin C, resulting in the decline of D_{HT}, leads to the enhancement of cumylperoxyl-scavenging activity. This is explained by the fact that **6H**, having six-fold lower D_{HT} as compared to artepillin C, showed more than two-fold higher $PhCMe_2OO^{\bullet}$-scavenging activity than artepillin C. Such an augmentation in radical-scavenging efficiency may have implications for reducing the excessive amount of radical scavenger used in *in vitro* as well as in *in vivo* studies.

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16 Density functional theory (DFT) calculations were performed on an 8CPU workstation (PQS, Quantum Cube QS8-2400C-064). Geometry optimizations were carried out using the Becke3LYP and 6-31G* basis set for the phenoxyl radical with the unrestricted Hartree–Fock (UHF) formalism as implemented in the Gaussian 03 program Revision C.02. The D_{HT} values were determined by the single point energy calculations at the B3LYP/6-31G* basis set with the restricted open shell Hartree–Fock (ROHF) formalism.

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