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Efficient radical scavenging ability of artepillin C, a major component of Brazilian propolis, and the mechanism

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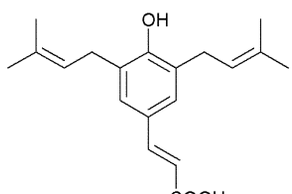
Hydrogen transfer from artepillin C to cumylperoxyl radical proceeds *via* one-step hydrogen atom transfer rather than *via* electron transfer, the rate constant of which is comparable to that of (+)-catechin, indicating that artepillin C can act as an efficient antioxidant.

Artepillin C [3-{4-hydroxy-3,5-bis(3-methyl-2-butenyl)phenyl}-2(*E*)-propenoic acid] (**1**), a major component (> 5%) of Brazilian propolis,[1] is a member of a class of 2,4,6-trisubstituted phenols that has recently been reported to show important biological activities, such as antitumor,[2] apoptosis-inducing,[3] immunomodulating,[4] and antioxidative activities.[5] It is known that hydrogen transfer from the phenolic hydroxyl group to active radical species, such as hydroxyl radical ($^{\bullet}OH$), superoxide anion ($O_2^{\bullet -}$), lipid peroxyl radical ($LOO^{\bullet}$), is responsible for the antioxidative activities of the phenolic compounds. However, little is known about the quantitative radical-scavenging ability of **1**, as well as the mechanism of hydrogen-transfer reactions from **1** to radical species. There are two possibilities in the mechanism of hydrogen-transfer reactions from phenolic compounds to radical species, *i.e.*, a one-step hydrogen atom transfer or electron transfer followed by proton transfer.[6] Recently, we have reported that the hydrogen transfer from (+)-catechin, one of the most powerful natural antioxidants, to cumylperoxyl radical proceeds *via* an electron transfer from (+)-catechin to cumylperoxyl radical, which is accelerated by the presence of scandium ion (Sc^{3+}), followed by proton transfer in an aprotic medium.[7] We herein report rates of hydrogen transfer from **1** to cumylperoxyl radical determined by the EPR technique in propionitrile (EtCN) at low temperature (203 K). Cumylperoxyl radical, which is much less reactive than alkoxyl radicals, is known to follow the same pattern of relative reactivity with a variety of substrates.[8–10] The effect of Sc^{3+} on the hydrogen transfer rates was also examined to distinguish between one-step hydrogen- or electron-transfer mechanisms for the radical-scavenging reactions of **1**.

Direct measurements of the rate of hydrogen transfer from **1**† to cumylperoxyl radical were performed in EtCN at 203 K by means of EPR. The photoirradiation of an oxygen-saturated EtCN solution containing di-*tert*-butyl peroxide (^{t}BuOO^{t}Bu) and cumene ($PhCHMe_2$) with a 1000 W high-pressure mercury lamp results in formation of cumylperoxyl radical ($PhCMe_2OO^{\bullet}$), which was readily detected by EPR. The cumylperoxyl radical is formed *via* a radical chain process shown in eqns. (1)–(3).[11–15] The photoirradiation of ^{t}BuOO^{t}Bu results in the homolytic cleavage of the O–O bond to produce ^{t}BuO$^{\bullet}$ [eqn. (1)],[16,17] which abstracts a hydrogen from cumene to give cumyl radical ($PhC^{\bullet}Me_2$) [eqn. (2)], followed by the facile addition of oxygen to cumyl radical [eqn. (3)]. The cumylperoxyl radical can also abstract a hydrogen atom from cumene in the propagation step to yield cumene hydroperoxide ($PhCMe_2OOH$), accompanied by regeneration of cumyl radical [eqn. (4)].[18,19] In the termination step, cumylperoxyl radicals decay by a bimolecular reaction to yield the corresponding peroxide and oxygen [eqn. (5)].[18,19] When the light is cut off, the EPR signal intensity decays, obeying second-order kinetics due to the bimolecular reaction in eqn. (5). In the presence of **1**, the decay rate of cumylperoxyl radical after cutting off the light becomes much faster than that in the absence of **1**. The decay rate in the presence of **1** ($2.8–5.6 \times 10^{-4}$ M) obeys pseudo-first-order kinetics. This decay process is ascribed to the hydrogen transfer from **1** to cumylperoxyl radical to produce the phenoxyl radical **1**$^{\bullet}$‡ and $PhCMe_2OOH$ [eqn. (6)]. The pseudo-first-order rate constants (k_{obs}) increase with increasing **1** concentration to exhibit first-order dependence on [**1**]. From the slope of the linear plot of k_{obs} *vs.* the concentration of **1** is determined the second-order rate constant (k_{HT}) for the hydrogen transfer from **1** to cumylperoxyl radical as 4.9×10^2 M^{-1} s^{-1} in EtCN at 203 K. This value is very close to the rate constant obtained for hydrogen transfer from (+)-catechin to cumylperoxyl radical in EtCN (6.0×10^2 M^{-1} s^{-1}),[7] indicating that, in aprotic medium, artepillin C is an excellent free radical scavenger, comparable to (+)-catechin.



1

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$$^{t}BuOO^{t}Bu \xrightarrow{h\nu} 2\,^{t}BuO^{\bullet} \quad (1)$$

$$PhCHMe_2 + {}^{t}BuO^{\bullet} \longrightarrow PhC^{\bullet}Me_2 + {}^{t}BuOH \quad (2)$$

$$PhC^{\bullet}Me_2 + O_2 \longrightarrow PhCMe_2OO^{\bullet} \quad (3)$$

$$PhCMe_2OO^{\bullet} + PhCHMe_2 \longrightarrow PhCMe_2OOH + PhC^{\bullet}Me_2 \quad (4)$$

$$2\,PhCMe_2OO^{\bullet} \longrightarrow (PhCMe_2O)_2 + O_2 \quad (5)$$

1 + $PhCMe_2OO^{\bullet}$ $\xrightarrow{k_{HT}}$ **1**$^{\bullet}$ + $PhCMe_2OOH$ (6)

If the hydrogen transfer from **1** to cumylperoxyl radical involves an electron-transfer process as the rate-determining step, the rate of hydrogen transfer would be accelerated by the presence of scandium ion.[7,20] This is checked by examining the effect of $Sc(OTf)_3$ (OTf = OSO_2CF_3) on the hydrogen-transfer rate from **1** to cumylperoxyl radical. No effect of Sc^{3+} on the k_{HT} values of the hydrogen-transfer reaction of **1** with cumylperoxyl radical used as a hydrogen abstracting agent was observed, as shown in Fig. 1. Thus, there may be no contribution of electron transfer from **1** to cumylperoxyl radical in the hydrogen-transfer reaction, which may thereby proceed *via* a one-step hydrogen atom-transfer process. On the other hand, the hydrogen transfer from (+)-catechin (**2**) to cumylperoxyl radical has been reported to proceed *via* electron transfer from **2** to cumylperoxyl radical, which is accelerated by the presence of Sc^{3+}, followed by proton transfer from the radical cation of **2** to cumylperoxylate (Scheme 1).[7] The difference in the hydrogen-transfer mechanism between **1** and **2** may be ascribed to the oxidation potentials of **1** and **2**. In fact, the oxidation potential of **1** (E^0_{ox} = 1.39 V *vs.* SCE) determined by second-harmonic alternating current voltammetry (SHACV)[21] with a Pt working electrode in acetonitrile, containing 0.1 M *n*-Bu_4NClO_4 as a supporting electrolyte, is significantly more positive than that of **2** (E^0_{ox} = 1.18 V *vs.* SCE). In such a case, the electron-transfer oxidation of **1** by cumylperoxyl radical, whose

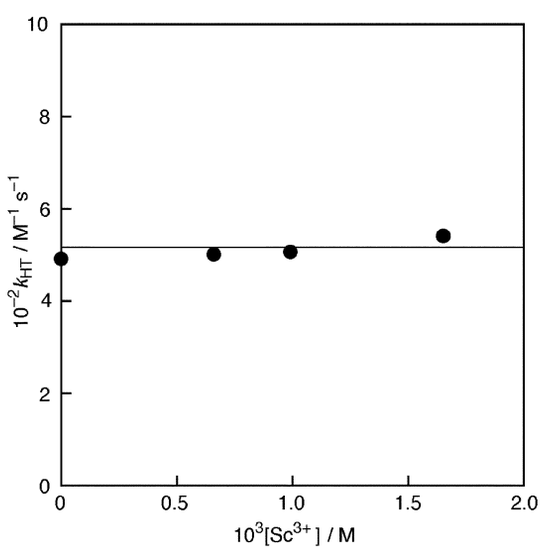
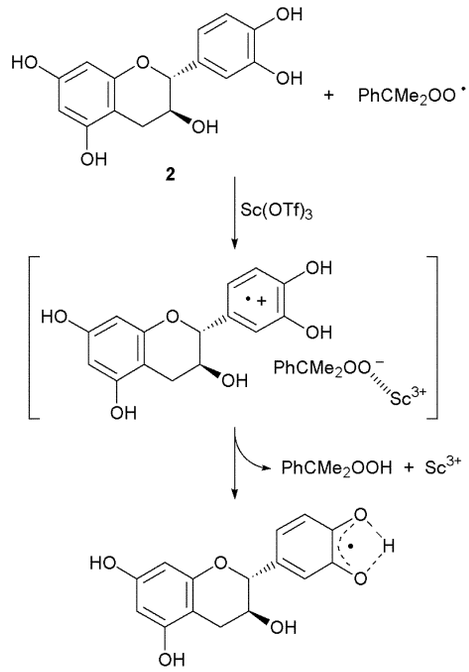


Fig. 1 Plot of k_{HT} *vs.* $[Sc^{3+}]$ in the hydrogen transfer from **1** to cumylperoxyl radical in the presence of $Sc(OTf)_3$ in EtCN at 203 K.



Scheme 1 Mechanism of hydrogen transfer from (+)-catechin (**2**) to cumylperoxyl radical *via* electron transfer.

reduction potential (E^0_{red}) is located at 0.65 V *vs.* SCE,[7] is less energetically feasible than that of **2**.

In conclusion, artepillin C shows an efficient radical-scavenging activity against cumylperoxyl radical in an aprotic medium, which is comparable to that of (+)-catechin. The absence of an effect of Sc^{3+} on the k_{HT} values demonstrates that the hydrogen transfer from artepillin C to cumylperoxyl radical proceeds *via* one-step hydrogen atom transfer rather than *via* an electron transfer followed by proton transfer.

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Notes and references

† Synthesis of **1** was carried out according to the procedure reported in the literature. See: Y. Uto, A. Hirata, T. Fujita, S. Takubo, H. Nagasawa and H. Hori. *J. Org. Chem.*, 2002, **67**, 2355.

‡ The EPR signal of phenoxyl radical **1**$^{\bullet}$ has successfully been detected in the photoreaction of **1** with $^{t}BuOO^{t}Bu$ in CH_2Cl_2, however, no hyperfine structure was observed because of its instability.

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