Protective Activities of Some Phenolic 1,3-Diketones against Lipid Peroxidation: Possible Involvement of the 1,3-Diketone Moiety

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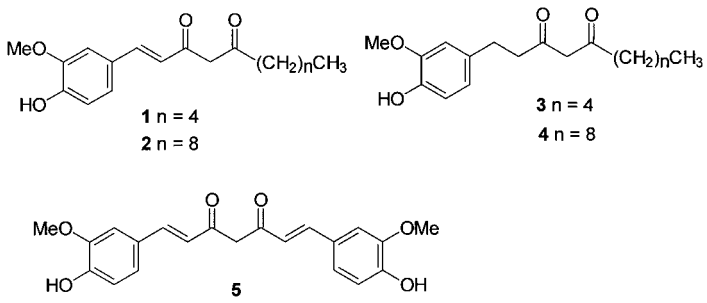
*The protective activities of four ginger-derived phenolic 1,3-diketones (**1** – **4**) and curcumin (**5**) against lipid peroxidation was studied by using different biologically relevant model systems and pulse radiolysis. The extraordinary activity of **5** vis-à-vis **1** – **4** against Fe^{2+}-mediated peroxidation may be attributed to the additional phenolic hydroxy group in the former, which lends it better iron-chelating and radical-scavenging properties. In iron-independent peroxidation, however, the ginger constituent [6]-dehydrogingerdione (**1**) showed activity comparable to that of **5**; this indicates its higher affinity for the lipid peroxide radical ($LOO^{\bullet}$), due to its higher hydrophobicity. A very high rate constant for the reaction between **1** and $Cl_3COO^{\bullet}$, measured by pulse radiolysis, not only confirmed this, but also established the superior antioxidant efficacy of **1** in comparison to vitamins E and C. This was also evident from the results obtained from a liposomal peroxidation study with **1** and vitamin C. This study also established a synergistic effect of the latter on the antioxidant activity of **1**. HPLC analysis of the products of the reaction between **1** and $Cl_3COO^{\bullet}$ revealed the formation of higher concentrations of ferulic acid (**7**), along with vanillin (**6**). The presence of ascorbate affected the generation of **7** more than it did that of **6**. On this basis, a mechanism for the antioxidant action of **1** has been proposed, which suggests the contribution of the phenolic group as well as the active methylene group of the 1,3-diketones.*

KEYWORDS:

diketones · lipids · peroxides · radicals · redox chemistry

Introduction

In recent years, the antioxidant properties of food constituents have been seriously noted by medical and nutritional experts, since oxidation of biological molecules has been postulated to induce a variety of pathological events such as atherogenesis,[1] carcinogenesis[2] and ageing.[3] These damaging events are caused by reactive oxygen species (ROSs), and various dietary compounds are known to prevent them, primarily through their ROS-scavenging potentials. Amongst dietary sources, spices and condiments possibly have the best potential, as these are widely used in traditional recipes as adjuncts to enhance the flavour, colour and taste of food preparations. One such spice, turmeric, is valued[4] for its various curative properties, mainly associated with its constituent curcuminoids.[5a,b] Recently, our reinvestigation into the antioxidant components of the ginger (*Zingiber officinale*) rhizome revealed[6] that, contrary to earlier reports,[7] the [6]- and [10]-dehydrogingerdiones (**1** and **2**) and their corresponding dihydro analogues (**3** and **4**) were superior antioxidants to the other ginger components, gingerols, dehydrogingerone and shogaol. Chemically, compounds **1** – **4** are similar to curcumin (**5**) (Scheme 1) and can be viewed as curcuminoids. Like curcumin, each of these compounds possesses a partially methylated catechol moiety, a 1,3-diketo functionality and (for **1** and **2**) extended conjugation. Hence, their superior antioxidant activity compared to the other ginger constituents was not surprising. Unlike **5**, which is a biphenol, however, each of the compounds **1** – **4** contains only one phenolic hydroxy group. It was therefore of interest to compare the antioxidant activities of **1** – **4** with that of **5** and study their modes of action, as phenols are known to exert this activity as free radical scavengers,[8a,b] metal chelators[9a,b] and cell membrane modifiers.



Scheme 1. *Chemical structures of the ginger compounds **1** – **4** and curcumin (**5**).*

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Results and Discussion

Cellular membranes, which perform a decisive role in the functional organization of the cell, are among the most important targets for oxidative damage, due to their high content of unsaturated fatty acids.[10] Adverse alterations in biomembranes can directly give rise to cytotoxicity and/or indirectly to genotoxicity.[11] The complex process of lipid peroxidation (LPO) is believed to be initiated by the ROSs produced in cells and tissues by partial reduction of oxygen during metabolism. Other prominent causative agents for LPO are ionizing radiation and transition metals (especially Fe^{2+} and Cu^{+}). The direct consequence of LPO is the loss of membrane integrity and inactivation of vital cellular components, including membrane-bound enzymes. In addition, the low molecular weight electrophilic products generated during the process can damage other biomolecules remote from the site of their generation.[12] Hence, the anti-LPO activities of the ginger components **1** – **4** were analysed by two biologically relevant test systems:[13] rat brain homogenate and liver mitochondria, both of which are highly susceptible to oxidation due to their elevated oxygen consumption.

Exposure of rat brain homogenate to Fe^{2+} ascorbate resulted in a significant increase in lipid peroxidation, as was evident from the increase in the absorbance at 532 nm (A_{532}) due to thiobarbituric acid reactive substrates (TBARSs). All the test compounds **1** – **4**, and curcumin **5**, inhibited the lipid peroxidation concentration dependently (Figure 1). Table 1 shows their

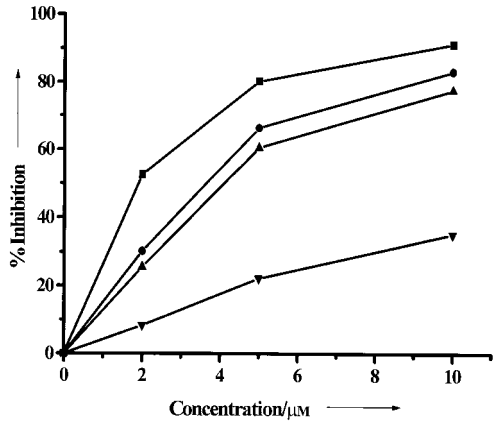


Figure 1. *Concentration-dependent inhibitory activities of **1** – **4** against Fe^{2+}-induced peroxidation of rat brain homogenate: ■ = **1**, ● = **2**, ▲ = **3** and ▼ = **4**. Values are mean ± standard error (n = 3).*

Table 1. *Protective activities of **1** – **5** against Fe^{2+}-induced lipid peroxidation.*

Compound	IC_{50} [μM]
1	1.76 ± 0.08
2	3.66 ± 0.39
3	3.10 ± 0.26
4	21.4 ± 0.51
5	0.4 ± 0.10
α-toc	9.2 ± 0.60
BHA	1.1 ± 0.50

IC_{50} values (the concentration at which 50% of activity is inhibited) along with those of two positive controls, α-tocopherol (α-toc) and butylated hydroxyanisole (BHA). Although compound **5** was the best inhibitor, the anti-LPO activities of **1** – **3** were far superior to that of α-toc, while compound **1** showed activity comparable to that of BHA. Interestingly, unlike in the case of curcuminoids,[14] the antioxidant activities of **1** and **2** were better than those of their corresponding hydrogenated derivatives **3** and **4**. The extraordinary efficacy of **5** in comparison to **1** – **4** seemed to originate from the additional phenolic hydroxy group present in the former. Besides increasing the radical scavenging ability, this group also made **5** a better iron chelator. This was evident from the results obtained in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and the iron chelation study.

Table 2 shows the DPPH radical scavenging activities of **1** – **5**, with α-toc as the positive control. The ginger compounds showed moderate scavenging activities compared to those of the control and **5**. In this assay, too, **5** showed radical scavenging

Table 2. *DPPH-scavenging activities of **1** – **5**.*

Compound	$IC_{0.200}$ [μM][a]
1	23.76 ± 0.77
2	42.6 ± 1.20
3	62.9 ± 0.97
4	91.66 ± 1.76
5	8.3 ± 0.55
α-toc	6.1 ± 0.46

[a] For definition of $IC_{0.200}$, see the Experimental Section.

potential far superior to that of **1**, the best candidate amongst the ginger constituents. In the chelation study, compound **1** was found to complex Fe^{2+}, since its absorbance at 363 nm (50 μM) gradually reduced with concentrations of Fe^{2+} increasing from 50 to 100 μM (data not shown). No further reduction in absorbance was observed on increasing the Fe^{2+} concentration beyond 100 μM; this suggests formation of a 1:2 complex between **1** and Fe^{2+}. In comparison, a similar experiment with **5** revealed the formation of a 1:4 complex. Thus, it was concluded that **5** had a better chelating action.

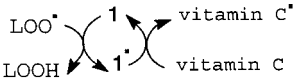
Although less potent than **5**, the low IC_{50} value for **1** against Fe^{+2}-mediated lipid peroxidation was impressive and indicative of a strong affinity for lipid peroxide radicals ($LOO^{\bullet}$). The anti-LPO activities of **1** and **5** were therefore assessed by an iron-independent technique, so as to offset the chelation factor. For this, peroxidation of rat liver mitochondria was carried out with *tert*-butylhydroperoxide (TBHP), an organic peroxide model widely used[15a,b] to induce oxidative stress in biomolecules. Table 3 shows the inhibitory effects of **1**, **4**, **5** and the positive control (α-toc), each at a concentration of 20 μM. It was observed that the protective activity of compound **1** (40% protection) was much better than that of α-toc (16% protection) and comparable to that of **5** (52% protection), while compound **4** gave least protection (10%). The results were in sharp contrast to those obtained in the iron-mediated peroxidation and DPPH-scaveng-

*Table 3. Protective activities of **1** and **5** against TBHP-induced lipid peroxidation.*

Compound	% Inhibition at 20 μM
1	39.16 ± 0.55
5	55.6 ± 1.50
4	16.23 ± 0.60
α-toc	10.3 ± 0.75

ing assay, in which **5** was found to be far superior than **1**. Thus, the LOO•-scavenging activity of **1** was almost 80% that of **5**. Considering that **5** has two radical scavenging phenolic sites, the above result substantiated our hypothesis that compound **1** should have a better binding affinity with lipid due to the higher hydrophobicity that arises from the alkyl chain.

The antioxidant/prooxidant activity of a test compound in iron-mediated lipid peroxidation in a biological system is critically governed by factors such as the concentration of endogenous reducing systems and/or redox couples. Hence, the true antioxidant activity of **1** was assessed by use of a well-defined, biologically relevant liposomal system, devoid of biological reductants such as ascorbate, NADPH, etc., and by carrying out the Fe^{2+}-mediated peroxidation at pH 5.0, at which value Fe^{2+} remains unoxidized,[16] for at least 3 h, during which considerable peroxidation took place. This study not only ensured minimization of the prooxidant effect of ascorbate, but also assisted in the quantitative comparison of the antioxidant activities of **1** and ascorbate, individually and in combination. Figure 2 shows the extent of liposomal peroxidation mediated by Fe^{2+} in the absence or presence of **1** and vitamin C, individually and in combination. The protection offered by vitamin C (15 μM) alone was considerably less than that offered by **1** even at lower concentrations (5 and 10 μM). More interestingly, the antioxidant activity of **1** increased drastically in the presence of vitamin C at increasing concentrations. These results strongly suggest that **1** scavenges the LOO• radical more quickly than vitamin C does, but the radical generated from **1** then reacts with vitamin C to regenerate **1**. This established a synergistic effect of vitamin C on the antioxidant activity of **1**, as shown schematically in Equation (1).



(1)

The above results were also confirmed by pulse radiolytic studies of the reaction between **1** and the trichloromethylperoxyl radical ($Cl_3COO•$). In spite of its higher reduction potential value (1.5 V)[17] compared to the physiologically relevant peroxides, the $Cl_3COO•$ radical is extensively used as a representative peroxyl radical because of the inherent simplicity in performing the experiments, and it was indeed used earlier to study a very important biophysical phenomenon, namely free radical interaction between vitamin E and vitamin C.[18] The radical can be generated in aerated water/isopropanol/acetone mixtures containing carbon tetrachloride.[18]

Figure 3 shows transient absorption spectra due to the reaction between $Cl_3COO•$ and **1** (1×10^{-4} M) obtained through pulse radiolysis at pH 9. The spectrum showed an absorption maximum at 360 nm attributable to a characteristic phenoxyl radical[19] and bleaching of the absorption at 430 nm for parent depletion. The reaction can be represented as in Equation (2), in

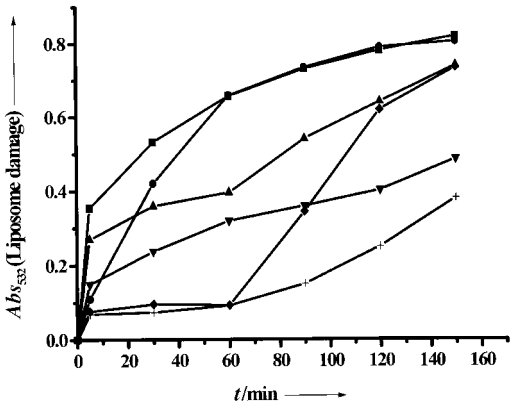


*Figure 2. Time-dependent inhibition of Fe^{2+}-induced liposomal peroxidation by **1**, vitamin C and their mixtures at pH 5 (40 mM tris(hydroxymethyl)aminomethane (Tris)-acetate buffer): ■ = without antioxidant, ● = with 15 μM vitamin C, ▲ = with 5 μM **1**, ▼ = with 10 μM **1**, ◆ = with 5 μM **1** and 15 μM vitamin C, + = with 10 μM **1** and 15 μM vitamin C. Values are mean ± standard error (n = 3).*

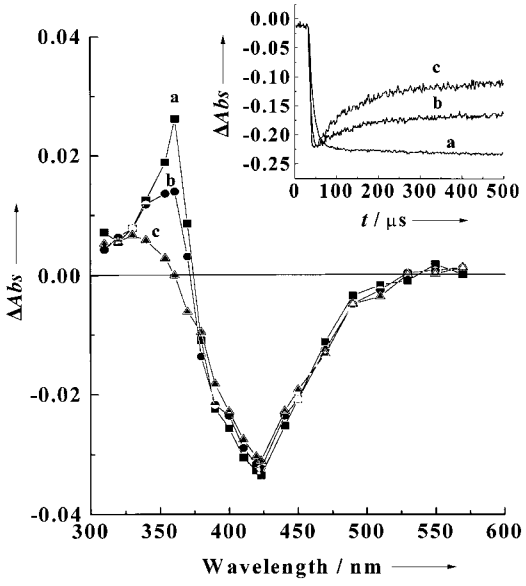
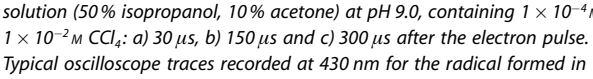


*Figure 3. Transient absorption spectra obtained from an air-saturated aqueous solution (50% isopropanol, 10% acetone) at pH 9.0, containing 1×10^{-4} M **1** and 1×10^{-2} M CCl_4: a) 30 μs, b) 150 μs and c) 300 μs after the electron pulse. Inset: Typical oscilloscope traces recorded at 430 nm for the radical formed in the reaction between 2×10^{-4} M **1** and $Cl_3COO•$ at pH 9: a) without vitamin C, b) with 5×10^{-6} M vitamin C and c) with 1.5×10^{-5} M vitamin C.*



(2)

which **1**• is a phenoxyl radical. The bimolecular rate constant for the formation of **1**• was $2 \times 10^9\ M^{-1} s^{-1}$, which is higher than that for the vitamin E radical in the same medium.[18] The decay of the absorption peak at 360 nm follows neither a clear first-order pathway nor a second-order one; this suggests that the phenoxyl radical has more than one channel for its decay.

The reaction between vitamin C and $Cl_3COO^•$ in the same medium produced the characteristic transient absorption peak at 360 nm.[20a–c] The bimolecular rate constant for its formation was calculated to be $1 \times 10^8\ M^{-1} s^{-1}$, which is much lower than that observed with **1** and in conformity with our results obtained with the liposomal system. Thus, the LOO•-scavenging potential of **1** was better than that of vitamin C even in homogeneous medium.

The regeneration of **1** by vitamin C was also studied by pulse radiolysis. Without vitamin C, the pattern of signal bleaching remained the same up to 500 μs, as evident in Figure 3 (inset). However, considerable bleaching recovery was observed even at a very low ascorbate concentrations and became higher with increasing concentrations of vitamin C. This also confirmed the repair of the radical **1**• by vitamin C.

The conclusion drawn from the pulse radiolysis experiment is based on the interaction between **1** and vitamin C in homogenous solution. In a real biological system, these two components would have to be located in separate phases, due to their different polarities, and so the above discussion appears to be somewhat inapplicable. However, similar results were also obtained in the liposomal peroxidation and substantiate our view that compound **1** is a LOO• scavenger superior to vitamin C. The liposome has been used previously as a biologically relevant system for studying the interaction between vitamins E and C.[16, 21]

In principle, **1** can exert its antioxidant action by conventional electron donation through the phenolic group and hydrogen donation from the active methylene group of its β-diketone moiety. Indeed, the latter possibility, proposed earlier for curcumin and its tetrahydro derivative,[22a] has recently been advocated strongly.[22b,c] However, the hydrogen-transfer mechanism has been disputed by Ross et al.[23] The antioxidative process is believed to proceed through two stages, as shown in Equations (3) and (4).

Radical Trapping

$$\mathbf{R}^• + \mathbf{AH} \longrightarrow \mathbf{RH} + \mathbf{A}^• \quad \text{where AH is the antioxidant} \qquad (3)$$

Radical Termination

$$\mathbf{A}^• \longrightarrow \mathbf{Nonradical\ Product} \qquad (4)$$

Most studies on antioxidant action are primarily focussed on the trapping stage, through use of a kinetic approach. However, the radical **A**• would definitely be converted into a nonradical product. Study of this would offer important mechanistic information, and could thereby settle the above controversy about the antioxidant action of phenolic β-diketones such as **1**.

For this purpose, **1** was treated with $Cl_3COO^•$ and the products formed were analysed by HPLC. At the outset, we anticipated formation of vanillin (**6**, $\lambda_{max} = 314$ nm) and ferulic acid (**7**, $\lambda_{max} = 310$ nm) from the reaction between **1** ($\lambda_{max} = 362$ nm) and the prototype peroxyl radical ($Cl_3COO^•$). The product peaks were therefore detected at 310 nm in the HPLC analysis. Best peak resolution for **1** ($R_t = 8.9$ min), **6** ($R_t = 1.51$ min) and **7** ($R_t = 0.69$ min) was observed with acetonitrile/water (60:40) as the eluent. The HPLC profile showed the formation of four products (Table 4), two of which were identified as **6** and **7** by comparison with authentic samples. The dose-dependent accumulation of **6** and **7** over a period of 1 h, in the presence and absence of vitamin C, is shown in Figure 4. This revealed that both products were formed, irrespective of the dose of irradiation, and that their accumulation increased steeply up to 15 min irradiation. Thereafter, although production of **7** continued, the concentration of **6** remained steady up to 30 min and subsequently started to reduce marginally. At all dose levels, however, **7** was produced in higher amounts than **6**. Surprisingly, the presence of vitamin C reduced the concentration of **7** drastically, while affecting the concentration of **6** to a much lesser extent.

Table 4. *HPLC profile of reaction products.*

Peak no.	Elution time [min]	Compound
1	0.69	ferulic acid (**7**)
2	1.51	vanillin (**6**)
3	2.15	–
4	2.95	–
5	8.9	[6]-dehydrogingerdione (**1**)

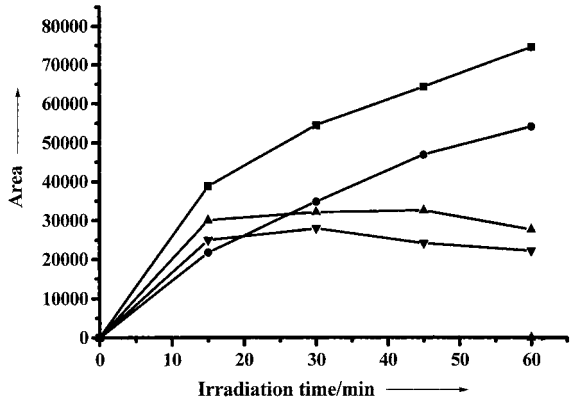
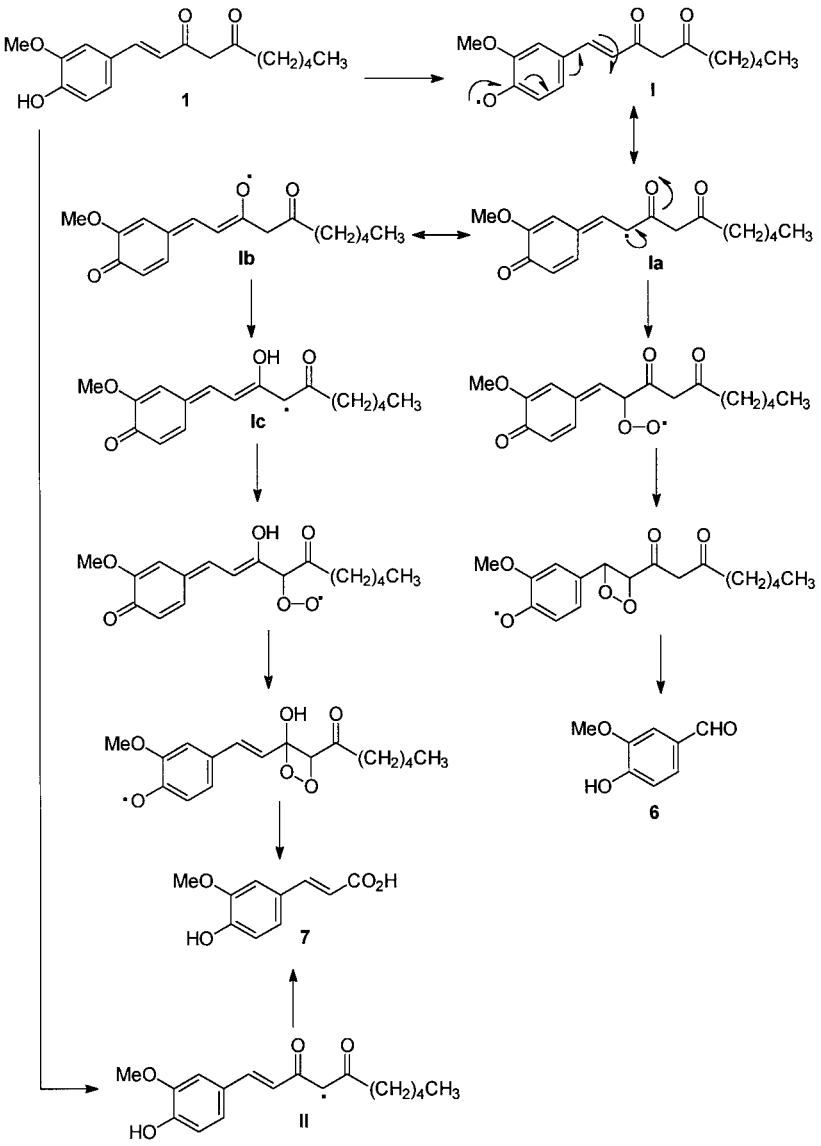


Figure 4. *Dose-dependent formation of **6** and **7** by the reaction between $Cl_3COO^•$ and **1** (300 μM) in the absence or presence of vitamin C (150 μM). ▲ = accumulation of **6** and ■ = accumulation of **7** in the absence of vitamin C; ▼ = accumulation of **6** and ● = accumulation of **7** in the presence of vitamin C. Each peak area was calculated from the 310 nm peak for **6** and **7**.*

Mechanistically, the formation of **6** and **7** from **1** can be explained schematically (Scheme 2). As indicated earlier, compound **1** can generate the phenoxyl radical (**I**) and/or the β-diketone methylene radical (**II**). The radical **I** has two more canonical forms, **Ia** and **Ib**, the latter subsequently furnishing



Scheme 2. *Proposed mechanism for the formation of **6** and **7** from the reaction between $Cl_3COO^{\bullet}$ and **1**.*

another radical intermediate (**Ic**). The canonical form **Ia**, being a carbon-centred radical, reacts very rapidly with molecular oxygen to produce the corresponding peroxide, which can furnish **6** by cyclization at its adjacent position and subsequent decomposition. Likewise, the carbon-centred intermediate **Ic** furnishes **7** by the corresponding sequence of reactions. As formation of **Ic** from **Ib** involves C–H bond breakage, its concentration should be less than, or at best equal to, that of **Ia**, which is merely a canonical form of **I**. In conformity with this logic, formation of equal amounts of similar intermediates has also been reported in the reaction between curcumin and azobisisobutyronitrile (AIBN).[24] Thus, the greater build-up of **7** than of **6** can not be explained by considering the intermediacy of **Ic** alone. A rational alternative pathway for the generation of **7** would be via the radical **II**. Hence, it is tempting to suggest that the methylene radical **II** is also produced during the reaction between **1** and $Cl_3COO^{\bullet}$ and accounts for the excess accumulation of **7** in the product mixture. This is in keeping with the recent report[22b] of the formation of a methylenic radical transient (absorption maximum at 490 nm) in the $CH_3^{\bullet}$ radical induced oxidation of curcumin. In view of the low reactivity of $Cl_3COO^{\bullet}$, we anticipated a smaller yield and hence a weaker absorption due to the methylenic radical in the pulse radiolysis experiment. This, coupled with the span of the bleaching spectrum (Figure 3) in the 420 – 500 nm region, probably masked the weak absorption of the 1,3-diketomethylenic radical.

In the presence of ascorbate, a portion of the phenoxyl radical **I** is repaired by hydrogen donation, which thus reduces the concentration of **6** produced in the subsequent steps. The canonical form **Ia**, responsible for producing **6**, is a carbon-centred radical and hence reacts very rapidly with oxygen. Vitamin C might not have sufficient time to repair **I** and, consequently, the reduction in the concentration of **6** is small. In contrast, formation of the intermediate **Ic**, responsible for producing **7**, must be relatively slow as it involves a bond-breakage (hydrogen-transfer) process. Hence, its progenitor **I** or its canonical form **Ib**, which are oxygen-centred radicals, are repaired by ascorbate, and the concentration of **7** is thereby significantly reduced. In a separate pulse radiolysis experiment, we found that the non-phenolic 1,3-diketo compound, $PhCOCH_2COPh$ reacts with $Cl_3COO^{\bullet}$ to furnish the diketomethylene radical ($\lambda_{max} = 460$ nm). The carbon-centred radical was not repaired by vitamin C (data not shown). In the light of this information, none of the carbon-centred radicals (canonical form **Ia** or intermediates **II** and **Ic**) involved in the present scheme appeared to have been repaired by vitamin C.

The above inferences are in line with the molecular model recently proposed[22c] to explain the antioxidant action of **5**. Indeed, the synergism between **1** and the water-soluble antioxidant vitamin C in the liposomal system can also be explained by the above model. It seems that the lipophilic radical **I** produced during the antioxidant action of **1** positions itself at the water-membrane interface. Subsequently, because of its high polarity and strong oxidizing capability, the oxygen-centred radical (**I** or its canonical form **Ib**) is repaired by vitamin C.]

In conclusion, the results of the experiments established **1** as a stronger antioxidant than vitamins C and E, with a high affinity particularly for the lipid peroxide radical. The antioxidant activity is enhanced in the presence of even small amounts of vitamin C, which repairs the phenoxyl radical generated by oxidation of **1**. Furthermore, the products generated by oxidation of **1** are also well known antioxidants, which should be beneficial in biological systems. Analysis of the oxidative products of **1** strongly suggested the involvement of both phenoxyl and 1,3-diketone moieties in the antioxidant action of **1**.

Experimental Section

Materials: Compounds **1** – **4** were isolated from ginger as described earlier.[7] For assaying the antioxidant activities, compounds **1** – **4** were dissolved in 10% aqueous ethanol, while **5** was taken up in 2% aqueous dimethylsulfoxide (DMSO); the results presented are measured against appropriate blanks. BHA, DPPH, Tris-acetate and Tris-HCl, α-tocopherol and L-α-phosphotidylcholine (L-α-lecithin) from dried egg yolk, vanillin and ferulic acid were purchased from Sigma (St.Louis, MO, USA). Vitamin C, ferrous ammonium sulfate, 2-thiobarbituric acid (all from Himedia Lab., India), trichloroacetic acid (TCA; from Thomas Baker, India) and TBHP (70%; from Lancaster, UK) were used.

Lipid (rat brain homogenate) peroxidation: Rat brain homogenate, prepared from the brains of freshly killed Wistar rats, was subjected to Fe^{2+}-induced lipid peroxidation as described previously,[25] with minor modifications. Briefly, the total reaction mixture (1.0 mL) contained Tris-HCl buffer (125 mM; pH 7.4) and brain homogenate (0.5 mg of protein per mL), with or without test compounds. The reaction was triggered by addition of ferrous ammonium sulfate (20 μM) and vitamin C (200 μM) and incubation at 37 °C for 30 min. The reaction was terminated by addition of TBA-TCA-HCl solution (2 mL; 0.37% TBA, 2.8% TCA, 0.25 M HCl) and boiling of the mixture at 100 °C for 10 min. The extent of lipid peroxidation was measured spectrophotometrically by recording the absorbance at 532 nm.

Iron chelation study: Iron chelation was determined by recording the absorption spectra of the test compounds (50 μM) from 190 – 600 nm, five minutes after addition of ferrous ammonium sulfate (0 – 350 μM). The chelation capacity of individual compounds was evaluated from the change and/or spectral shift of the absorbance.

DPPH assay: The DPPH-scavenging assay was carried out[26] by monitoring the absorbance of an ethanolic solution of DPPH (100 μM) at 517 nm in the presence and absence of the test compounds. The concentration ($IC_{0.200}$) of the test compounds at which absorbance decreased by 0.200 of a unit during a 30 min observation was taken as the free radical scavenging potency.

TBHP-induced mitochondrial lipid peroxidation: For the preparation of mitochondria, livers of male Wistar rats (250 ± 20 g) were excised and homogenized in a sucrose solution (0.25 M) containing ethylenediaminetetraacetate (EDTA; 1 mM). The homogenate was centrifuged at 3000 × *g* for 10 min to remove the cell debris and nuclear fraction and the supernatant was centrifuged three times at 10000 × *g* for 10 min. The sedimental mitochondria pallet was washed three times with potassium phosphate buffer (pH 7.4; 0.05 M) to remove sucrose. The protein content was estimated by Lowry's method and the pellets were resuspended in the phosphate buffer at a final concentration of 20 mg mL^{-1}. The mitochondrial lipid peroxidation was initiated by addition of TBHP (final concentration: 1 mM) to the reaction mixture, which also contained the following components at the final concentration stated: mitochondrial fraction (4.0 mg of protein per mL) and test compounds (20 μM) or vehicle (0.1% ethanol or 0.25% DMSO) in potassium phosphate buffer (pH 7.4; 100 mM). The mixture was incubated at 37 °C for 20 minutes, TCA-TBA-HCl solution was added and the absorbance at 532 nm was monitored as described previously.

Iron(II)-mediated liposomal peroxidation: Fe^{2+}-induced oxidation of the liposome was carried out by the reported procedure.[16] In brief, a solution of phosphotidylcholine (32 mg) in chloroform (0.5 mL) was concentrated with N_2 purging; the residue was dissolved in cold water, vortexed for 1 min and stored at 4 °C for 1 h. The solution was then sonicated for 2 min, and peroxidation of the liposome was carried out within 24 h of preparation. Peroxidation of the reaction mixture (0.5 mL) containing the liposome (0.24 mg) in Tris-acetate buffer (pH 5.0; 40 mM) was carried out by initiation with ferrous ammonium sulfate (10 μM), incubation for 0 – 120 mins and subsequent termination with HCl (0.5 mL, 0.25 M) and TBA (0.5 mL, 1% solution in 50 mM NaOH). The solution was heated at 100 °C for 15 min, and the TBARS thus formed were extracted with *n*BuOH (1 mL) and their absorbance at 532 nm was recorded. The experiments were carried out in the absence or presence of **1** (5 – 10 μM), or ascorbate (15 μM) or a combination of both.

Pulse radiolysis: Aqueous solutions were prepared in nanopure water (conductivity < 0.06 μs) from a Barnstead nanopure cartridge filtration system. An alkaline pH value was obtained by addition of NaOH only. The pulse radiolysis system, using pulses of 7 MeV electrons, has been described previously.[27] The width of the pulse was selected at 50 ns and the dose was 12 Gy per pulse. The kinetic spectrophotometric system covered the wavelength range 250 – 800 nm. The dosimetry was carried out by using an air-saturated aqueous solution containing 0.05 M KSCN ($G\varepsilon = 21522\ M^{-1}cm^{-1}$ per 100 eV for $(SCN)_2^{\bullet -}$ at 500 nm).[28] The bimolecular rate constants for the reaction between **1** and the $Cl_3COO^{\bullet}$ radicals were calculated from the slope of the plot of the pseudo first order build-up rate constant of the transient species at 360 nm and bleaching at 420 nm against the concentration of **1**. The concentration range used for the measurement of rate constants was 0.1 – 0.4 mM. The uncertainty in the rate constant measurement is ± 10%.

Product analysis for the reaction between 1 and $Cl_3COO^{\bullet}$: Reaction mixtures (each 1 mL) containing *tert*-BuOH (3.5 M), CCl_4 (30 mM) and **1** (300 μM) in the presence or absence of ascorbate (150 μM) were taken in different tubes. These were subjected to γ radiolysis by irradiating for 0, 15, 30, 45 and 60 min, by using a γ chamber with a ^{60}Co source (dose rate: 570 Gy h^{-1}). Product analysis for the reaction was carried out with a Bruker HPLC instrument under the following conditions: RP-18 column (Merck, Germany); eluent: acetonitrile/water (6:4), flow rate: 1 mL min^{-1}; detection wavelength: 310 nm, injection volume: 20 μL. Authentic samples of ferulic acid and vanillin were also analysed under the same conditions, and the peak areas were calculated by use of Bruker HPLC software.

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