Evaluation of the Antioxidant and Prooxidant Actions of Gallic Acid and Its Derivatives

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The antioxidant and prooxidant activities of gallic acid and its propyl, methyl, and (where solubility allowed) lauryl esters were investigated. Gallic acid (GA), propyl gallate (PG), and gallic acid methyl ester (GM) were able to scavenge hypochlorous acid at a rate sufficient to protect α-1-antiproteinase against inactivation by this molecule. When dissolved in ethanol, gallic acid lauryl ester (GL), PG, and GM decreased the peroxidation of ox brain phospholipids. GA had only a weak inhibitory effect. GM, GL, and PG reacted with $CCl_3O_2^\bullet$ (trichloromethyl peroxyl radical) with rate constants of 1.23×10^7, 2.33×10^7, and 1.67×10^7 M^{-1} s^{-1}, respectively. Gallic acid was much less reactive with a rate constant of 4.47×10^5 M^{-1} s^{-1}. By contrast to these antioxidant properties, GA, GM, and PG accelerated damage to the sugar deoxyribose in the presence of ferric–EDTA and H_2O_2. GA also accelerated DNA damage by a ferric–bleomycin system. GM was less effective but GL had no effect. Reaction of NBT and cytochrome c by $O_2^{\bullet-}$ was only slightly inhibited by PG, GM, and GA, indicating that their rates of reaction with $O_2^{\bullet-}$ are low. Our data confirm the antioxidant actions of gallic acid lauryl, propyl, and methyl esters. However, they also show that both the prooxidant and antioxidant actions of "proposed antioxidants" should be fully characterized.

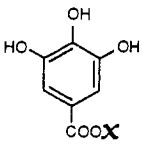
INTRODUCTION

The role of free radical reactions in food preservation has continued to receive attention in recent years (for recent reviews, see Aruoma and Halliwell, 1991; St. Angelo, 1992; Aruoma, 1993a). The term "antioxidant" is often implicitly restricted to compounds acting as chain breaking inhibitors of lipid peroxidation. The free radical reaction of peroxidation is often a major problem for food manufacturers whose interests include maintenance of both nutritional qualities and shelf life of lipid containing foods (reviewed by Löliger, 1991).

Control of nonenzymic lipid peroxidation is often achieved by adding lipid soluble antioxidants to food during processing (Hudson, 1991; Ericksson, 1987; Loliger, 1991). These antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate and "natural" antioxidants such as vitamin E, rosemary extracts, and flavonoids. However, there are some concerns about the adverse effects of synthetic antioxidant food additives (Stich, 1991; Ito et al., 1985, 1986; Hudson, 1990; Kirkpatrick and Lauer, 1986; Inatani et al., 1983; St. Angelo, 1992; Wurtzen, 1990; Aruoma et al., 1992; Kahl and Hildebrandt, 1986; Kahl, 1991).

Gallic acid, GA (3,4,5-trihydroxybenzoic acid), often obtained by alkaline or acid hydrolysis of tannins or from hydrolysis of spent broths from *Penicillium glaucum* or *Aspergillus niger*, is used in the manufacturing of gallic acid methyl ester (GM), gallic acid lauryl ester (GL), and propyl gallate (PG) (see Figure 1) which are widely used food antioxidant additives. Thus, gallic acid and its derivatives are often present in the diet.

Recent studies have shown that several phenolic food antioxidant additives can accelerate oxidative damage *in vitro*, to DNA, proteins, and carbohydrates, despite their antioxidant actions toward lipids (Laughton et al., 1989; Aruoma et al., 1990, 1992). Indeed, many natural products are mutagenic in some tests *in vitro* (Pueyo and Ariza, 1993). It is thus important to characterize the effects of antioxidants upon all biological molecules not just lipids (Halliwell, 1990; Aruoma, 1993b,c). In the present paper, we have characterized in detail the antioxidant (protection against reactive oxygen species, ROS) and the prooxidant effects of GA, GM, GL, and PG, using established assays (Halliwell, 1990; Aruoma et al., 1992).



X = H	Gallic acid
X = CH_3	Gallic acid methyl ester
X = $CH_2CH_2CH_3$	Gallic acid propyl ester (Propyl gallate)
X = $CH_2(CH_2)_9CH_3$	Gallic acid lauryl ester

Figure 1. Structure of compounds used in this study.

MATERIALS AND METHODS

Chemicals were of the highest quality available and were purchased from Sigma Chemical Co. (Poole, Dorset) or from BDH Chemical Co. (Gillingham, Dorset).

Reactions with Hypochlorous Acid. Reaction with hypochlorous acid (HOCl) was studied using the elastase assay essentially as described in Wasil et al. (1987) but with some modifications (Aruoma et al., 1992). For the assay, 75 μM HOCl (produced immediately before use by adjusting NaOCl to pH 6.2 with dilute H_2SO_4) and the compounds to be tested were incubated in a final volume of 1.0 mL in phosphate-buffered saline, pH 7.4, containing 140 mM NaCl, 2.7 mM KCl, 16 mM Na_2HPO_4, and 2.9 mM KH_2PO_4. To the reaction mixture was

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added α_1-antiproteinase (Sigma type A9024) (4 mg/mL). This allows any HOCl remaining to inactivate α_1AP. After 20 min of further incubation, 0.05 mL of 5 mg/mL elastase (Sigma type E0258) was added. Any HOCl remaining is diluted out to the point at which it cannot affect elastase, by addition of 2 mL of phosphate buffered saline. This mixture was allowed to stand a further 30 min to allow any α_1AP still active to inhibit elastase. The elastase activity remaining was measured by adding elastase substrate (5 mg/mL, *N*-succinyltriala-*p*-nitroanilide) and monitoring increases in absorbance at 410 nm.

Peroxidation of Phospholipid Liposomes. The ability of compounds to inhibit lipid peroxidation at pH 7.4 was tested using ox brain phospholipid liposomes essentially as described in Quinlan et al. (1988) and in Aruoma et al. (1992). The experiments were conducted in a physiological saline buffer (3.4 mM Na_2HPO_4–NaH_2PO_4, 0.15 M NaCl), pH 7.4. Assay mixtures contained in a final volume of 1 mL were made up with PBS, 0.5 mg/mL phospholipid liposomes, 100 μM $FeCl_3$, varying concentrations of the compounds dissolved either in water or in ethanol (ethanol does not affect the outcome of the lipid peroxidation assay), and 100 μM ascorbate (added last to start the reaction). Incubations were at 37 °C for 60 min. At the end of this incubation period, 0.1 mL of 2% (w/v) butylated hydroxytoluene (BHT) was added to each mixture followed by addition of 1 mL each of 1% (w/v) thiobarbituric acid (TBA) and 2.8% (w/v) trichloroacetic acid. The solutions were heated in a water bath at 80 °C for 20 min to develop the $(MDA)_2$–TBA adduct. Addition of BHT to the reaction mixtures minimizes erroneous increases in color due to iron ion-dependent hydroperoxide decomposition during the acid heating stage. The $(TBA)_2$–MDA chromogen was extracted into 2 mL of butan-1-ol and the extent of peroxidation measured, in the organic layer as absorbance at 532 nm.

Reactions with Trichloromethyl Peroxyl Radicals. Reaction with trichloromethyl peroxyl radical was conducted using the Linear Accelerator Facility at the Paterson Institute, Christie Hospital, Manchester, U.K. Reaction mixtures contained 1% v/v CCl_4 and 50% v/v isopropyl alcohol in 10 mM KH_2PO_4–KOH pH 7.4 and 0.05% w/v of the compounds studied. The absolute rate constants were calculated from the reaction kinetics (see for example, Packer et al., 1978; Monig et al., 1985).

Reaction with Superoxide Radical. Generation of $O_2^{\bullet-}$ by the hypoxanthine–xanthine oxidase system (McCord and Fridovich, 1969) was conducted essentially as described in Halliwell (1985) and in Aruoma et al. (1992). Reaction mixtures contained, in a final volume of 3 mL, 0.1 mL of 30 mM hypoxanthine (dissolved in minimum potassium hydroxide solution), 0.1 mL of 0.3 mM EDTA, either 0.1 mL of 3 mM cytochrome *c* or 0.1 mL of 3 mM nitroblue tetrazolium, and 88 mM (final concentration) KH_2PO_4–KOH buffer (pH 7.4). In each case, the reaction was started by adding 0.3 mL of xanthine oxidase (Sigma X1875, freshly diluted in the above phosphate buffer to give one unit of enzyme activity per milliliter) and the rate of cytochrome *c* or NBT reduction measured at 550 and 560 nm, respectively, at 25 °C. In separate experiments, the effects of 0.2 mM of the compounds were tested in the systems containing either cytochrome or nitroblue tetrazolium.

Bleomycin-Dependent DNA Damage. The bleomycin assay (Gutteridge et al., 1981) was assayed using a modified method described in Aruoma (1993b) and in Smith et al. (1992). Reaction mixtures contained, in a final volume of 1.0 mL, the following reagents at the final concentrations stated: DNA (0.2 mg/mL), bleomycin (0.05 mg/mL), $FeCl_3$ (0.025 mM), $MgCl_2$ (5 mM), KH_2PO_4–KOH buffer pH 7.0 (30 mM), and ascorbate (where used 0.24 mM) *or* the compounds tested at varying concentrations. Compounds were either dissolved in water with addition of minimum NaOH to ensure solubilization or dissolved in ethanol. Control experiments showed that ethanol itself has no effect on the bleomycin assay. Reaction mixtures were incubated at 37 °C for 1 h. At the end of the incubation period, 0.1 mL of 0.1 M EDTA was added to stop the reaction (the iron–EDTA complex is unreactive in the bleomycin assay). The coloration was obtained by adding 1 mL of 1% (w/v) TBA and 1 mL of 25% (v/v) HCl followed by heating in a water-bath maintained at 80 °C for 15 min. The chromogen formed was extracted into butan-1-ol and the absorbance was measured at 532 nm.

Table I

additions to reaction mixture	elastase activity ΔA_{410}, min^{-1}
elastase only	0.520
+ α_1AP	0.003
+ HOCl	0.520
+ α_1AP and HOCl	0.380
α_1AP, HOCl, gallic acid[a]	
0.2 mM	0.000
0.4 mM	0.0009
0.8 mM	0.0001
0.16 mM	0.0005
α_1AP, HOCl, gallic acid methyl ester	
0.2 mM	0.0010
0.4 mM	0.0003
0.8 mM	0.0002
0.16 mM	0.0004
α_1AP, HOCl, propyl gallate	
0.2 mM	0.0858
0.4 mM	0.0014
0.8 mM	0.0005
0.16 mM	0.0004

[a] The compounds at the concentrations stated were first incubated with HOCl, before adding α_1AP and elastase as described under Materials and Methods. For this set of experiments, the compounds were dissolved in double-distilled water. Lauryl ester could not therefore be tested here.

Deoxyribose Assay To Assess Prooxidant Action. The deoxyribose assay is a simple method which allows determination of the rate constant for reaction between antioxidants and hydroxyl radicals (Halliwell et al., 1987). This assay has also been adapted to assess prooxidant actions (Laughton et al., 1989, Aruoma et al., 1990, 1992). The assay was conducted as described in Aruoma (1993b). Details of the reaction mixture components are given in the table legends.

RESULTS AND DISCUSSION

In foods, not only lipids but also DNA, proteins, and carbohydrates can be damaged by reactive oxygen species (ROS). The ROS often encountered in biological systems include the radicals hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), and peroxyl ($RO_2^{\bullet}$) and the nonradicals, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). HOCl is a major active constituent of chlorine-based bleaches often used to disinfect equipment with which food will come into contact. The ability of gallic acid and its esters to protect against or aggravate oxidative damage was evaluated.

Scavenging of Hypochlorous Acid. The protein α_1-antiproteinase (α_1AP) is the major inhibitor of serine proteases in human body fluids and is highly sensitive to inactivation by HOCl. For example, α_1AP inhibits the hydrolytic enzyme elastase. When the α_1AP is inactivated by hypochlorous acid, it loses its ability to inhibit elastase (Clark et al., 1981). Thus the ability of a compound to protect α_1-AP against inactivation by HOCl has been adopted as a method for assessing the antioxidant action of such compounds toward HOCl (Wasil et al., 1987; Aruoma et al., 1992).

Table I shows that incubation of HOCl (78 μM) with varying concentrations of gallic acid prior to addition to α_1AP resulted in protection of the α_1AP activity. Gallic acid methyl ester (GM) and propyl gallate (PG) were also effective. Solubility problems precluded the testing of gallic acid lauryl ester since it has limited solubility in water. The effect of increasing concentrations of HOCl on the spectrum of the compounds is shown in Figure 2. It is clear for a 0.32 mM solution of GA, GM, and PG that concentrations of HOCl up to 0.5 mM diminished significantly the λ_{max} at 270 nm (Figure 2A–C). None of the compounds tested interfered with the ability of α_1AP to

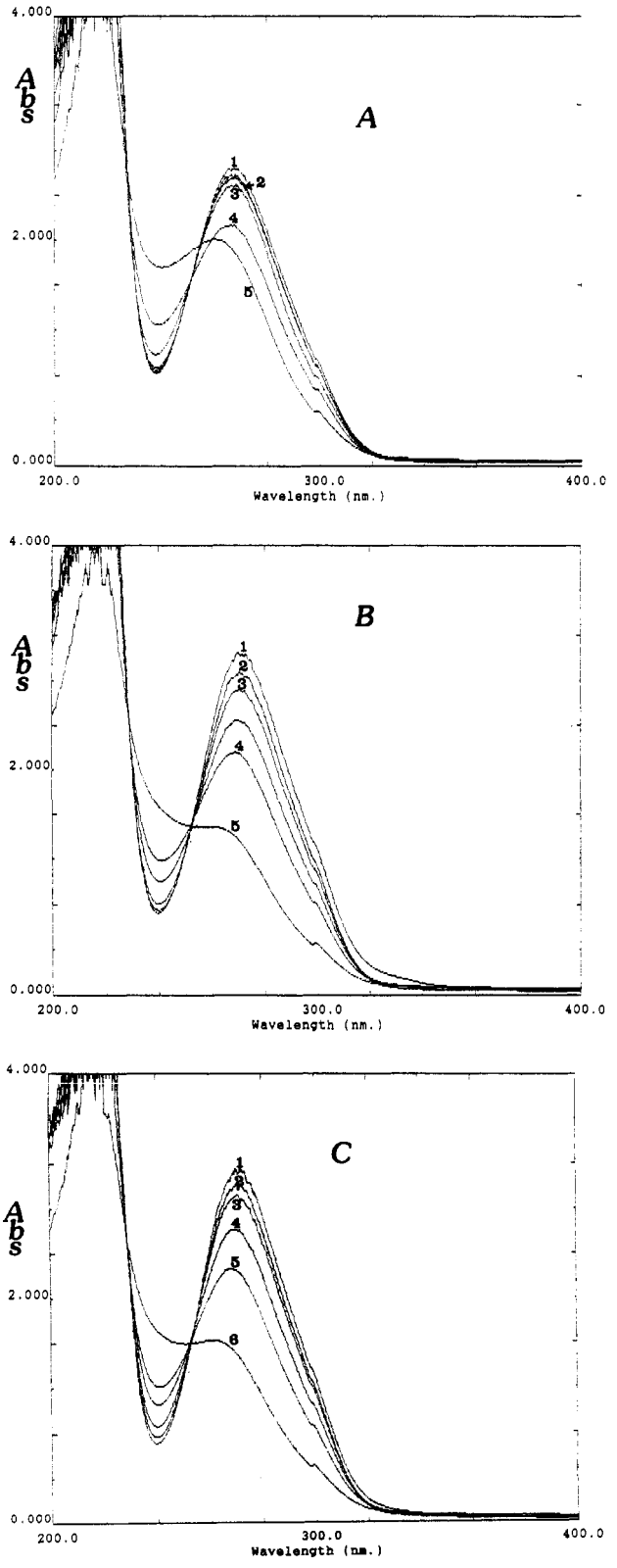

Figure 2. Effects of increasing concentrations of HOCl on the spectrum of gallic acid and its derivatives. GA, GM, and PG were at a final concentration of 0.32 mM in a final volume of 3 mL containing phosphate-buffered saline as described in the Materials and Methods section. Line 1 contained no HOCl; line 2, 0.05 mM HOCl; line 3, 0.10 mM HOCl; line 4, 0.2 mM HOCl; line 5, 0.3 mM HOCl; and line 6 contained 0.5 mM HOCl. Graphs A, gallic acid; B, gallic acid methyl ester; and C, propyl gallate. The instrument used was a Shimadzu UV-2101PC scanning spectrophotometer.

inhibit elastase nor did they inhibit elastase directly under our assay conditions.

Table II. Inhibition of Peroxidation in the Liposomal System Using Ox Brain Phospholipids[a]

compds at the respective concn tested, μM	extent of peroxidation A_{532}			
	compd dissolved in aq medium	% inhibition	compd dissolved in ethanol	% inhibition
none	1.84		2.13	
GA				
5	1.85	0	NT	
12	1.85	0	1.94	9
25	1.80	2	NT	
50	1.52	17	1.19	44
GL				
5	1.88	NT	NT	
12	1.88	NT	0.61	71
25	1.79	NT	NT	
50	1.73	NT	0.51	76
GM				
5	1.23	33	NT	
12	0.96	48	0.92	57
25	0.89	52	NT	
50	0.59	68	0.74	65
PG				
5	1.58	14	NT	
12	0.90	51	0.52	76
25	0.51	72	NT	
50	0.47	74	0.40	81

[a] The experiments were conducted as described under Materials and Methods. Compounds were either dissolved in ethanol or in aqueous medium and tested at the final concentrations shown. The values are the means from triplicate experiments, in which absorbances varied by no more than 5%. NT = not tested in the assay system. Limited concentrations of the compounds (12 and 50 μM) were used to test the effect of using ethanol as the dissolution medium. GL was not tested in aqueous solution because of its limited solubility.

Scavenging of HOCl by the active components of rosemary extract has been reported (Aruoma et al., 1992). The present data suggest that food antioxidants containing phenolic functional groups could provide protection against HOCl, which is produced *in vivo* by the neutrophil derived enzyme myeloperoxidase at sites of inflammation (Weiss, 1989) and is used in many bleaches.

Inhibition of Phospholipid Liposome Peroxidation and Scavenging of Trichloromethyl Peroxyl Radicals. Ox-brain phospholipid liposomes undergo rapid nonenzymic peroxidation when incubated in the presence of $FeCl_3$ and ascorbic acid. The data in Table II show that GM and PG markedly decreased the peroxidation of ox-brain phospholipids when the compounds were dissolved in ethanol or in aqueous medium. GA was much less effective.

Peroxyl radicals are important intermediates in lipid peroxidation. The trichloromethyl peroxyl radical, $CCl_3O_2^{\bullet}$, is a reactive organic radical frequently used in studies to assess the ability of a compound to react with peroxyl radicals (Packer et al., 1978; Monig et al., 1985; Lal et al., 1988; Aruoma et al., 1992). $CCl_3O_2^{\bullet}$ was generated by radiolysis of an aqueous mixture of propan-2-ol and CCl_4:

$$e^-aq + CCl_4 \rightarrow {}^{\bullet}CCl_3 + Cl^- \quad (1)$$

$$OH^{\bullet} + CH_3CHOHCH_3 \rightarrow H_2O + CH_3{}^{\bullet}COHCH_3 \quad (2)$$

$$CH_3{}^{\bullet}COHCH_3 + CCl_4 \rightarrow CH_3COCH_3 + H^+ + {}^{\bullet}CCl_3 \quad (3)$$

$${}^{\bullet}CCl_3 + O_2 \rightarrow CCl_3O_2^{\bullet} \quad (4)$$

The abilities of gallic acid and its derivatives to react with $CCl_3O_2^{\bullet}$ are shown in Table III. GM, GL, and PG reacted rapidly with $CCl_3O_2^{\bullet}$. The values for Trolox C, a water soluble analogue of vitamin E, carnosic acid (Aruoma et al., 1992), and quercetin, a naturally occurring lipid antioxidant, are given for comparison. The low rate constant for $CCl_3O_2^{\bullet}$ scavenging by gallic acid is consistent

Table III. Reactions with Trichloromethyl Peroxyl Radicals[a]

compds	rate constants of reaction k_2 $CCl_3O_2^\bullet$, M^{-1} s^{-1}
gallic acid	4.47×10^5
gallic acid lauryl ester	2.33×10^7
gallic acid methyl ester	1.23×10^7
propyl gallate	1.67×10^7
Trolox C	2.23×10^8
carnosol	$1\text{–}3 \times 10^6$
carnosic acid	2.7×10^7
quercetin	3.9×10^7

[a] The rate constants quoted for GA, GL, GM, and quercetin are averages of two independent measurements which varied by less than 5%. Values for propyl gallate and Trolox C were abstracted from Aruoma (1993a) and those for carnosol and carnosic acid from Aruoma et al. (1992).

Table IV. Reaction of Gallic Acid and Its Derivatives with Superoxide Radical[a]

systems and compds tested	calcd rates and % inhibition of reduction of cytochrome c and nitroblue tetrazolium	
	ΔA, s^{-1}	% inhibition
Cytochrome c System		
HX, XO	15.8×10^{-3}	
+ GA	17.08×10^{-3}	0
+ GM	10.08×10^{-3}	36
+ PG	11.72×10^{-3}	26
+ SOD	1.4×10^{-3}	91
Nitroblue Tetrazolium System		
HX, XP	1.56×10^{-3}	
+ GA	7.4×10^{-4}	53
+ GM	5.76×10^{-4}	63
+ PG	9.05×10^{-4}	42
+ SOD	0	100

[a] Experiments were performed using the cytochrome c and NBT assays as described under Materials and Methods. Representative data are shown. The compounds were tested in the respective assay systems at the final concentrations of 0.2 mM. Superoxide dismutase was used at a final concentration of 500 units/mL. GL increased the baseline in each of the systems. This compound was not tested in line with the other compounds hence the omission of the data.

with the report of Cholbi et al. (1991) that gallic acid has no inhibitory effect on CCl_4-induced microsomal lipid peroxidation.

Reaction with Superoxide. A mixture of hypoxanthine and xanthine oxidase at pH 7.4 generates $O_2^{\bullet-}$ which can be measured by its ability to reduce ferricytochrome c to ferrocytochrome c, measured as a rise in absorbance at 550 nm (McCord and Fridovich, 1969), or by the reduction of nitroblue tetrazolium to formazan, measured at 560 nm. This assay has been adopted to assess the ability of antioxidants to react with $O_2^{\bullet-}$ (Halliwell, 1985; Aruoma, 1993b).

The compounds were tested and dissolved in either water or ethanol, which does not normally interfere with the assay system (Aruoma et al., 1992). Some problems were encountered in this assay with GL because of precipitation out of solution, and so it was not tested. GM, GA, and PG alone did not reduce NBT directly. However, all three decreased the reduction of NBT by $O_2^{\bullet-}$ (Table IV). The measured rates of reaction (measured as increases in absorbance at 560 nm) were significantly less than the control rate (Table IV). Superoxide dismutase completely inhibited the reduction of NBT by $O_2^{\bullet-}$. Table IV also shows the reaction of $O_2^{\bullet-}$ with cytochrome c and the effects of the compounds. PG, GM, and GA did not reduce cytochrome c directly. Again PG and GM decreased cytochrome c reduction by $O_2^{\bullet-}$. Inhibition of NBT reduction was generally greater than that of cytochrome c reduction. This is because $O_2^{\bullet-}$ reacts much faster with cytochrome c than it does with NBT, so a given concentration of added $O_2^{\bullet-}$ scavenger competes less efficiently in the cytochrome c system and exerts less inhibition (Halliwell, 1985). However, the fact that 0.2 mM concentrations were needed to produce significant inhibition even in the NBT system shows that the rate constant for reaction with $O_2^{\bullet-}$ is $<10^5$ M^{-1} s^{-1} (Halliwell, 1985).

Table V. Deoxyribose Damage in the Presence of Gallic Acid and Its Derivatives[a]

additions to RM, μM	damage to deoxyribose A_{532}, nm		
	RM + DR	omit DR	RM + DR + BSA
none	0.433	0.064	0.163
ASC			
50	0.939	0.070	0.210
100	1.190	0.071	0.267
GA			
25	1.269	0.077	0.198
100	1.980	0.083	0.343
GM			
25	0.791	0.069	0.171
100	1.256	0.070	0.206
PG			
25	0.865	0.068	0.166
100	1.300	0.074	0.172

[a] Reaction mixtures (RM) contained in a final volume of 1.2 mL, the following reagents at the final concentration stated: 10 mM KH_2PO_4–KOH buffer (pH 7.4), 2.8 mM H_2O_2, 2.8 mM deoxyribose (where used), 50 μM $FeCl_3$ premixed with 100 μM EDTA before addition to the reaction mixture, and 25 or 100 μM of compounds tested dissolved in water, added to start the reaction. Ascorbate (50 or 100 μM) where used, was added to start the reaction. The tubes were incubated at 37 °C for 1 h. Products of $OH^\bullet$ attack upon deoxyribose were measured as described in Halliwell et al. (1987). Bovine serum albumin (BSA) and transferrin (TF) were included in the assay mixtures at final concentrations of 10 and 5 mg/mL, respectively. Values are the means from triplicate measurements that had less than 10% variation. DR = deoxyribose.

In control experiments, the formation of uric acid at 290 nm was measured using the same assay mixtures but omitting NBT and cytochrome c. Although PG, GM, and GA raised background A_{290}, they did not change the rate of uric acid formation. Hence the inhibition by GA, GM, and PG is not due to direct inhibition of xanthine oxidase and must be due to direct scavenging of $O_2^{\bullet-}$ by the compounds.

Prooxidant Action and Protection against It. Antioxidants that have been shown to protect lipids against free radical chain reactions of peroxidation can be evaluated for their ability to damage carbohydrates using 2-deoxy-D-ribose as a substrate. In the so-called deoxyribose assay (Halliwell et al., 1987) a mixture of $FeCl_3$–EDTA, hydrogen peroxide (H_2O_2) and ascorbic acid at pH 7.4 generates hydroxyl radicals ($OH^\bullet$) (eqs 5 and 6) which can be detected by their ability to degrade the sugar deoxyribose into fragments that, on heating with thiobarbituric acid at low pH, generate a pink chromogen

$$Fe^{3+}\text{–EDTA} + \text{ascorbate} \rightarrow Fe^{2+}\text{–EDTA} + \text{oxidized ascorbate} \quad (5)$$

$$Fe^{2+}\text{–EDTA} + H_2O_2 \rightarrow Fe^{3+}\text{–EDTA} + OH^\bullet + OH^- \quad (6)$$

In this system, addition of ascorbic acid greatly increases the rate of $OH^\bullet$ generation by reducing iron and maintaining a supply of Fe^{2+} (eq 5). This ability to reduce Fe^{3+} and stimulate deoxyribose degradation has been adopted as one measure of prooxidant properties of actual and proposed food antioxidant additives (Laughton et al., 1989; Aruoma et al., 1992; Aruoma, 1993b). The data in Table V show that not only PG (Aruoma et al., 1990) but also

Table VI. DNA Damage by Ferric Bleomcyin[a]

compds added	concn, mM	DNA damage A_{532}, nm A	B
none, control		0.030	0.0
ascorbate[b]	0.24	0.99	0.90
gallic acid	0.005	0.12	0.11
	0.012	0.18	0.27
	0.025	0.18	0.36
	0.050	0.29	0.76
gallic acid lauryl ester	0.005	0	0
	0.012	0.06	0
	0.025	0.09	0
	0.050	0.09	0
gallic acid methyl ester	0.005	0.07	0.11
	0.012	0.14	0.10
	0.025	0.12	0.16
	0.050	0.14	0.25
propyl gallate	0.005	0.10	0.12
	0.012	0.21	0.26
	0.025	0.20	0.36
	0.050	0.27	0.44

[a] Experiments were performed as described under Materials and Methods. The table shows representative data in which the mean values quoted varied by no more than 10% in three separate experiments: A = compounds dissolved in aqueous medium and B = compounds dissolved in absolute ethanol. [b] This is the positive control in this experiment. The ability of GA, GL, GM, and PG (in the absence of ascrobate) to promote DNA degradation is shown.

GA and GM were prooxidant at the concentrations tested. The prooxidant effect of food antioxidant additives *in vitro* can be attenuated by albumin (Smith et al., 1992). Indeed, bovine serum albumin protected deoxyribose against the prooxidant actions of GA, GM, and PG (Table V). However, it is likely that the protein itself is damaged, and proteins in the food matrix could be similarly damaged.

The bleomycin assay (Gutteridge et al., 1981) has also been adapted for assessing the prooxidant effects of proposed food antioxidants (Aruoma, 1993c). The antitumor antibiotic bleomycin binds iron ions and to DNA. The bleomycin–iron complex degrades DNA in the presence of O_2 and a suitable reducing agent such as ascorbic acid to give base propenals. Such degradation can be measured almost quantitatively by the TBA test (Gutteridge et al., 1981).

Table VI shows that PG and GA promoted DNA damage in the system in a concentration dependent manner, whereas GL and GM were less effective. The mechanism of DNA damage in the bleomycin–Fe^{3+} system does not exclusively involve $OH^{\bullet}$ (Gajewski et al., 1991). Nevertheless, previous observations (Aruoma et al., 1990; Aruoma, 1993c) suggest that some compounds that exhibit positive prooxidant action in the deoxyribose assay do not necessarily exert the same effect in the bleomycin assay. This has been illustrated by vanillin, carnosol, carnosic acid, and a number of flavonoids (Aruoma et al., 1990, 1992; Aruoma, 1993c; Laughton et al., 1989).

Conclusion. The data presented in this paper suggest that the gallic acid derivatives GM and PG can react with reactive oxygen species to protect lipids against damage. However, they can exert prooxidant effects toward DNA and carbohydrates in the assays we have used, presumably by interacting with iron. Such effects might be attenuated by the presence of proteins, but the proteins might then be damaged. The solubility of the compounds determined which of the assays could be performed. Gallic acid lauryl ester is only soluble in organic solvents and as such could only be tested in lipid peroxidation, $CCl_3O_2^{\bullet}$ scavenging, bleomycin, and HOCl systems. Our data show the need for a full evaluation of both antioxidant and prooxidant actions of food antioxidants (Halliwell, 1990; Aruoma, 1993d). The alleged benefits of gallic acids may, of course, involve mechanisms which are different from their ability to react with important reactive oxygen species. However, increased consumption of gallic acids and its derivatives should be viewed with caution in view of their potential prooxidant properties.

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