JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Gallic Esters of Sucrose as Efficient Radical Scavengers in Lipid Peroxidation

CLAIRE DUFOUR,*,† ERIC DA SILVA,† PIERRE POTIER,‡ YVES QUENEAU,‡ AND OLIVIER DANGLES†

UMR A 408 INRA-Université d'Avignon et des Pays de Vaucluse, Sécurité et Qualité des Produits d'Origine Végétale, Agroparc, F-84914 Avignon Cedex 9, France; and UMR 143 CNRS-Béghin-Say, C.E.I., 27 bld du 11 novembre 1918, BP 2132, F-69603 Villeurbanne, France

Three tests of increasing complexity were used to assess the antioxidant activity of five synthetic gallic esters of sucrose bearing 3, 6, 7, or 8 galloyl units. In addition, two of these compounds had 1 or 2 hydrocarbon (C10−C12) acyl chains. Reaction with the DPPH radical led to the evaluation of the number of radicals trapped per galloyl unit *n* (3−4), as well as the apparent second-order rate constant for H atom donation *k* (1200−1500/M/s). These results indicated similar contribution and reactivity of all the galloyl units. Inhibition of the AAPH-initiated peroxidation of linoleic acid in a micellar medium confirmed the additive contribution of the galloyl units, whereas the presence of the hydrocarbon acyl chains had no influence. These results suggest an inhibition of initiation at high antioxidant levels and an underlying prooxidant effect of the galloyl radicals at low concentrations. Finally, LDL peroxidation was inhibited in proportion to the number of galloyl units, in agreement with the preceding tests.

KEYWORDS: Antioxidant; lipid peroxidation; DPPH; galloylsucrose; gallotannin; LDL; quercetin

INTRODUCTION

Gallotannins are plant polyphenols containing a polyol core acylated by gallic acid (*1*). The most common polyol core in gallotannins is D-glucose, although other monosaccharides (D-hamamelose (*2*) or D-fructose (*3*)) and a nonsugar polyol core (quinic acid) have been found. However, disaccharides may exist as evidenced by the isolation of several monogalloylsucroses extracted from rhubarbs in China and North Korea (*4*). Hydrolyzable tannins are potent scavengers of reactive oxygen species (alkylperoxyl and hydroxyl radicals, superoxide, and singlet oxygen) usually involved in degenerative deseases (*2*, *5*). Galloylation in tea catechins was also found to increase tannin antioxidant activity (*6*−*8*). Oxidation largely affects the quality of food and processed fruits and vegetables. In particular, lipid autoxidation in oils and fats is responsible for off-flavors and decreased nutritional quality. Preservation is achieved by addition of natural antioxidants such as ascorbate and tocopherols (*9*).

Because sucrose is widely consumed in the human diet, new antioxidants were synthesized bearing several gallic acid units on the disaccharide core (*10*). The antioxidant activity of tri-, hexa-, and octagalloylsucroses was first examined in the DPPH test, then in the inhibition of lipid peroxidation in aqueous micelles of sodium dodecyl sulfate (SDS) and in low-density lipoproteins (LDL). The influence of a lipid chain was further evaluated with monodecanoylsucrose and didodecanoylsucrose of gallic acid. It is currently believed that the oxidative modification of LDL is an early event in the pathogenesis of atherosclerosis (*11*).

MATERIALS AND METHODS

Materials. Diphenylpicrylhydrazyl (DPPH), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), linoleic acid, methyl gallate, quercetin, α-tocopherol, and sodium dodecyl sulfate (SDS) of the highest quality available (95−99%) were purchased from Sigma-Aldrich (l'Isle d'Abeau, France). LDL was available from Sigma as a 6.3 mg protein/mL solution. Sodium dihydrogenphosphate (99%) was from Analyticals Carlo Erba. Water was purified through a Millipore Milli-Q system (Bedford, MA) and methanol was HPLC grade.

Synthesis of Galloylsucroses. *NMR Analysis*. ^{1}H and ^{13}C NMR spectra were recorded on 300 and 500 MHz Bruker apparatus at 27 °C. Chemical shifts (δ) are given in ppm (solvent as the internal reference), coupling constants (*J*) are given in Hz. ^{1}H signals were assigned from 1D- and 2D-COSY spectra and ^{13}C signals were from DEPT.

Protected SG7Ac (Ac = decanoyl). 3,4,5-Tribenzyloxybenzoic acid (465 mg, 1.05 mmol), prepared in a two-step procedure including total benzylation ($PhCH_2Br$, K_2CO_3, DMSO) and subsequent saponification of the benzylester (KOH, EtOH), was dissolved in CH_2Cl_2 (4 mL) under N_2. Oxalyl chloride (0.138 mL, 1.58 mmol) and *N*,*N*-dimethylformamide (1 drop) were added, and the solution was stirred for 4 h at room temperature. After removal of the solvent, the acyl chloride was dissolved into CH_2Cl_2 (2 mL) and the resulting solution was added to a solution of 1′-*O*-decanoylsucrose (*12*) (50 mg, 0.1 mmol), triethyl-

* To whom correspondence should be addressed. Tel: (33) 4 32 72 25 15. Fax: (33) 4 32 72 24 92. E-mail: cdufour@avignon.inra.fr.
† UMR A 408 INRA-Université d'Avignon et des Pays de Vaucluse.
‡ UMR 143 CNRS-Béghin-Say.

10.1021/jf011683k CCC: $22.00
Published on Web 05/10/2002

amine (0.2 mL), and 4-(dimethylamino)pyridine (12 mg) in THF (2 mL) at 5 °C under N_2. The mixture was stirred at room temperature for 2 h. After concentration, the crude mixture was dissolved into CH_2Cl_2, washed with 0.2 M HCl, and dried over Na_2SO_4. After concentration and chromatography on silica gel (eluent CH_2Cl_2/hexane, 4:1), the product was isolated (R_f (CH_2Cl_2) = 0.51, 269 mg, yield 78%).

^{13}C NMR (75 MHz, $CDCl_3$, δ): 173.4 (1 C, CO); 166.1−165.5 (7 C, Ar−CO); 153.3−152.9 (14 C, c,c′); 143.7−143.0 (7 C, d); 137.9−136.8 (21 C, Ph); 128.8−128.1 (105 CH, Ph); 125.1−124.0 (7 C, a); 109.5−109.3 (14 CH, b,b′); 106.1 (1 C, C2′); 91.8 (1 CH, C1); 79.7, 77.7 × 2, 75.5, 72.6, 70.2, 69.6 (CH, C2, C3, C4, C5, C3′, C4′, C5′); 75.7−75.5 (benzylic CH_2); 71.9−71.4 (benzylic CH_2); 64.5, 64.2, 63.3 (3 CH_2, C1′, C6′, C6); 34.4 (1 CH_2, CH_2CO); 32.3, 29.9−29.7, 23.1 (6 CH_2, hydrocarbon chain); 25.3 (1 CH_2, CH_2CH_2CO); 14.6 (1 CH_3).

HRMS−FAB (*m*/*z*): [M + Li]$^+$, calcd for $C_{218}H_{194}O_{40}Li$, 3458.3306; found, 3458.3454.

SG7Ac. Protected SG7Ac (377 mg, 0.11 mmol) and 10% palladium on charcoal (200 mg) were stirred in THF (4 mL) at room temperature under a H_2 flux for 6 h. After filtration on Celite and concentration, the solid residue was chromatographed on a C18 reversed-phase silica gel eluted with a H_2O/MeOH gradient, thus yielding SG7Ac as a gray solid (R_f (ethyl acetate/butanone/HCO_2H/H_2O, 20:2:1:1) = 0.41, 167 mg, yield: 98%).

^{1}H NMR (300 MHz, CD_3OD, δ): 7.35; 7.14; 7.10; 7.03; 6.90 (5 s, 14 H, CH Ar); 6.0 (d, 1 H, J = 3.6, H1); 5.86 (t, 1 H, J = 10.1, H3); 5.81−5.74 (m, 2 H, H4′, H3′); 5.65 (t, 1 H, J = 9.9, H4); 5.29 (dd, 1 H, J = 10.3, 3.7, H2); 4.74−4.56 (m, 5 H, H6′a, H6a, H6′b, H5′, H5); 4.41−4.36 (m, 2 H, H1′a, H6b); 4.28 (d, 1 H, J = 11.9, H1′b); 2.13 (t, 2 H, J = 7.3, CH_2CO); 1.44 (m, 2 H, CH_2CH_2CO); 1.18 (m, 12 H, 6 CH_2); 0.83 (t, 3 H, J = 6.5, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$, δ): 173.9 (1 C, CO); 167.1−165.9 (7 C, Ar−CO); 145.4 (14 C, c,c′); 145.2 (7 C, d); 120.3−119.2 (7 C, a); 109.5 (14 CH, b,b′); 104.8 (1 C, C2′); 91.0 (1 CH, C1); 80.0 (1 CH, C5′); 77.8 (1 CH, C3′); 76.1 (1 CH, C4′); 71.4 (1 CH, C2); 70.7 (1 CH, C3); 69.6 (1 CH, C5); 68.5 (1 CH, C4); 65.2 (1 CH_2, C1′); 64.5 (1 CH_2, C6′); 61.7 (1 CH_2, C6); 33.7 (1 CH_2, CH_2CO); 32.0, 29.5, 29.4 × 2, 29.0, 22.7 (6 CH_2, hydrocarbon chain); 24.9 (1 CH_2, CH_2CH_2CO); 13.5 (1 CH_3).

HRMS−FAB (*m*/*z*): [M + H]$^+$, calcd for $C_{71}H_{69}O_{40}$, 1561.3365; found, 1561.3400.

Protected SG6Ac2 (Ac = dodecanoyl). Starting material 1′,6′-*O*-didodecanoylsucrose (*13*) was submitted to a procedure similar to that used for the preparation of protected SG7Ac. The product was isolated after chromatography on silica gel (eluent CH_2Cl_2/hexane, 4:1). R_f (CH_2Cl_2/hexane, 4:1) = 0.25, 334 mg, yield 97%.

^{13}C NMR (75 MHz, acetone-d_6, δ): 173.4, 172.9 (2 C, CO); 165.6−165.5 (6 C, Ar−CO); 153.1−152.9 (12 C, c,c′); 143.4−142.8 (6 C, d); 138.2−137.2 (18 C, Ph); 128.8−128.1 (90 CH, Ph); 124.8−124.0 (6 C, a); 109.2−109.1 (12 CH, b,b′); 105.5 (1 C, C2′); 91.4 (1 CH, C1); 79.6 (1 CH, C5′); 78.1 (1 CH, C3′); 76.6 (1 CH, C4′); 75.1, 71.1 (18 benzylic CH_2); 72.3 (1 CH, C2); 71.7 (2 CH, C3, C5); 69.6 (1 CH, C4); 64.6 (1 CH_2, C1′); 64.1 (1 CH_2, C6′); 63.6 (1 CH_2, C6); 34.1 (2 CH_2, CH_2CO); 30.1, 29.5−29.3 (16 CH_2, hydrocarbon chain); 25.2 (2 CH_2, CH_2CH_2CO); 14.0 (2 CH_3).

HRMS−FAB (*m*/*z*): [M + Li]$^+$, calcd for $C_{204}H_{198}O_{37}Li$, 3246.3772; found, 3246.3860.

SG6Ac2. The procedure was similar to that used for the preparation of SG7Ac. R_f (ethyl acetate/butanone/HCO_2H/H_2O, 30:2:1:1) = 0.5, 138 mg, yield 92%.

^{1}H NMR (500 MHz, CD_3OD, δ): 7.30 (s, 2 H, CH Ar at 3′ or 4); 7.18 (s, 2 H, CH Ar at 4′ or 3); 7.12 (s, 2 H, CH Ar at 6 or 2); 7.03 (s, 2 H, CH Ar at 2 or 6); 6.91 (s, 2 H, CH Ar at 4′ or 3); 6.89 (s, 2 H, CH Ar at 3′ or 4); 6.06 (d, 1 H, J = 3.7, H1); 5.88 (t, 1 H, J = 10.0, H3); 5.85 (t, 1 H, J = 6.2, H4′); 5.71 (d, 1 H, J = 6.2, H3′); 5.66 (t, 1 H, J = 10.0, H4); 5.23 (dd, 1 H, J = 3.7, 10.3, H2); 4.54 (dd, 1 H, J = 12.3, 2.0, H6a); 4.51 (m, 1 H, H5); 4.47 (d, 1 H, J = 11.7, H1′a); 4.43−4.39 (m, 3 H, H6′a,b, H5′); 4.25 (dd, 1 H, J = 12.3, 2.3, H6b); 4.22 (d, 1 H, J = 11.7, H1′b); 2.36 (t, 2 H, J = 7.4, CH_2CO at 6′); 2.23 (t, 2 H, J = 7.4, CH_2CO at 1′); 1.53 (m, 4 H, 2 CH_2CH_2CO); 1.25 (m, 32 H, 16 CH_2); 0.90 (t, 6 H, J = 7.0, 2 CH_3).

^{13}C NMR (125 MHz, CD_3OD, δ): 174.4 (1 C, CO at 6′); 173.8 (1 C, CO at 1′); 167.0 (2 C, Ar−CO at 3′ and 4); 166.4 (1 C, Ar−CO at 6); 166.3 (1 C, Ar−CO at 2); 166.1, 165.9 (2 C, Ar−CO at 3 and 4′); 145.4 (12 C, c,c′); 139.6−139.0 (6 C, d); 120.2−119.1 (6 C, a); 109.8−109.5 (12 CH, b,b′); 104.2 (1 C, C2′); 90.5 (1 CH, C1); 79.0 (1 CH, C5′); 77.7 (1 CH, C3′); 75.4 (1 CH, C4′); 71.4 (1 CH, C2); 70.6 (1 CH, C3); 69.5 (1 CH, C5); 68.4 (1 CH, C4); 65.7 (1 CH_2, C1′); 63.1 (1 CH_2, C6′); 61.8 (1 CH_2, C6); 33.8 (2 CH_2, CH_2CO); 33.1, 29.8−29.1, 22.7 (16 CH_2, hydrocarbon chain); 25.1 (2 CH_2, CH_2CH_2CO); 13.5 (2 CH_3).

HRMS−FAB (*m*/*z*): [M + H]$^+$, calcd for $C_{78}H_{91}O_{37}$, 1619.5239; found, 1619.5245.

Absorption Spectra. Spectra were recorded on a Hewlett-Packard 8453 diode-array spectrometer equipped with a magnetically stirred quartz cell (optical path length 1 cm). The temperature in the cell was kept constant by means of a thermostated water-bath.

H Atom Transfer to DPPH. To 2 mL of a freshly prepared solution of DPPH in MeOH (2×10^{-4} M) placed in the spectrometer cell at 25 °C were added small volumes (30−35 or 70 μL) of a galloylsucrose solution (10^{-3} M in galloyl units) in MeOH. Recording of the absorbance was started before addition of the antioxidant and continued for, respectively, 50 s (cycle time 0.5 s) for the determination of the kinetic parameters and 300 s (cycle time 3 s) for the determination of the total stoichiometry. The curve-fittings of absorbance at 515 nm vs time plots were carried out as reported below.

Data Analysis. To achieve a quantitative description, the following simple model was used. An antioxidant of stoichiometry N is regarded as N independent antioxidant subunits AOH which all transfer a H atom to DPPH with the same second-order rate constant k. Hence, Beer's law (eq 1) and sets of differential kinetic equations (eq 2 and 3) with initial conditions on concentrations were input in the model.

$$A = A_0[\mathrm{DPPH}]/[\mathrm{DPPH}]_0 \quad (1)$$

$$-\mathrm{d}[\mathrm{AOH}]/\mathrm{d}t = k[\mathrm{AOH}][\mathrm{DPPH}] \quad (2)$$

$$-\mathrm{d}[\mathrm{DPPH}]/\mathrm{d}t = k[\mathrm{AOH}][\mathrm{DPPH}] \quad (3)$$

where A is the visible absorbance at time t, A_0 is the initial absorbance, and $[\mathrm{DPPH}]_0$ is the initial DPPH concentration. The initial condition on the concentration in subunits AOH is $[\mathrm{AOH}]_0 = Nc_0$ where c_0 is the initial antioxidant concentration. Curve-fittings were achieved through least-squares regression using the Scientist program (MicroMath, Salt Lake City, UT) and yielded optimized values for the kinetic rate constant k and the stoichiometry N. Stoichiometries per galloyl unit n ($N = n \times$ number of galloyl units in the antioxidant) and standard deviations are reported from triplicates (five replicates for methyl gallate).

The total stoichiometry N_t for the antioxidant was evaluated after 300 s using $N_t\, c_0 = [\mathrm{DPPH}]_0\, (A_0 - A_f)/A_0$ where A_f is the absorbance at the end of the run. The total stoichiometry per galloyl unit n_t is reported as the average from duplicates.

Inhibition of Linoleic Acid Peroxidation. Solutions of SDS (0.1 M) and AAPH (0.08 M) were prepared in 50 mM phosphate buffer (pH 7.4). A freshly prepared solution of linoleic acid (2 mL; 2.5×10^{-3} M) in the SDS medium was placed in the spectrometer cell and equilibrated at 37 ± 0.1 °C. The initial level of hydroperoxides was kept below 2% in all the experiments. Freshly prepared AAPH (25 μL) was added and the monitoring of the absorbance at 234 nm was started. After the peroxidation rate had kept constant over 10 min, 25 μL of a solution of galloylsucrose or quercetin or α-tocopherol in MeOH (typical concentration range $10^{-5} - 10^{-3}$ M in galloyl unit and quercetin or $10^{-6} - 10^{-4}$ M in α-tocopherol) were added. From the slope of the linear absorbance vs time plots (evaluated in the 3−10 min interval), the uninhibited peroxidation rate (R_{uninh}) was calculated using 26100/M/cm as the molar absorption coefficient of the linoleic acid hydroperoxides at 234 nm (*14*). The inhibited peroxidation rate (R_{inh}) was similarly calculated throught a linear treatment of the curve in the 11−18 min interval (1−8 min after addition of the antioxidant). Data were further analyzed according to the classic rate law for inhibited autoxidation of linoleic acid (eq 4) with k_p and k_{inh} being, respectively,

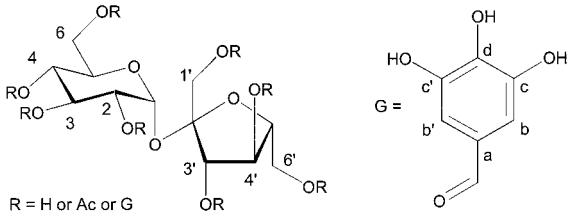

Figure 1. Structures of the galloylsucroses (Ac═C_9H_{19}–CO or $C_{11}H_{23}$–CO) (see **Table 1** for substitutions).

the propagation and inhibition rate constants, [LH] and [AH] the linoleic acid and the antioxidant concentrations, R_i the initiation rate, and n the stoichiometry of the antioxidant (*14*). The antioxidant efficiency *AE* is defined as the ratio between the rate constants of chain break (k_{inh}) and chain propagation (k_p) uncorrected for n (eq 5). From the slope of the R_{inh} vs 1/[AH] plot in the initial inhibition period, the antioxidant efficiency can be extracted. R_i was calculated from $R_i = 2ek_d$[AAPH] with e set at 0.49 for the efficiency of radical production from AAPH, k_d set at 3.72×10^{-7}/s for the decomposition rate of the initiator, and [AAPH] the initial AAPH concentration (*14*).

$$R_{inh} = \frac{k_p[LH]R_i}{nk_{inh}[AH]} + R_i \quad (4)$$

$$AE = \frac{nk_{inh}}{k_p} = \frac{[LH]R_i}{slope} \quad (5)$$

Inhibition of LDL Peroxidation. LDL suspensions (100 μg protein/mL) were prepared daily from the commercial LDL solution in 0.01 M phosphate buffered saline (PBS, pH 7.4) containing 0.15 M NaCl. The galloylsucroses (20 μL, 10^{-3} M galloyl unit in MeOH) and quercetin (20 μL, 10^{-3} M in MeOH) were incubated for 30 min with 1.9 mL of the LDL suspension at room temperature. The mixture was then warmed to 37 °C and the peroxidation was initiated by the addition of 50 μL of 0.32 M AAPH in PBS. The hydroperoxide formation was monitored at 246 nm over 90 min (cycle time 90 s) as described above. Experimental absorbance values are reported every 1000 s and corrected for AAPH absorption. Blank trials (20 μL of MeOH in place of the antioxidant) were performed before each inhibition experiment. Standard deviations for the replicates were below 2%.

RESULTS AND DISCUSSION

The antioxidant activity of five synthetic galloylsucroses (**Figure 1**) was assessed in systems of increasing complexity and compared to that of methyl gallate. Two typical phenolic antioxidants, quercetin (3,3′,4′,5,7-pentahydroxyflavone) and α-tocopherol, were also investigated for comparison purposes. The data were analyzed in terms of intrinsic reactivity and efficiency of the antioxidants in protecting biological targets (polyunsaturated fatty acids, LDL) from degradation by peroxyl radicals.

Quenching of the DPPH Radical. The decay of the visible absorbance of DPPH that follows the addition of the antioxidant lasts for several minutes and is relatively fast during the first 50 s. This fast step was kinetically analyzed to obtain the number of labile H atoms transferred from the antioxidant to DPPH (stoichiometry N) as well as the corresponding apparent rate constant (k) assuming that the N H atoms are all transferred at the same rate. In addition, from the monitoring of the reaction over 300 s, i.e., until no significant decay in the visible absorbance took place, the total stoichiometry N_t was evaluated. The stoichiometries n and n_t, reported per galloyl unit, are similar for the five galloylsucroses (**Table 2**). The galloyl group remains accessible to the DPPH radical even for the most hindered galloylsucroses SG7Ac and SG8. In the fast kinetic step, there is no apparent discrimination between the hydrogens

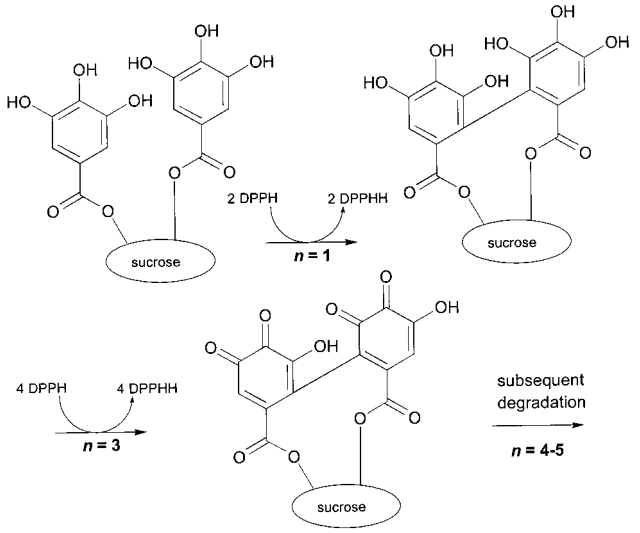

Figure 2. Possible pathway for the oxidative degradation of galloylsucroses during trapping of the DPPH radical.

Table 1. Substitution Pattern of the Galloylsucroses Used in This Study; Structures are Shown in **Figure 1**

R[a] in	O2	O3	O4	O6	O1′	O3′	O4′	O6′
SG3	H	H	H	H	H	G	G	G
SG6	G	G	H	H	G	G	G	G
SG6Ac2	G	G	G	G	Ac	G	G	Ac
SG7Ac	G	G	G	G	Ac	G	G	G
SG8	G	G	G	G	G	G	G	G

[a] R = H, G (galloyl), and Ac (decanoyl for SG7Ac and dodecanoyl for SG6Ac2).

Table 2. H Atom Transfer Reaction from Methyl Gallate and Galloylsucroses to DPPH in MeOH at 25 °C at Two Different DPPH/Galloyl Unit Ratios (Stoichiometries (n_t, n) and Second-Order Rate Constant (k) as Defined in Materials and Methods)

	DPPH/galloyl unit ratio					
	12–14			6		
antioxidant	n_t	n	$k \pm$ SD ($M^{-1}s^{-1}$)	n_t	n	$k \pm$ SD ($M^{-1}s^{-1}$)
methyl gallate	5.21	4.64 ± 0.08	1667 ± 69	4.75	4.36 ± 0.02	1350 ± 46
SG3	4.40	3.50 ± 0.11	1497 ± 130	3.48	3.02 ± 0.05	1480 ± 25
SG6	3.75	3.09 ± 0.04	1304 ± 50	3.45	2.93 ± 0.05	1141 ± 12
SG6Ac2	4.22	3.57 ± 0.03	1429 ± 43	3.76	3.37 ± 0.03	1337 ± 6
SG7Ac	4.38	3.77 ± 0.06	1604 ± 91	3.83	3.37 ± 0.03	1468 ± 17
SG8	3.76	3.41 ± 0.08	1462 ± 67	3.37	2.90 ± 0.05	1302 ± 24

abstracted as seen from the constant value of n. This value was calculated to be close to 3 suggesting a total reactivity of the three hydroxyl groups borne by the aromatic nucleus. As expected, n_t is higher than n for a given galloylsucrose, the difference being attributable to the residual H atom-donating activity of the oxidation products formed during the fast step. A possible mechanism for galloyl unit oxidation is outlined in **Figure 2**. Coupling reactions may occur intramolecularly in the galloylsucroses upon recombination of two adjacent galloyl radicals formed by single H atom abstraction from a OH group ($n = 1$). A biaryl dimer (C−C coupling) has already been isolated from the reaction of DPPH with gallic acid (*5*). Alternatively, C−C coupling may also occur via nucleophilic attack of a galloyl moiety on the orthoquinone formed by double H atom abstraction from an adjacent galloyl moiety (*15*). Subsequent fast H atom abstractions from the remaining OH groups in the biaryl moiety would lead to a bis-orthoquinone

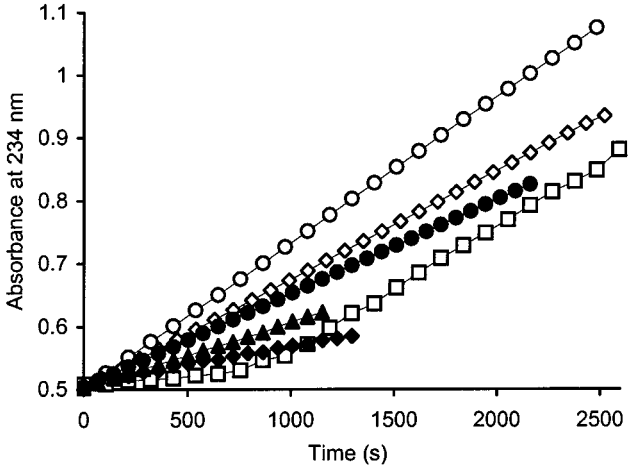

Figure 3. Formation of lipid hydroperoxides initiated by AAPH (1 mM) in SDS micelles at 37 °C. Inhibition by SG3 (●, 5.04×10^{-7} M), SG6 (▲, 4.91×10^{-7} M), SG7Ac (◆, 4.50×10^{-7} M), quercetin (◊, 4.76×10^{-7} M), and α-tocopherol (□, 3.53×10^{-7} M). Control (○, no antiox.).

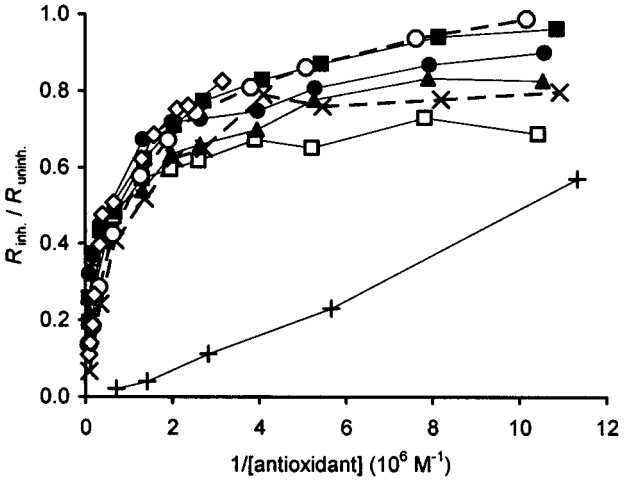

Figure 4. Changes in the relative rate of inhibited peroxidation as a function of the reciprocal of the concentration (expressed in galloyl unit for the sucroses) for linoleic acid peroxidation in SDS micelles. Inhibitors: methyl gallate (□), SG3 (●), SG6 (■), SG8 (▲), SG6Ac2 (×), SG7Ac (○), quercetin (◊), and α-tocopherol (+).

moiety in agreement with the estimated stoichiometry per galloyl unit of ca. 3 at the end of the fast step. Subsequent solvent addition and ring opening reactions may then form degradation products with additional OH groups responsible for the residual H atom-donating activity (n_t ca. 4). Methyl gallate, taken as a reference, was found to give higher stoichiometries. Values of n and n_t close to 5 suggested a more complete degradation in the DPPH reaction, probably due to a higher reactivity of this small molecule compared to that of the galloyl units attached to sucrose. The parameter k is an apparent rate constant for the abstraction by DPPH of the labile H atoms from the antioxidant and its oxidation products formed during the fast step. The validity of our model (correlation coefficients >0.99 for the curve fittings of the kinetic traces) and the close k values (in the range 1300−1700/M/s) obtained for the series of galloylsucroses investigated, suggest a common mechanism and that the steps of H atom abstraction actually take place at similar rates. In addition, these rate constants appear to be higher than values measured for quercetin ($n = 3$; $k = 583$/M/s) and its 3-glucoside rutin ($n = 2$; $k = 718$/M/s) usually classified as good antioxidants (*16*). Close stoichiometries for quercetin and sucrose-borne galloyl units are in agreement with a previous DPPH test with quercetin and 6-*O*-galloylfructose (*2*).

Rate constants obtained in the DPPH test are solvent-dependent. A decrease in the DPPH radical reactivity was correlated with the hydrogen bond accepting ability of the solvent. For phenols and catechols, the kinetic solvent effect was found to be independent of the nature of the abstracting radical (*17*). Although alkoxyl and peroxyl radicals are more highly reactive than the DPPH nitrogen-centered radical, this test may parallel the reaction of antioxidants with oxygen-centered radicals in a given solvent (*18*).

Inhibition of Linoleic Acid Peroxidation. Owing to the major role played by lipids in various biological and food processes, antioxidants are better challenged with inhibition of lipid peroxidation. The various galloylsucroses were thus tested for their ability to inhibit the radical-induced chain peroxidation of linoleic acid in a micellar medium. Comparison was made with methyl gallate, the chain-breaking antioxidant α-tocopherol, and the dietary flavonoid quercetin. Accumulation of the conjugated hydroperoxides resulting from linoleic acid peroxidation was monitored at 234 nm (**Figure 3**). The concentration in hydroperoxides increases linearly as the result of a constant decomposition rate of the AAPH initiator (*19*). Addition of the various sucrose-derived antioxidants was found to inhibit the formation of hydroperoxides with a close-to-linearity pattern being conserved over the inhibited period. Furthermore, for a similar antioxidant level, the inhibited peroxidation rate decreased proportionally to the number of galloyl units borne by the sucrose common core. Galloylsucrose antioxidants behaved similarly to quercetin in lowering the rate of hydroperoxide formation. Conversely, α-tocopherol almost completely quenched the peroxidation. A lag phase can be defined during which peroxidation is very slow. When α-tocopherol approaches consumption, peroxidation resumes and quickly reaches its rate before inhibition. Unlike α-tocopherol, the galloylsucroses and quercetin do not display a clear-cut lag phase.

Comparison of the galloylsucrose antioxidant efficiencies was obtained by plotting the relative rate of inhibited peroxidation as a function of the reciprocal of the antioxidant concentration expressed in galloyl unit (**Figure 4**). Surprisingly, all the galloylsucroses displayed a similar pattern, suggesting a low influence of the fatty acid chain and accessibility of all the galloyl units for quenching of the peroxyl radicals. The reactivity of the galloyl unit is conserved despite the steric hindrance offered by the most substituted sucroses. No intramolecular interaction between the planar aromatic nuclei appears to lower the reactivity as well as the level of participation of each galloyl unit in agreement with the rate constants and stoichiometries measured in the reaction with DPPH.

The curves presented in **Figure 4** prove to be different for α-tocopherol and the polyphenols. In the case of α-tocopherol, the initial rate of inhibited peroxidation R_{inh} rises linearly as a function of the reciprocal of the initial antioxidant concentration AH_0. This is the so-called classic behavior of typical chain-breaking antioxidants in which termination of the radical chain is accounted by antioxidant H atom donation to the lipid peroxyl radical (*14*). The slope of the plot is then inversely proportional to the antioxidant efficiency (*AE*) which is defined as nk_{inh}/k_p with n the number of radicals trapped by the antioxidant, and k_{inh} and k_p being, respectively, the rate constants of chain break and chain propagation.

In the case of the galloylsucroses, the R_{inh} vs $1/AH_0$ plots are linear at low antioxidant concentrations only. In the range of galloyl unit concentrations 1.3×10^{-7}− 3.8×10^{-7} M^{-1}, the antioxidant efficiency was found to be 2175 for α-toco-

pherol, 4398 for methyl gallate, and in the range of 2900−3930 per galloyl unit in the sucroses. For higher concentrations, strong deviations from linearity suggested a decrease in the antioxidant efficiency although it was supposed to be constant. The invalidity of the classic model for galloylsucroses may have several causes. Attempts to locate the antioxidants in a biphasic system showed that hydrophobic tocopherol is within the micelle (*19*), whereas the hydrophilic sucroses and quercetin must be located in the aqueous phase. Hydrosoluble AAPH was found to associate with SDS, probably by electrostatic interactions between the amidino groups of AAPH and the anionic surface of the micelle (*19*). As a consequence, the lipid peroxyl radicals, less accessible to the sucrose antioxidant than to tocopherol, may propagate the peroxidation process and decompose by recombination leading to the failure of eq 5.

Deviations from the classic law have been already pointed out during the investigation of the inhibition of peroxidation by tea flavanols (*6*), flavonoids in organic solvents (*21*−*22*), and in SDS micelles (*23*). Galloylsucroses share with flavonoids a catechol unit, clearly assessed as the antioxidant active moiety (*24*). Polyphenols with a catechol unit react faster with oxygen-centered radicals than do simple phenols (*17*, *25*) suggesting that these polyphenols are intrinsically better antioxidants. In a micellar medium and at high antioxidant concentrations, methyl gallate and galloylsucroses may exert their antiperoxidizing activity essentially via quenching of the AAPH-derived peroxyl radical. At low antioxidant concentrations, the intercept of the linear portion of the plot (R_i/R_{uninh}) is much higher than anticipated from the classic law. Such distortions can be accounted for by assuming that the aryloxyl radicals derived from the antioxidant via reaction with peroxyl radicals, in addition to evolving toward inert nonradical products, are reactive enough to abstract labile H atoms from the polyunsaturated fatty acid, thereby propagating the peroxidation chain (prooxidant effect) (*6*, *23*). This side-reaction does not take place with the sterically hindered α-tocopheryl radical and this makes α-tocopherol a better inhibitor of lipid peroxidation (lower initial R_{inh} value at a given antioxidant concentration) than flavonoids and the galloylsucroses investigated here.

Moreover, for life and food processes, the antioxidant efficiency may be better evaluated by titration of the hydroperoxides after a long period of inhibition. In such conditions, polyphenols may be more efficient than α-tocopherol as evidenced in **Figure 3** by the crossing of the corresponding curves at a high inhibition period. Comparison of the rates of inhibition just after the addition of a given antioxidant (Pryor's method) (*14*) is indeed distorted by mechanism differences. Thus, antioxidants such as flavonoids and galloylsucroses should be classified as retardants when compared to the chain-breaker α-tocopherol.

Inhibition of LDL Peroxidation. The influence of galloylsucroses, gallic acid, and quercetin on AAPH-induced LDL peroxidation was evaluated during the initial stage of peroxidation. Although Cu^{2+} has been widely used for initiation, azo initiators have the advantage of generating localized well-defined radicals at a constant rate. Kinetic quantitative studies are then possible in the early stage of LDL oxidation (*26*).

Hydroperoxide formation was evaluated at 246 nm, which is the best compromise between high hydroperoxide absorption (λ_{max} 234 nm) and low apoprotein absorption. The ratio between the absorbance at 245 nm and the absorbance at 234 nm has been reported to be 0.6 for conjugated dienyl hydroperoxides (*27*). The kinetics of hydroperoxide formation in the AAPH-initiated LDL peroxidation does not display a clear-cut lag phase

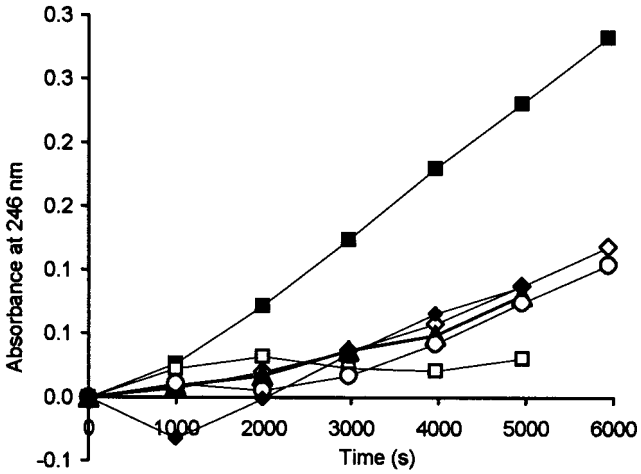

Figure 5. Inhibition of LDL (0.1 mg/mL protein) peroxidation by methyl gallate (□, 1.07×10^{-5} M), SG6 (◇, 1.7×10^{-6} M), SG7Ac (▲, 1.47×10^{-6} M), SG6Ac2 (○, 1.69×10^{-6} M), and quercetin (◆, 1.01×10^{-5} M). Uninhibited curve (■). Absorbance of initiator AAPH (8×10^{-3} M) subtracted.

(**Figure 5**). A control experiment without LDL showed that AAPH decomposition resulted in a linear absorption increase in the 200−250 nm region although slower than that in the presence of LDL. Thus, correction from the AAPH contribution should be made when monitoring hydroperoxides at relatively high AAPH concentrations. In AAPH-initiated LDL peroxidation, Bowry and Stocker (*28*) showed that the hydroperoxides begin to accumulate during the depletion of the endogenous antioxidants α-tocopherol and ubiquinol-10, and that the rate of peroxidation increases after total consumption of the antioxidants. This behavior is different in the Cu^{2+}-induced LDL peroxidation where a lag phase has been correlated to α-tocopherol protection (*11*).

At a constant concentration in galloyl unit, the galloylsucroses SG6, SG6Ac, and SG6Ac2 display similar inhibition levels, indicating that the reactivity of the galloyl moiety is unaltered. Furthermore, the lack of influence of the hydrocarbon chain suggests a weak interaction between the antioxidant and the LDL particle. A similar accessibility of the different galloyl groups could result from their location in the aqueous phase where they would directly trap the peroxyl radicals generated from AAPH. Methyl gallate displays a slightly different pattern supporting the results in the micellar system. A higher stoichiometry, as shown in the DPPH test, associated with a smaller size may slightly favor the antiperoxidizing activity in both systems. For comparison, caffeic acid and its quinic ester, chlorogenic acid, proved to be equally efficient inhibitors in metmyoglobin- and AAPH-induced LDL peroxidation (*29*−*30*) showing no influence of the hydroxylated matrix. Finally, the galloylsucroses appear as efficient as quercetin in inhibiting LDL peroxidation.

In conclusion, the galloylsucroses investigated here may be valuable antioxidants for increasing the shell life of foodstuffs. Readily prepared from cheap naturally occurring compounds (gallic acid and sucrose), they could favorably compete with synthetic antioxidants commonly used for this purpose. In model systems, they compare well with the potent dietary antioxidant quercetin.

LITERATURE CITED

(1) Haslam, E. Polyphenols - structure and biosynthesis. In *Practical Polyphenols;* University Press: Cambridge, 1998; pp 10−83.

(2) Masaki, H.; Atsumi, T.; Sakurai, H. Hamamelitannin as a new potent active oxygen scavenger. *Phytochemistry* **1994**, *37*, 337−343.
(3) Chevalley, I.; Marston, A.; Hostettmann, K. A new gallic acid fructose ester from *Saxifraga stellaris*. *Phytochemistry* **1999**, *50*, 151−154.
(4) Kashiwada, Y.; Nonaka, G.-I.; Nishioka, I. Galloylsucroses from rhubarbs. *Phytochemistry* **1988**, *27*, 1469−1472.
(5) Yoshida, T.; Mori, K.; Hatano, T.; Okumura, T.; Uehara, I.; Komagoe, K.; Fujita, Y.; Okuda, T. Studies on inhibition mechanism of autoxidation by tannins and flavonoids. V. Radical-scavenging effects of tannins and related polyphenols on DPPH radical. *Chem. Pharm. Bull.* **1989**, *37*, 1919−1921.
(6) Jia, Z. S.; Zhou, B.; Wu, L. M.; Liu, Z. L. Antioxidant synergism of tea polyphenols and α-tocopherol against free radical induced peroxidation of linoleic acid in solution. *J. Chem. Soc., Perkin Trans. 2* **1998**, 911−915.
(7) Salah, N.; Miller, N. J.; Paganga, G.; Tijburg, L.; Bolwell, G. P.; Rice-Evans, C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch. Biochem. Biophys.* **1995**, *322*, 339−346.
(8) Gardner, P. T.; McPhail, D. B.; Duthie, G. G. Electron spin resonance spectroscopy assessment of the antioxidant potential of teas in aqueous and organic media. *J. Sci. Food Agric.* **1998**, *76*, 257−262.
(9) Johnson, L. E.; Mergens, W. J. Added ascorbates and tocopherols as antioxidants and food improvers. In *Nutrient Additions to Food;* Bauernfeind J. C., Lachance, P. A., Eds.; Food and Nutrition Press: Trumbull, CT, 1991; pp 433−458.
(10) Potier, P.; Maccario, V.; Giudicelli, M. B.; Queneau, Y.; Dangles, O. Gallic esters of sucrose as a new class of antioxidants. *Tetrahedron Lett.* **1999**, *40*, 3387−3390.
(11) Esterbauer, H.; Gebicki, J.; Pulh, H.; Jurgens, G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biol. Med.* **1992**, *13*, 341−390.
(12) Potier, P.; Bouchu, A.; Gagnaire, Y.; Queneau, Y. Proteinase N-catalysed regioselective esterification of sucrose and other mono- and di-saccharides. *Tetrahedron Asymmetry* **2001**, *12*, 2409−2419.
(13) Potier, P.; Bouchu, A.; Descotes, G.; Queneau, Y. Lipase-catalysed selective synthesis of sucrose mixed diesters. *Synthesis* **2001**, 458−462.
(14) Pryor, W. A.; Cornicelli, J. A.; Devall, L. J.; Tait, B.; Trivedi, B. K.; Witiak, D. T.; Wu, M. A rapid screening test to determine the antioxidant potencies of natural and synthetic antioxidants. *J. Org. Chem.* **1993**, *58*, 3521−3532.
(15) Quideau, S.; Feldman, K. S. Ellagitannin chemistry. *Chem. Rev.* **1996**, *96*, 475−503.
(16) Dangles, O.; Fargeix, G.; Dufour, C. One-electron oxidation of quercetin and quercetin derivatives in protic and non protic media. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1387−1395.
(17) Barclay, L. R. C.; Edwards, C. E.; Vinqvist, M. R. Media effects on antioxidant activities of phenols and catechols. *J. Am. Chem. Soc.* **1999**, *121*, 6226−6231.
(18) Valmigli, L.; Banks, J. T.; Lusztyk, J.; Ingold, K. U. Solvent effects on the antioxidant activity of vitamin E. *J. Org. Chem.* **1999**, *64*, 3381−3383.
(19) Niki, E. Free radical initiators as source of water- or lipid-soluble peroxyl radicals. *Methods Enzymol.* **1990**, *186*, 100−108.
(20) Barclay, L. R. C.; Baskin, K. A.; Locke, S. J.; Schaefer, T. D. Benzophenone-photosensitized autoxidation of linoleate in solution and sodium dodecyl sulfate micelles. *Can. J. Chem.* **1987**, *65*, 2529−2540.
(21) Roginsky, V. A.; Barsukova, T. K.; Remorova, A. A.; Bors, W. Moderate antioxidant efficiencies of flavonoids during peroxidation of methyl linoleate in homogeneous and micellar solutions. *J. Am. Oil Chem. Soc.* **1996**, *73*, 777−786.
(22) Foti, M.; Ruberto, G. Kinetic solvent effects on phenolic antioxidants determined by spectrophotometric measurements. *J. Agric. Food. Chem.* **2001**, *49*, 342−348.
(23) Dangles, O.; Dufour, C.; Fargeix, G. Inhibition of lipid peroxidation by quercetin and quercetin derivatives: antioxidant and prooxidant effects. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1215−1222.
(24) Jovanovic, S. V.; Steenken, S.; Hara, Y.; Simic, M. G. Reduction potentials of flavonoid and model phenoxyl radicals. Which ring in flavonoid is responsible for antioxidant activity? *J. Chem. Soc., Perkin Trans. 2* **1996**, 2497−2504.
(25) Belyakov, V. A.; Roginsky, V. A.; Bors, W. Rate constants for the reaction of peroxyl free radical with flavonoids and related compounds as determined by the kinetic chemiluminescence method. *J. Chem. Soc., Perkin Trans. 2* **1995**, 2319−2336.
(26) Noguchi, N.; Gotoh, N.; Niki, E. Dynamics of the oxidation of LDL induced by free radicals. *Biochim. Biophys. Acta* **1993**, *1168*, 348−357.
(27) Schnitzer, E.; Pinchuk, I.; Bor, A.; Fainaru, M.; Lichtenberg, D. The effect of albumin on copper-induced LDL oxidation. *Biochim. Biophys. Acta* **1997**, *1344*, 300−311.
(28) Bowry, V. W.; Stocker, R. Tocopherol-mediated peroxidation. The prooxidant effect of vitamin E on the radical-initiated oxidation of human LDL. *J. Am. Chem. Soc.* **1993**, *115*, 6029−6044.
(29) Castelluccio, C.; Paganga, G.; Melikian, N.; Bolwell, G. P.; Pridham, J.; Sampson, J.; Rice-Evans, C. Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. *FEBS Lett.* **1995**, *368*, 188−192.
(30) Laranjinha, J. A. N.; Almeida, L. M.; Madeira V. M. C. Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low-density lipoprotein peroxidation. *Biochem. Pharmacol.* **1994**, *48*, 487−494.

Received for review December 20, 2001. Revised manuscript received March 21, 2002. Accepted March 22, 2002.

JF011683K