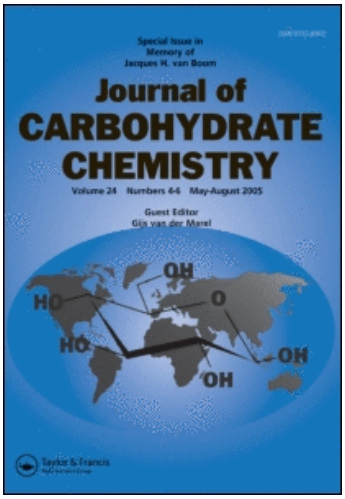

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PREPARATION AND ANTIOXIDANT ACTIVITIES OF PHENOLIC ESTERS AND ETHERS OF L-ASCORBIC ACID[1]

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ABSTRACT

Esterification of L-ascorbic acid in concentrated sulfuric acid with 0.33 moles of gallic acid gave a 90% yield of a 2:5 mixture of L-ascorbyl 5- and 6-gallate esters. The 6-gallate ester (**1**) was purified from the 5-gallate ester (**2**) by fractional crystallization in 40% yield. Three other aromatic derivatives of L-ascorbic acid also were prepared. The order of antioxidant activities of the L-ascorbyl derivatives determined by thiobarbituric acid number in methyl linolenate at 37 °C was as follows: 6-gallate (**1**) > 6-(3',4'-dihydroxy)benzoate (**3**) ≈ 6-(4'-hydroxy)phenyl ether (**4**) >6-(4'methoxy)phenyl ether (**5**).

INTRODUCTION

Propyl gallate and L-ascorbyl 6-palmitate, both[3] generally-recognized-as-safe (GRAS) by the U.S. Food and Drug Administration, have been used[4-6] for many years in foods to inhibit oxidative deterioration. Structurally, the antioxidant portions of those two preservatives are the gallate and ascorbyl moieties. Because antioxidants differ in their effectiveness in various foods and often display synergism, we decided to join the two active moieties into one molecule, L-ascorbyl 6-gallate (**1**). In the reaction used to synthesize **1**, L-ascorbyl 5-gallate (**2**) was formed in low yield. We decided also to prepare L-ascorbyl 6-(3',4'-dihydroxy)benzoate (**3**), L-ascorbyl 6-(4'-hydroxy)phenyl ether (**4**), and L-ascorbyl

6-(4'-methoxy)phenyl ether (**5**), and to compare their antioxidant activities in methyl linolenate.

RESULTS AND DISCUSSION

Preparation of 6-derivatives

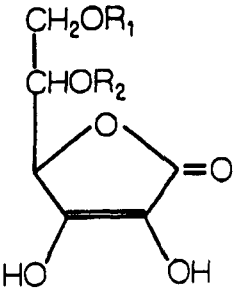
L-Ascorbyl 6-gallate (**1**) (Fig. 1) was produced using the conventional method[7] to prepare 6-fatty acid esters of L-ascorbic acid, except excess L-ascorbic acid rather than excess fatty acid was used to shift the reaction equilibrium towards formation of **1**. It was difficult to isolate and purify **1** from unreacted gallic acid but not from L-ascorbic acid.

The optimum reaction time for formation of **1** in concentrated sulfuric acid at 25-30 °C was 26-44 hours. Under those conditions, the 6-gallate ester (**1**) was isolated in analytically pure form in 40% yield by fractional crystallization. The 5-gallate ester (**2**) was a secondary product in the esterification reaction. The 5-ester was isolated in only 75% purity, apparently because of the ease of acyl migration from 0-5 to 0-6. L-Ascorbyl 6-(3',4'-dihydroxy)benzoate (**3**) also was obtained in crystalline yield of 44%.

The yields of the aromatic ethers (**4** and **5**) of L-ascorbic acid were only 7% and 10% using the procedure of Andrews.[8] Those yields might be improved by adding 6-bromo-6-deoxy-L-ascorbic acid to an alkaline solution of the phenolic compounds. However, our first objective was to test the antioxidant properties of **4** and **5**, and no effort was made to improve yields.

Stability of L-ascorbyl 6-gallate (1) in solution

Dissolving 30 mM 6-gallate (**1**) in water at pH 5 and 8 and storing the solutions at 25 °C resulted in partial acyl migration from 0-6 to 0-5. The proportion of the 5-gallate ester was at most 7% of the combined esters at pH 5, and ~20% at pH 8 as determined by HPLC-UV. Loss of ester (**1** plus **2**) at 25 °C was first-order, and the ester was almost twice as stable at pH 8 ($t_{½}$ 88d) compared to pH 5 ($t_{½}$ 45d). Over 90% of the loss of **1** at pH 5 was accounted for by hydrolysis to gallic acid, but less than 50% at pH 8. Acyl migration was not observed in a 15 mM ethanolic solution of **1** at 25 °C, and again the loss of **1** was first order with $t_{½}$ ~50 days. HPLC-UV showed two unknown reaction products, one of which was probably ethyl gallate.



1 R_1 = 3',4',5'-$(HO)_3C_6H_2CO$, R_2 = H
2 R_1 = H, R_2 = 3',4',5'-$(HO)_3C_6H_2CO$
3 R_1 = 3',4'-$(HO)_2C_6H_3CO$, R_2 = H
4 R_1 = 4'-$(HO)C_6H_4$, R_2 = H
5 R_1 = 4'-$(CH_3O)C_6H_4$, R_2 = H

Figure 1. Derivatives of L-ascorbic acid.

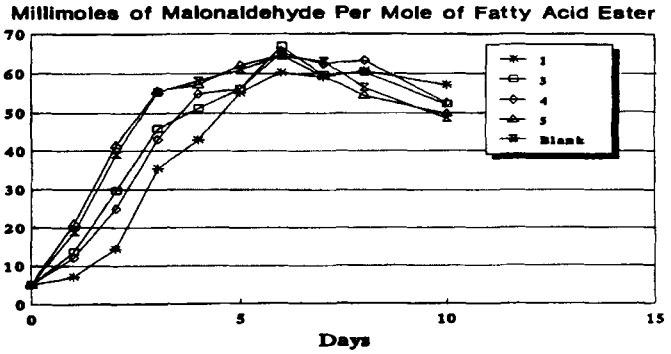


Figure 2. Antioxidant activities of aromatic ethers and esters of L-ascorbic acid determined in methyl linolenate at 37° C by TBA method.

Antioxidant activity

In a screening test[9] for antioxidant activity in methyl linolenate at 37 °C (Fig. 2), 6-(4'-methoxy)phenyl ether (**5**) showed the lowest, 6-gallate ester (**1**) the highest, and 6-(3',4'-dihydroxy)benzoate ester (**3**) and 6-(4'-hydroxy)phenyl ether (**4**) intermediate activities. In a separate test on refined soybean oil containing 0.23 mM antioxidant, which was added in a small amount of ethanolic solution, the days to reach a peroxide value of 70 meq/kg in the oil held at 60° C in a forced-draft oven were: blank, 9.6; L-ascorbic acid, 13.3; L-ascorbyl 6-palmitate, 14.3; L-ascorbyl 6-gallate, 18.0; propyl gallate, 19.8; and gallic acid, 20.5. Refined soy oil contains ~ 700 mg/L (1.5 mM) total tocopherols. Assuming that the added protection beyond 9.6 days could be attributed to the additives, we concluded that the

6-gallate ester was a somewhat less effective antioxidant than propyl gallate, but more effective than L-ascorbyl 6-palmitate and L-ascorbic acid.

EXPERIMENTAL

General methods. Solvents were evaporated at <40° C under reduced pressure. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Specific rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were determined on a U-3201 UV/VS spectrophotometer (Hitachi). Both ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker WM-400 instrument with chemical shifts referenced to tetramethylsilane (TMS) or calculated from the signals of the solvents. Mass spectra were recorded on a Hewlett-Packard 5989A MS instrument.

High performance liquid chromatography (HPLC) was carried out using a Knauer pump fitted with a loop injector (20 μL), a C-18 reverse-phase column (250 x 4.6 mm), an integrating recorder, and a UV detector. For HPLC-UV analysis, samples (20 μL) were injected and components eluted with a 85:15 (v/v) mixture of 0.1 M acetate buffer (pH 4.7) and methanol containing 1.0 mM tetrabutylammonium phosphate (TBAP) and 0.2 mM EDTA. The column was maintained at 35 °C, the flow rate was 0.8 mL/min, and the detector was set at 250 nm. The retention times for **1**, **2**, and gallic acid were approximately 23.4, 11.1, and 6.3 min, respectively.

Column chromatography was carried out on silica gel (230-400 mesh, Fisher Scientific Co.). Thin-layer chromatography was performed on aluminum sheets coated with silica gel 60 F_{254} (Whatman Ltd.), and components were detected either by viewing under short-wavelength UV light or by spraying with acid molybdate.[12]

L-Ascorbyl 6-gallate (1) and L-ascorbyl 5-gallate. (2). The literature method[7] for preparing L-ascorbyl 6-palmitate was used with modification. A mixture of L-ascorbic acid (10.56 g, 60 mmol), gallic acid (3.40 g, 20 mmol), and concentrated sulfuric acid (50 mL) was stirred at 25 °C for 24 h. The reaction mixture was poured onto a mixture of crushed ice (300 g), sodium chloride (50 g), and ethyl acetate (150 mL) with rapid stirring. The water phase was extracted with ethyl acetate (7 x 100 mL), and the combined extracts were washed with brine (3 x 50 mL). The brine layers were combined and then extracted with ethyl acetate (3 x 50 mL); finally, the combined organic phases were dried over magnesium sulfate. Assay

of the reaction products by HPLC-UV showed 1.94 mmol (10%) of unreacted gallic acid, 13.1 mmol (65%) of **1**, and 5.2 mmol (26%) of **2**, assuming that **1** and **2** had identical extinction coefficients. The solvent was removed by evaporation to give a syrup (6.2 g), which crystallized from acetone-ethyl acetate to afford crude yellow crystals (3.1 g). Recrystallization from acetone gave pure **1** as colorless crystals (2.62g, 40%), mp 190-192°; $[\alpha]_D^{25}$+50.6° (*c* 0.54, MeOH); U.V. λ_{max}(MeOH)(ε): 274 nm (15700), 220 nm (34500); ^{1}H NMR (MeOH-d_4): δ 7.10 (s, 2 H, H-2' and H-6'), 4.82 (d, 1 H, J 2.0 Hz, H-4), 4.41 (dd, 1 H, J 11.2, 6.9 Hz, H-6_a), 4.36 (dd, 1 H, J 11.2, 5.8 Hz, H-6_b), 4.21 (ddd, 1 H, J 6.9, 5.8, 2.0 Hz, H-5); ^{13}C NMR. (MeOH-d_4): δ 173.3 (C-1), 168.1 (C-7'), 154.1 (C-3), 146.5 (C-3' and C-5'), 139.9 (C-4'), 121.2 (C-1'), 120.1 (C-2), 110.2 (C-2' and C-6'), 77.4 (C-4), 68.2 (C-5), and 66.1 (C-6).

Anal. Calcd for $C_{13}H_{12}O_{10}H_2O$: C, 45.09; H, 4.05. Found: C, 45.15; H, 4.12.

After isolation of **1**, the mother liquor was subjected to column chromatography on silica gel using chloroform / methanol / acetic acid (8 / 2 / 0.05, v / v) to give a light yellow syrup (1.8 g, fractions 10-19). The syrup, which was decolorized with charcoal, was found by HPLC-UV to be 75% **2** contaminated with 17% of **1** and 8% of gallic acid. After storage at 4 °C for several months syrupy **2** became a colorless solid, mp 52-55 °C. Attempts to purify the crystals were unsuccessful. The crystals gave $[\alpha]_D^{25}$ -5.5° (*c* 1.0, MeOH); ^{1}H NMR. (MeOH-d_4): δ 6.95 and 6.94 (s each, 2 H, H-2' and H-6'), 5.37 (ddd, 1 H, J 6.7, 6.7, 2.0 Hz, H-5), 5.04 (d, 1 H, J 2.0 Hz, H-4), 3.83 (d, 1 H, J 6.7 Hz, H-6_a), 3.81 (d, 1H, J6.7Hz, H_{6b}), and signals of low intensity that matched those of **1**. ^{13}C NMR (MeOH-d_4): δ 172.8 (C-1), 166.8 (C-7'), 153.6 (C-3), 146.1 (C-3' and C-5'), 139.7 (C-4'), 120.6 (C-1'), 119.7 (C-2), 110.0 (C-2' and C-6'), 75.2 (C-4), 71.3 (C-5), 60.9 (C-6) and signals of low intensity that matched those of **1**.

L-Ascorbyl 6-(3',4'-dihydroxy)benzoate (3). The title compound (**3**) was prepared and isolated by the procedure used to produce the 6-gallate ester (**1**). HPLC-UV analysis of the reaction mixture showed that the major product was the 6-ester (**3**). The minor product, which was assumed to be the 5-ester, was not isolated. The syrupy product crystallized from acetone/chloroform, and the first batch of crystals (3.42 g) was recrystallized from acetone to give **3** as colorless crystals (2.77 g, 44%), mp 231-233 °C (dec.), $[\alpha]_D^{25}$+39.8° (*c* 1.0, MeOH); ^{1}H NMR (acetone-d_6): δ 7.39 (d, 1 H, J 2.0 Hz, H-2'), 7.35 (dd, 1 H, J 8.2, 2.0 Hz,

H-6'), 6.75 (d, 1 H, J 8.2 Hz, H-5'), 4.75 (d, 1 H, J 1.9 Hz, H-4), 4.31 (dd, 1 H, J 1.9, 6.7 Hz, H-6_a), 4.24 (dd, 1 H, J 1.9, 5.8 Hz, H-6_b), and 4.16 (ddd, 1 H, J 1.9, 6.7, 5.8 Hz, H-5); ^{13}C NMR (CD_3OD): δ 173.2 (C-1), 167.9 (C-7'), 154.1 (C-3), 151.8 (C-3'), 146.1 (C-4'), 123.9 (C-5'), 122.3 (C-1'), 120.1 (C-2), 117.5 (C-6'), 115.9 (C-2'), 77.3 (C-4), 68.2 (C-5), and 66.1 (C-6).

Anal. Calcd for $C_{13}H_{12}O_9$: C, 50.00; H, 3.85. Found: C, 50.20; H, 3.52.

L-Ascorbyl 6-(4'-hydroxy)phenyl ether (4) and L-ascorbyl 6-(4'-methoxy)phenyl ether (5). The procedure to prepare 6-*O*-phenyl-L-ascorbic acid[8] was used with modification. To a stirred solution of sodium carbonate (6.36 g, 60 mmol) in water (25 mL) at 25 °C was added crystalline (mp 176-178 °C) 6-bromo-6-deoxy-L-ascorbic acid (4.78 g, 20 mmol) in small portions, followed by hydroquinone (4.4 g, 40 mmol). The solution thickened to a white paste, which gradually turned yellow after being stirred for 6.5 h under nitrogen. The mixture was adjusted to pH 0.5 with 3 M hydrochloric acid, the solution extracted with ethyl acetate (4 x 90 mL), and the organic phases were combined. The combined organic phase was washed with brine (3 x 50 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, the residue dissolved in acetone, and the solution mixed with diatomaceous earth (5 g) and dried under vacuum. The dried powder was extracted with hot benzene in a Soxhlet extractor to remove unreacted hydroquinone. Then the powder was extracted with ethyl ether, and the ether evaporated to give crude **4** (1.31 g). Crude **4** was decolorized and crystallized from ethyl ether to give a colorless solid (0.4 g, 7%) with mp 105-108 °C (dec.); $[\alpha]_D^{25}$+54.0° (*c* 0.8, MeOH); ^1H NMR (acetone-d_6): δ 6.90 (m, 4 H, Ar-H), 4.90 (d, 1 H, J 1.8 Hz, H-4), 4.25 (m, 1 H, H-5), 4.07 (m, 2 H, H-6); ^{13}C NMR (acetone-d_6): δ 170.1 (C-1), 152.3 (C-4'), 152.0 (C-1'), 150.9 (C-3), 119.4 (C-2), 116.1 (C-2', C-3', C-5' and C-6'), 75.5 (C-4), 69.6 (C-5), and 67.4 (C-6); EIMS *m/z*: 268 (M+).

Anal. Calcd for $C_{12}H_{12}O_7 \cdot H_2O$: C, 50.35; H, 4.90. Found: C, 50.57; H, 4.75.

Compound (**5**) was prepared in the same manner as **4** except the starting phenol was 4-methoxyphenol and the isolation method was different. After removal of ethyl acetate in vacuum, the syrupy residue was triturated with benzene to effect crystallization of **5**. The crystals (0.54g, 10%) were recrystallized twice, first from nitromethane, then from acetone-benzene to give pure **5**, mp 189-192 °C (dec.); $[\alpha]_D^{25}$ +68.0°(*c* 0.76, MeOH); ^1H NMR

(acetone-d_6): δ 6.90 (m, 4 H, H-2', H-3', H-5' and H-6'), 4.90 (d, 1 H, J 1.9 Hz, H-4), 4.28 (m, 1 H, H-5), 4.12 (m, 2 H, H-6), 3.74 (s, 3 H, OCH_3); ^{13}C NMR (acetone-d_6): δ 170.2 (C-1), 154.8 (C-4), 153.4 (C-1), 150.9 (C-3), 119.6 (C-2), 116.2 (C-3' and C-5'), 115.1 (C-2' and C-6'), 75.7 (C-4), 69.7 (C-5), 67.6 (C-6), and 55.5 (OCH_3); EIMS *m/z*: 282 (M+).

Anal. Calcd for $C_{13}H_{14}O_7$: C, 55.32; H, 4.96. Found: C, 55.14; H, 5.02.

Autoxidation determined by thiobarbituric acid (TBA). The method of Dahle et al.[9] was followed with methyl linolenate (Aldrich Chemical Co.) as substrate. The samples were prepared for autoxidation by adding 0.1 mL anhydrous ethanol containing 0.1 mg of test compound to 0.5 mL of methyl linolenate. After being mixed well with stirring, the ethanol was evaporated. Autoxidation was conducted in a forced-draft oven at 37 °C, and individual samples were removed at timed intervals for TBA assay. Standard curves were run using tetraethoxypropane (Aldrich Chemical Co.), which yields one equivalent of malonaldehyde under the conditions of the assay.[9]

Stability of 1 in water and ethanol solutions. Ester **1** was dissolved in 0.2 M sodium phosphate buffer at pH 8 and in 0.1 M potassium hydrogen phthalate buffer at pH 5 in 10 mL flasks. The buffered solutions were held at 25 °C, and aliquots (80 μL) were taken to monitor **1**, **2** and gallic acid by HPLC-UV. The ethanol solution (10 mL, 15mM) of **1** was stored and assayed in the same manner.

REFERENCES AND NOTES

1. This is Contribution No. 94-152J from the Kansas Agricultural Experiment Station.
2. Current Address: Proctor and Gamble Co., Health Care Center, PO Box 10078, Mason, OH 45040-9462.
3. Code of Federal Regulations (1996), **Title 21**, Parts 500-599, Subpart D. Paragraphs 582.3149 and 582.3660.
4. R.J. Lewis, Sr. *Food Additives Handbook*, Van Nostrand Reinhold, New York, 1989, pp 69 and 370.
5. S.P. Kochhar, and J.B. Rossell, *Food Antioxidants*, B.J.F. Hudson, Ed.; Elsevier Science Publishing Co., Inc., New York, 1990, pp 19-64.
6. J. Giese, *Food Tech.* **50**, 73 (1996).
7. R.C. Cousins, P.A. Seib, R.C. Hoseney, C.W. Deyoe, Y.T. Liang, and D.W. Lillard, Jr., *J. Am. Oil Chem. Soc.*, **54**, 308 (1977).
8. G.C. Andrews, *Carbohydr. Res.*, **134**, 321 (1984).
9. L.K. Dahle, E.G. Hill, and R.T. Holman. *Arch. Biochem. Biophys.*, **98**, 253 (1962).
10. P. Cunniff, ed. *Official Methods of Analysis of AOAC*, 16th Ed., Vol II, Method No. 41.1.16, AOAC, Inc., Gaithersburgh, MD, 1996, p. 9B.

11. H.-D. Belitz and W. Grosch, *Food Chemistry*, Springer-Verlag, New York, 1987, p189.
12. W. Meyer zu Reckendorf, *Tetrahedron* **19,** 2033(1963).