Design of Novel Hybrid Vitamin C Derivatives: Thermal Stability and Biological Activity

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Novel hybrid L-ascorbic acid (vitamin C) derivatives with other biologically active substances, 5-hydroxy-2-hydroxymethyl-beta-pyrene (kojic acid) and z-tocopherol (vitamin E), linked at the C-2 or C-3 hydroxyl group were synthesized, and their thermal stability and inhibitory effects on tyrosinase activity, active oxygen species (AOS), and free radicals were estimated in vitro. It was found that a hydrophilic derivative, 2-O-(5-hydroxy-4H-pyran-4-one-2-methyl)-L-ascorbic acid (I), exhibited good thermal stability and inhibitory activities against tyrosinase-catalyzed melanin formation, AOS, and free radicals compared to vitamin C and its conventional derivatives (such as the 2-phosphate, 6-steareate and 2,6-dipalmitate, and 2-O-octadeclascorbic acid), as well as vitamin E, kojic acid, and arbutin. It is apparent that I has the biological properties of vitamin C and kojic acid, and acts synergistically. The hydroxyl groups at the C-3 position of the vitamin C moiety and the C-5 position of the kojic acid moiety are critical for the biological activities. We consider that the kojic acid moiety of I counterbalances the diminution of the biological activity due to shielding of the biologically important C-2 hydroxyl group of the vitamin C moiety. In addition, the thermal stability was significantly improved relative to not only vitamin C but also kojic acid. Further, a lipophilic derivative, 3-O[(z-tocopheryl-2-hydroxypropyl)-L-ascorbic acid, 2, was far more stable than vitamin C and its typical lipophilic derivatives. Compound 2 exhibited almost the same inhibitory activities against tyrosinase-catalyzed melanin formation, AOS, and free radicals as typical lipophilic derivatives, although these biological activities of 2 were lower than those of vitamin C.

Key words L-ascorbic acid; kojic acid; z-tocopherol; melanogenesis; tyrosinase; active oxygen species

Recently, depletion of the ozone layer in the stratosphere has allowed an increasing amount of solar ultraviolet (UV) radiation (290—400 nm) to reach the Earth’s surface. UV radiation has been shown to generate active oxygen species (AOS) such as superoxide anion radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen or free radicals in living organisms.3 AOS and free radicals derived from the biological utilization of O2 are responsible for oxidative injury to enzymes, lipid membranes, protein, and DNA in living cells,2 and participate in the development or exacerbation of various kinds of diseases.3 Furthermore, oxidative stress or damage induced by free radicals is related to the aging process called dermatoheliosis.4 Tyrosinase (tyrosine hydroxylase, dopa oxidase, and 5,6-dihydroxyindole oxidase) activity is enhanced by AOS, resulting in excess melanin formation,5 which is a natural biological protecting mechanism, though melanin peroxidizes phospholipid in the presence of Fe2+, resulting in cell death among dopaminergic neurons.6 Under normal conditions, cells and tissues are protected from the attack of AOS and free radicals by various enzymes such as superoxide dismutase (SOD), catalase, and peroxidase, as well as z-tocopherol (vitamin E), glutathione, and L-ascorbic acid (vitamin C).3,7 It is known that vitamin C (3) scavenges AOS and free radicals as a chain-breaking antioxidant9 and inhibits tyrosinase-catalyzed melanin formation by reduction of the intermediate, dopaquinone (z-aminoo-3,4-dioxo-1,5-cyclohexadiene-1-propionic acid), as well as cumelanin, in melanogenesis.9 Furthermore, there is considerable evidence that vitamin C is important in the prevention of a large number of chronic diseases, such as cancer, cerebral apoplexy, diabetes, atopic dermatitis, myocardial infarction, and AIDS.10 These characteristic biological activities of vitamin C are derived from the enediol structure with its strong electron-donating ability. Vitamin C is reversibly metabolized to dehydro-L-ascorbic acid via monodehydro-L-ascorbic acid in a series of oxidative processes (Chart 1). The redox reactions among these three ascorbates are closely related to their biological activity as vitamin C.

The well-known susceptibility of vitamin C to thermal and oxidative degradation has led to interest in derivatives with increased stability in vitro, while maintaining the inherent biological activity in vivo. In particular, the chemical modification of hydroxyl groups of vitamin C is of interest, and numerous stable derivatives of vitamin C have been reported.11-21 Generally, the partial modification of the enediol system gives two isomers, both of which show markedly decreased reducing power and are therefore stabilized against oxidation, and vitamin C activities tend to be reduced with increasing number of substituents in the molecule.11 Among these derivatives, the magnesium salt of ascorbic acid 2-phosphate (APC-3, 14) is available as a hydrophilic antioxidant9,12,13 (Fig. 1). Furthermore, 2-O-µ-D-glucopyranosylascorbic acid (AA-2G) has good stability and a potent inhibitory effect on tyrosinase in vivo.14 Ascorbic acid 6-steareate (15) and 2,6-dipalmitate (16) have been widely used as lipophilic antioxidants15 (Fig. 1). These compounds are relatively stable in vitro and exhibit vitamin C activity after enzymatic degradation to free vitamin C by phosphatase, glucosidase, or esterase in vivo. 3-O-(1-Dodecylcarboxylmethyl)ascorbic acid (HX-0112)16 and 2-O-octadeclascorbic acid (CV-3611, 17)17 have been developed as new lipophilic antioxidants, which exhibit vitamin C activity without enzymatic degradation.

It is well-known that vitamin E (11) acts as a synergistic antioxidant in conjunction with vitamin C to suppress lipid

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peroxidation in the cell membrane. Recently, two more effective inhibitors of tyrosinase, kojic acid (5-hydroxy-2-hydroxymethyl-β-pyrene, 6) and arbutin (hydroquinone-β-D-glucopyranoside, 18) have been reported (Fig. 1). Kojic acid, isolated from fermentative products of Aspergillus species, inhibits tyrosinase due to chelation of its copper, which is indispensable for tyrosinase activity. Arbutin, isolated from dried leaves of Bergenia crassifolia Fritsch (Saxifragaceae), suppresses the expression and synthesis of tyrosinase.

Based on this background, we designed and synthesized a novel series of hybrid vitamin C derivatives linking these biologically active substances (kojic acid and vitamin E) at the C-2 or C-3 hydroxyl group, which are crucial for both stability and biological activity, with a view to simultaneously improving the stability and preventing diminution of activity. The stability and inhibitory effects of the products on tyrosinase activity, and on AOS and free radicals in vitro, were evaluated.

**Results and Discussion**

**Synthesis** 2-O-(5-Hydroxy-4H-pyran-4-one-2-methyl)-L-ascorbic acid (1) was synthesized in five steps from kojic acid (6) as shown in Chart 2. Selective protection of the hydroxyl group at the C-5 position as the benzyl ether was carried out by Kawase’s method to afford 7 in 87% yield. Successive chlorination of 7 by using the CCl₄-PPh₃ system exclusively gave 8 in 73% yield, whereas chlorination with thionyl chloride resulted in partial allylic rearrangement. Condensation of 8 with 3-O-benzyl-5,6-0-isopropylidene-L-ascorbic acid (5) in the presence of K₂CO₃ in dimethyl formamide (DMF) afforded 9 in 92% yield. Removal of protecting groups was achieved by acid hydrolysis followed by catalytic hydrogenation to afford the hydrophilic derivative 1 in 98% and 84% yield, respectively.

Compound 5 was synthesized in two steps from L-ascorbic acid (3) as shown in Chart 3. The hydroxyl groups at the C-5 and C-6 positions were protected with acetone by Jung’s method to yield 5,6-O-isopropylidene-L-ascorbic
acid (4) in 75% yield.\textsuperscript{25} Compound 4 was protected as the benzyl ether by using benzyl bromide in the presence of K$_2$CO$_3$ in DMF to give 5 in 47% yield.

3-O-[3-[[3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-yl]oxy]-2-hydroxypropyl]-L-ascorbic acid (2) was synthesized in three steps from vitamin E (11) as shown in Chart 4. Alkylation of 11 with S-(+)-epichlorohydrin in the presence of NaOH in dioxane gave the corresponding glycidyl ether (12) in 92% yield. Use of a lower substrate concentration (<1 m) gave the chlorohydrin derivative simultaneously. Condensation of 12 with the vitamin C derivative 4 in the presence of NaHCO$_3$ and a catalytic amount of 4-dimethylaminopyridine (DMAP) in dioxane afforded 13 in 60% yield. Subsequent removal of the protecting group was achieved by acid hydrolysis to yield the lipophilic derivative 2 almost quantitatively.

Physicochemical, Biological, and Photophysical Prop-
Chart 5. Mechanism of Melanogenesis

Table 1. Stability of Vitamin C Derivatives in Aqueous Solution

<table>
<thead>
<tr>
<th>Compound</th>
<th>Remaining (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>1 month</th>
<th>2 months</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.4</td>
<td>85.1</td>
<td>80.0</td>
<td></td>
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<tr>
<td>2</td>
<td>88.5</td>
<td>82.3</td>
<td>79.8</td>
<td></td>
</tr>
<tr>
<td>14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
<td>98.9</td>
<td>95.5</td>
<td></td>
</tr>
<tr>
<td>15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.5</td>
<td>17.3</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td>16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.2</td>
<td>22.3</td>
<td>21.7</td>
<td></td>
</tr>
<tr>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.2</td>
<td>77.7</td>
<td>37.1</td>
<td></td>
</tr>
<tr>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.2</td>
<td>80.8</td>
<td>46.9</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The test compound was dissolved in EtOH: H₂O = 1:1 (v/v) to give a concentration of 1 mol%.<br> <sup>b</sup> The resulting solution was stored at 60 °C for 3 months, and the decrease in concentration was measured by HPLC. <sup>c</sup> Reagents (Wako Pure Chemical Industries Ltd.) were used without purification. <sup>d</sup> Reagents (Tokyo Chemical Industry Co., Ltd.) were used without purification.

Table 2. Inhibitory Activity against Tyrosinase-Catalyzed Oxidation of L-Tyrosine

<table>
<thead>
<tr>
<th>Inhibitor&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Inhibition (%)&lt;sup&gt;f&lt;/sup&gt;</th>
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<tr>
<td>1</td>
<td>90.6</td>
</tr>
<tr>
<td>2</td>
<td>43.0</td>
</tr>
<tr>
<td>14&lt;sup&gt;i&lt;/sup&gt;</td>
<td>57.1</td>
</tr>
<tr>
<td>15&lt;sup&gt;i&lt;/sup&gt;</td>
<td>31.4</td>
</tr>
<tr>
<td>16&lt;sup&gt;i&lt;/sup&gt;</td>
<td>39.7</td>
</tr>
<tr>
<td>17&lt;sup&gt;i&lt;/sup&gt;</td>
<td>37.1</td>
</tr>
<tr>
<td>18&lt;sup&gt;i&lt;/sup&gt;</td>
<td>50.1</td>
</tr>
<tr>
<td>19&lt;sup&gt;i&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>20&lt;sup&gt;i&lt;/sup&gt;</td>
<td>52.4</td>
</tr>
<tr>
<td>21&lt;sup&gt;i&lt;/sup&gt;</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>3 + 6&lt;sup&gt;j&lt;/sup&gt;</td>
<td>84.7</td>
</tr>
<tr>
<td>3 + 11&lt;sup&gt;j&lt;/sup&gt;</td>
<td>48.5</td>
</tr>
</tbody>
</table>

<sup>e</sup> A 10⁻³ m sample solution was added to 0.3 mg/ml l-tyrosine aqueous solution in buffer solution (pH 6.8). <sup>f</sup> The resulting solution was added to 1.0 mg/ml tyrosinase aqueous solution, and incubated at 37°C for 20min. The absorbance of the reaction mixture was measured at 475 nm, and the inhibition was calculated. <sup>i</sup> Reagents (Wako Pure Chemical Industries Ltd.) were used without purification. <sup>j</sup> This was synthesized by Kato's method. <sup>k</sup> Reagents (Tokyo Chemical Industry Co., Ltd.) were used without purification. <sup>l</sup> A mixed solution of 3 (5 x 10⁻⁴ m) and another substrate (5 x 10⁻⁵ m) was added to 0.3 mg/ml l-tyrosine aqueous solution in buffer solution (pH 6.8).

The inhibitory activity of test compounds was estimated at 10⁻³ M. It was found that 1 inhibited potent inhibitory activity (inhibition = 90.6%) compared to 3 and its conventional derivatives 14, 15, 16, and 17 (inhibition < 60%). Compound 14 was remarkably superior to arbutin (18) (inhibition = 52.4%), but slightly inferior to 6 (inhibition = 100%). Furthermore, it was found that 1 exhibited almost the same inhibitory activity as a combined system of 3 and 6 corresponding to an equal molar amount to 1 (inhibition = 84.7%). On the other hand, the inhibitory activity of 2 (inhibition = 43.0%) was slightly lower than that of 3 (inhibition = 50.1%) or that of a combined system of 3 and 11 corresponding to an equal molar amount to 2 (inhibition = 48.5%).

The results on inhibitory activity against tyrosinase-catalyzed oxidation of L-dopa are shown in Table 3. The inhibitory activity of test compounds was evaluated at 3 x 10⁻⁴ M against tyrosinase, and the 50%-inhibition concentration (IC₅₀) was also estimated. It was found that 1 exhibited almost equivalent inhibitory activity (inhibition...
potent inhibitory activity against tyrosinase-catalyzed oxidation of both L-tyrosine and L-dopa. It seems that its high inhibitory activity on melanogenesis is caused by the synergistic effect of the vitamin C and kojic acid moieties; the 3-enol structure of the vitamin C moiety presumably reduces dopaquinone to regenerate dopa, while the 5,6-enol structure of the kojic acid moiety deactivates tyrosinase due to chelation of its copper$$^{22}$$ (Fig. 2). This synergistic effect was found in a combined system of 3 and 6 (Tables 2 and 3). Consequently, the action of the kojic acid moiety counterbalances the diminution of the tyrosinase-inhibitory activity that arises from blocking of the 2-hydroxyl group of the vitamin C moiety. On the other hand, it seems that the low inhibitory activity of 2 is caused by the blocking of the key 3-hydroxyl group of the vitamin C moiety. Vitamin E (11) did not suppress tyrosinase-catalyzed oxidation of L-tyrosine and L-dopa at all, as shown in Table 3.

The reducing activity of 1 and 2 was measured by use of a stable radical, 2,2'-diphenyl-1-picyrlhydrazyl (DPPH), in vitro, and the results are shown in Table 4. At a high concentration (10$$^{-3}$$M) corresponding to ten molar amounts with respect to DPPH, 1 and 2 both exhibited

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Table 3. Inhibitory Activity against Tyrosinase-Catalyzed Oxidation of L-Dopa

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Inhibition (%)$^{(b)}$</th>
<th>IC$_{50}$, $10^{-3}$ M$^{(a)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43.9</td>
<td>0.37</td>
</tr>
<tr>
<td>2</td>
<td>11.4</td>
<td>23.49</td>
</tr>
<tr>
<td>14$^{(c)}$</td>
<td>5.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>15$^{(d)}$</td>
<td>3.5</td>
<td>0.93</td>
</tr>
<tr>
<td>16$^{(e)}$</td>
<td>18.9</td>
<td>11.68</td>
</tr>
<tr>
<td>17$^{(f)}$</td>
<td>6.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3$^{(g)}$</td>
<td>38.2</td>
<td>0.47</td>
</tr>
<tr>
<td>6$^{(h)}$</td>
<td>39.2</td>
<td>0.18</td>
</tr>
<tr>
<td>18$^{(i)}$</td>
<td>12.8</td>
<td>20.00</td>
</tr>
<tr>
<td>11$^{(j)}$</td>
<td>&lt;1.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3 + 6$^{(k)}$</td>
<td>44.2</td>
<td>0.36</td>
</tr>
<tr>
<td>3 + 11$^{(l)}$</td>
<td>26.8</td>
<td>1.02</td>
</tr>
</tbody>
</table>

$^{(a)}$ Sample solutions ($10^{-4}$, $3 \times 10^{-4}$, and $10^{-3}$ M) were added to 0.5 mg/ml L-dopa aqueous solution in buffer solution (pH 6.8). $^{(b)}$ The resulting solution was added to 1.0 mg/ml tyrosinase aqueous solution, and incubated at 37°C for 1 min. The absorbance of the reaction mixture was measured at 475 nm, and the inhibition was calculated. $^{(c)}$ The 50%-inhibitory concentration was calculated from the plot of inhibition against log M. $^{(d)}$ Reagents (Wako Pure Chemical Industries Ltd.) were used without purification. $^{(e)}$ This was synthesized by Kato's method.$^{19}$ $^{(f)}$ Reagents (Tokyo Chemical Industry Co., Ltd.) were used without purification. $^{(g)}$ A mixed solution of 3 ($5 \times 10^{-4}$ M) and another substrate ($5 \times 10^{-4}$ M) was added to 0.3 mg/ml L-tyrosine aqueous solution in buffer solution (pH 6.8).

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Table 4. Reducing Activity against 2,2'-Diphenyl-1-picyrlhydrazyl

<table>
<thead>
<tr>
<th>Scavenger</th>
<th>Inhibition (%)$^{(b)}$</th>
<th>IC$_{50}$, $10^{-3}$ M$^{(a)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>97.2</td>
<td>10.0</td>
</tr>
<tr>
<td>2</td>
<td>95.3</td>
<td>50.1</td>
</tr>
<tr>
<td>15$^{(c)}$</td>
<td>97.0</td>
<td>18.8</td>
</tr>
<tr>
<td>16$^{(d)}$</td>
<td>68.6</td>
<td>281.8</td>
</tr>
<tr>
<td>17$^{(e)}$</td>
<td>96.4</td>
<td>89.1</td>
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<tr>
<td>3$^{(f)}$</td>
<td>97.2</td>
<td>17.8</td>
</tr>
<tr>
<td>6$^{(g)}$</td>
<td>14.0</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>11$^{(h)}$</td>
<td>97.0</td>
<td>11.6</td>
</tr>
</tbody>
</table>

$^{(a)}$ The test compound in DMF was added to $10^{-3}$ M DPPH in ETOH to prepare $10^{-5}$, $10^{-3}$, $10^{-1}$, and $10^{-3}$ M sample solutions. $^{(b)}$ The resulting solution was stirred at 25°C for 20 min. The absorbance (OD) of the reaction mixture was measured at 517 nm, and the inhibition was calculated. $^{(c)}$ The 50%-inhibitory concentration was calculated from the plot of OD against --log M. $^{(d)}$ Reagents (Wako Pure Chemical Industries Ltd.) were used without purification. $^{(e)}$ This was synthesized by Kato's method.$^{19}$ $^{(f)}$ Reagents (Tokyo Chemical Industry Co., Ltd.) were used without purification.

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Fig. 2. Proposed Inhibition Mechanism of Tyrosinase-Catalyzed Melanin Formation by 1
almost the same reducing activity as 3, a conventional derivative 15, and a typical antioxidant, 11 (inhibition >95%). In addition, it was found that the IC$_{50}$ of 1 (10$^{-5}$ M) was almost the same as that of 3 (1.8 × 10$^{-5}$ M), 15 (1.9 × 10$^{-5}$ M), and 11 (1.2 × 10$^{-5}$ M). Furthermore, 1 exhibited far higher reducing activity than other conventional derivatives, 16 and 17. It is apparent that its high reducing activity is not caused by the kojic acid moiety, since kojic acid 6 exhibited little reducing activity towards free radicals, as shown in Table 4. On the other hand, the IC$_{50}$ of 2 (5 × 10$^{-5}$ M) was far higher than that of 3, namely the reducing activity was low. It seems that this low reducing activity is caused by shielding of the most active sites against free radicals, the 3-hydroxyl group of the vitamin C moiety and the 6-hydroxyl group of the vitamin E moiety, although 11 exhibited considerable reducing activity (IC$_{50}$ = 1.2 × 10$^{-5}$ M).

The scavenging activity of 1 and 2 against AOS was measured by the nitro blue tetrazolium (NBT) method depending on enzymatic superoxide anion generation in vitro, and the results are shown in Table 5. The typical scavenger SOD (Cu, Zn-form; M.W. ca. 32000) almost completely scaved AOS generated by the xanthine-xanthine oxidase (XOD) system at 10$^{-6}$ M. However, the scavenging activity of other test compounds was too low to allow calculation of the IC$_{50}$ at concentrations of less than 10$^{-3}$ M. Therefore, the scavenging activity of each compound was estimated in terms of the inhibition at 10$^{-6}$ M. We found that 1 exhibited almost the same scavenging activity (inhibition = 7.0%) as 3 (inhibition = 5.0%), as did its conventional derivatives 15, 16, and 17. It seems that its high scavenging activity arises from a synergistic effect of vitamin C and kojic acid, which exhibited only a slight scavenging activity (inhibition = 3.1%). On the other hand, the scavenging activity of 2 (inhibition = 1.8%) was far lower than that of 3. This may be because of shielding of the most active sites, i.e., the 3-hydroxyl group of the vitamin C moiety and the 6-hydroxyl group of the vitamin E moiety, although 11 exhibited considerable scavenging activity (inhibition = 10.8%).

The UV absorption spectra of vitamin C (3), kojic acid (6), and 1 are shown in Fig. 3. Compounds 3 and 6 exhibited characteristic absorptions at 258, 218 and 268 nm respectively. Compound 1 exhibited absorptions corresponding those of 3 and 6 at 262 and 217 nm, respectively.

**Conclusion**

To improve the thermal stability and simultaneously to avoid diminution of the activity of vitamin C, we designed a novel series of hybrid vitamin C derivatives in which these biologically active substances (kojic acid and vitamin E) are linked at the C-2 or C-3 hydroxyl group, which predominantly influence both stability and activity. Among these derivatives, 1 exhibited high thermal stability, moderate inhibitory activity against tyrosinase-catalyzed melanin formation, and moderate scavenging activity towards AOS and free radicals in vitro compared to other typical inhibitors and scavengers. It is apparent that 1 has the biological properties of both vitamin C and kojic acid. We regard 1 as a promising hydrophilic vitamin C derivative with high thermal stability and the characteristic biological activity of vitamin C.

The lipophilic derivative 2 was far more stable than vitamin C and its typical lipophilic derivatives. Furthermore, 2 exhibited almost the same inhibitory activity against tyrosinase-catalyzed melanin formation and scavenging activities towards AOS and free radicals as those of typical lipophilic derivatives, although these activities of 2 were weaker than those of vitamin C. It seems that the low biological activity of 2 is caused by the masking of the biologically active sites, the 3-hydroxyl group of the vitamin C moiety and the 6-hydroxyl group of the vitamin E moiety.

**Experimental**

All of the solvents and reagents used were of reagent grade; in cases where further purification was required, the standard procedures were followed. TLC was performed on precoated Silica-gel 60F$_{254}$ plates (Art. 5554, E. Merck). Silica gel (300—200 mesh, Wakoigel C-300) was used for Silica-gel chromatography, and the ratio of silica gel to the compound was in the range of 30:1—100:1. Elemental analyses were performed by the Advanced Center for Chemical Analysis of Ehime
3.0-Benzyl-2-O-(5-benzyloxy-4H-pyr-4-an-1-2-methyl)-t-ascorbic Acid (10) A solution of 9 (17.6 g, 33.8 mmol) in THF (50 mL) was treated with 35% HCl (5 mL) at room temperature. The mixture was stirred for 1 h at room temperature. The reaction was quenched by the addition of saturated NaHCO₃ solution. An aqueous layer was extracted with AcOEt. The combined layers were washed with brine and dried over anhydrous Na₂SO₄. After the solvent was evaporated in vacuo, the residue was chromatographed (SiO₂: hexane: AcOEt = 1:4) to give 10 (15.9 g, 98% yield): mp 150–152°C. 1H-NMR (CDCl₃) δ: 3.85 (2H, m, J₁₂₃ = 6.72 Hz), 4.22 (1H, ddd, J₃₄₅ = 5.66 Hz, J₄₅₆ = 6.72 Hz), 4.55 (1H, d, J₃₄₅ = 5.66 Hz), 4.35 (2H, s, kjoic acid C-5,CH₃ 5.04 (2H, s, benzyl), 5.39 (2H, s, benzyl), 6.41 (1H, s, kjoic acid-C-6), 7.35 (5H, m, aromatic), 7.49 (1H, s, kjoic acid-C-3). 13C-NMR (CDCl₃) δ: 61.9 (C-6), 68.4 (C-5), 68.5 (kjoic acid C-5,CH₃), 72.3, 74.7, 75.7 (C-4), 112.5 (kjoic acid-C-3), 121.84 (C-14), 127.4, 127.6, 127.7, 127.8, 128.0, 128.1, 128.4, 128.5, 128.7, 137.6, 137.8, 138.5, 139.9 (kjoic acid-C-6), 146.1 (kjoic acid-C-5), 155.3 (C-2), 162.6 (kjoic acid-C-2), 166.8 (C-1), 173.9 (kjoic acid-C-1). Anal. Calcd for C₃₀H₂₄O₉C: 65.0, H: 5.0. Found: C: 64.6; H: 4.99.

2-O-(5-Hydroxy-4H-pyran-4-one-2-methyl)-t-ascorbic Acid (1) A solution of 10 (15.9 g, 33.1 mmol) in MeOH (100 mL) containing 5% Pd-C (1.5 g) was stirred for 3 h at room temperature under a hydrogen atmosphere. After the hydrogenation had been completed, the filtrate was evaporated to dryness in vacuo. The residue was dissolved in H₂O and this solution was washed with AcOEt. The aqueous layer was evaporated in vacuo and the residue was recrystallized from acetone to give 11 (3.8 g, 84% yield): mp 222–224°C. 1H-NMR (H₂O, 400 MHz) δ: 262.0 (3.96), 217.0 (3.9). 1H-NMR (CDCl₃) δ: 3.45 (2H, m, J₁₂₃ = 6.72 Hz), 3.80 (1H, ddd, J₃₄₅ = 5.66 Hz, J₆₇₈ = 6.66 Hz, J₆₇₈ = 6.72 Hz, 4.83 (3H, s, kjoic acid-C-6), 6.12 (1H, s, kjoic acid-C-5), 6.18 (1H, s, kjoic acid-C-3), 6.98 (1H, s, kjoic acid-C-1), 9.01–9.40 (1H, s, OH), 13.34 (kjoic acid-C-1). 13C-NMR (CDCl₃) δ: 61.8 (C-6), 68.4 (C-5), 68.5 (kjoic acid C-5,CH₃), 74.9 (C-4), 112.6 (kjoic acid-C-3), 119.2 (C-2), 139.8 (kjoic acid-C-4), 146.0 (kjoic acid-C-5), 160.4 (C-4), 162.6 (kjoic acid-C-2), 169.2 (C-1), 173.8 (kjoic acid-C-1). IR (KBr) cm⁻¹: 3440, 3325, 3170, 2930, 2926, 1764, 1760, 1648, 1640, 1577, 1215, 1138, 1044. Anal. Calcd for C₃₀H₂₄O₉C: 64.0, H: 4.91, C: 68.1, H: 4.99, C: 68.5. 

2-Hydroxy-5,7,8-tetramethyl-6-(oxiranylmethoxy)-2,4,8,12-trimethyltridecyl-2H-1-benzopyran (12) Finely powdered NaOEt (0.2 g, 4 ml) was added to a mixture of 11 (1.8 g, 41 mmol) and (S)-(+)-p-chlorohydrin (1.9 g, 20.5 mmol) in dioxane (3 ml). The mixture was heated under reflux and vigorously stirred for 1 h, then diluted with H₂O (2-fold) and extracted with ether. The combined layers were thoroughly washed with brine, dried over anhydrous Na₂SO₄, and evaporated in vacuo. The residue was chromatographed (SiO₂: hexane: AcOEt = 10:1) to give 12 (1.8 g, 92% yield): 1H-NMR (CDCl₃) δ: 11.9 (C-5,Me), 12.1 (C-7,Me), 13.0 (C-8,Me), 19.7 (C-4,Me), 19.8 (C-8,Me), 20.6 (C-4), 21.1 (C-2), 22.7 (C-13,Me), 22.8 (C-2,Me), 24.5 (C-6), 24.9 (C-10), 28.0 (C-12), 31.0 (C-3), 32.8 (C-4), 32.9 (C-8), 37.4 (C-3, 5, 7, 9), 37.5 (C-11), 40.4 (C-1), 64.2 (OCH₂), 69.8 (OCH₂), 70.3 (OCH), 74.7 (C-2), 117.8 (C-7), 123.4 (C-8), 125.7 (C-5), 127.4 (C-14), 127.5 (C-6), 146.2 (C-9), 147.5 (C-4,6), 158.2 (C-1), 160.5 (C-11), 162.6 (kjoic acid-C-2), 166.8 (C-1), 173.6 (kjoic acid-C-3). Anal. Calcd for C₃₀H₂₄O₉C: 78.86; H: 11.18. Found: C: 78.7; H: 10.94.

3-O-[3-[3,4-Dihydro-2,5,7,8-tetramethyl-2-(4,4,12-trimethyltridecyl)-2H-1-benzopyran-6-yloxy]-2-hydroxypropyl]-5,6-Isopropylidendene-1,4-Ascorbic Acid (13) A mixture of 4 (4.3 g, 19.9 mmol), 12 (8.1 g, 16.6 mmol), and 4-dimethylaminopropionic acid (0.2 g, 1.6 mmol) in dioxane (40 ml) was treated with NaHCO₃ (1.2 g, 16.7 mmol) and the whole was washed with reflux and vigorously stirred for 24 h. The reaction mixture was washed with H₂O (2-fold) and extracted with AcOEt. The combined layers were thoroughly washed with brine, dried over anhydrous Na₂SO₄, and evaporated in vacuo. The residue was chromatographed (SiO₂, hexane: AcOEt = 5:1) to give 13 (7.0 g, 60% yield): 1H-NMR (CDCl₃) δ: 11.9 (tocopherol C-5,Me), 12.4 (tocopherol C-7,Me), 13.0 (tocopherol C-9,Me), 19.4 (tocopherol C-4,Me), 19.6 (tocopherol C-6,Me), 20.9 (tocopherol C-4), 20.9 (tocopherol C-2), 22.5 (tocopherol C-13,12,Me), 23.4 (tocopherol C-2,Me), 24.3 (tocopherol C-6), 24.7 (tocopherol C-10), 25.4 (acetone), 25.5 (acetone), 27.8 (tocopherol C-12), 31.1 (tocopherol C-3), 32.7 (tocopherol C-4), 32.9 (tocopherol C-8), 37.3 (tocopherol C-7, 5, 7, 9), 39.3 (tocopherol C-11), 40.3 (tocopherol C-1), 63.0 (OCH₂), 68.1 (OCH₂), 69.2 (OCH₂), 69.8 (C-6), 72.2 (C-5), 74.7 (CH₃), 124.9 (C-14), 127.4 (C-7), 129.0 (C-2), 123.0 (tocopherol C-8), 125.8 (tocopherol C-5), 127.4 (tocopherol C-4a), 147.3 (tocopherol C-6a), 148.0 (tocopherol C-8a), 151.1.
buttermilk (≥0.5 units/mg protein) in 0.1% (w/v) bovine serum albumin (BSA) aqueous solution (1.6×10^{-2} mol/l) was added to the above mixture, and the whole was incubated at 25°C. The enzymatic reaction was stopped at the end of 20 min with 0.6 mol CuCl_{2} aqueous solution (0.1 mol/l), and the absorbance of the reaction mixture was measured at 560 nm, the absorbance maximum of diformazan. The difference in absorbance from the control, without test compound, was taken as the scavenging activity. The results are shown in Table 5.

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References


