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# Antioxidant Constituents from Rhubarb: Structural Requirements of Stilbenes for the Activity and Structures of Two New Anthraquinone Glucosides

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**Abstract**—The methanolic extracts from five kinds of rhubarb were found to show scavenging activity for DPPH radical and $\cdot O_2^-$. Two new anthraquinone glucosides were isolated from the rhizome of *Rheum undulatum* L. together with two anthraquinone glucosides, a naphthalene glucoside, and 10 stilbenes. In the screening test for radical scavenging activity of rhubarb constituents, stilbenes and a naphthalene glucoside showed activity, but anthraquinones and sennosides did not. In addition, most stilbenes inhibited lipid peroxidation of erythrocyte membrane by *tert*-butyl hydroperoxide. Detailed examination of the scavenging effect on various related compounds suggested the following structural requirements; 1) phenolic hydroxyl groups are essential to show the activity; 2) galloyl moiety enhances the activity; 3) glucoside moiety reduces the activity; 4) dihydrostilbene derivatives maintain the scavenging activity for the DPPH radical, but they show weak activity for $\cdot O_2^-$. In addition, several stilbenes with both the 3-hydroxyl and 4′-methoxyl groups inhibited xanthine oxidase. 

## Introduction

Rhubarbs, the rhizomes of *Rheum palmatum* L., *R. tanguticum* Maxim., *R. officinale* Baill., *R. coreanum* Nakai, and *R. undulatum* L., are used in remedies for blood stagnation syndrome (called 'Oketsu syndrome' in Japanese traditional medicine) as well as a purgative agent in Japanese, Korean, and Chinese traditional medicines. Among them, the rhizome of *R. undulatum*, a Korean rhubarb, is considered to have less purgative effect but more potent effect on Oketsu syndrome than other kinds of rhubarbs.[1] Previously, the nitric oxide (NO) production inhibitory activity in lipopolysaccharide-activated mouse macrophages, anti-platelet aggregation in rabbit platelets, and anti-allergic and anti-inflammatory effects in mice and rats were reported as its anti-Oketsu effect.[1–3] Many studies about the pharmacological properties and bioactive constituents of the former four rhubarbs, which are listed in Japanese Pharmacopoeia XIII, have been reported, but those of the latter rhubarb have not been studied sufficiently.

Active oxygen species and free radicals react with biomolecular constituents (e.g., lipids, protein, and DNA) to cause certain clinical diseases, such as cerebral ischemia, atherosclerosis, inflammation, diabetes, and cancer,[4–8] which are regarded as damage associated with Oketsu syndrome in Chinese traditional medicine. We examined the scavenging effects of the methanolic extract from the dried rhizome of *R. palmatum*, *R. tanguticum*, *R. officinale*, *R. coreanum*, and *R. undulatum* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and superoxide anion radical ($\cdot O_2^-$) generated by the xanthine–xanthine oxidase system and/or on lipid peroxidation by *tert*-butyl hydroperoxide (*t*-BuOOH) in the erythrocyte membrane ghost system. As a result, these extracts showed DPPH radical and $\cdot O_2^-$ scavenging activities and/or inhibition of lipid peroxidation. From the rhizome of *R. undulatum*, of which neither chemical nor pharmacological studies have been adequately reported, we isolated 10 known stilbene constituents from the active fraction together with a known naphthalene glucoside and two known and two new anthraquinone glucosides. This study dealt with the isolation and characterization of active constituents from the rhizome of *R. undulatum*, and structural requirements of active constituents for antioxidant activity.

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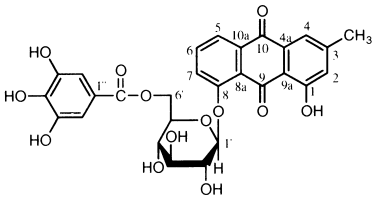
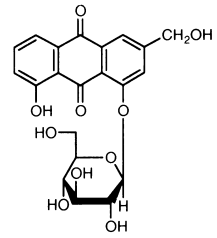
## Results and Discussion

### Isolation of chemical constituents from the rhizome of *R. undulatum*

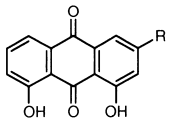
The dried rhizome of *R. undulatum* (5.8 kg, cultivated in Korea) was extracted with methanol at room temperature. The methanolic extract (33.8% from the natural medicines) was subjected to Diaion HP-20 column chromatography ($H_2O$→MeOH→acetone) to give the $H_2O$-eluted fraction (13.3%), MeOH-eluted fraction (18.7%), and acetone-eluted fraction (1.8%). The MeOH-eluted fraction was subjected to silica gel column chromatography [$CHCl_3$–MeOH (10:1, v/v)→(4:1)→ $CHCl_3$–MeOH–$H_2O$ (10:3:1, lower layer)→MeOH] to give five fractions (Fr. 1–Fr. 5). Each fraction was subjected to ODS column chromatography (MeOH–$H_2O$) and finally HPLC [YMC-pack R&D ODS-5-A, 250×20 mm i.d., MeOH–$H_2O$ or $CH_3CN$–$H_2O$] to give two new anthraquinone glucosides, chrysophanol 8-*O*-β-D-(6′-galloyl)-glucopyranoside (**1**, 0.092%) and aloe-emodin 1-*O*-β-D-glucopyranoside (**2**, 0.0065%) together with two anthraquinone glucosides: chrysophanol 1-*O*-β-D-



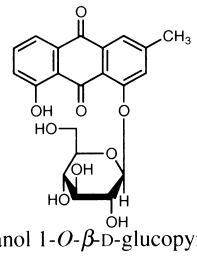
chrysophanol 8-*O*-β-D-(6'-galloyl)-glucopyranoside (**1**)



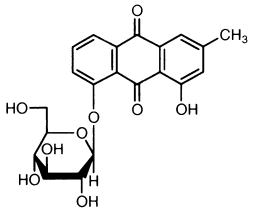
aloe-emodin 1-*O*-β-D-glucopyranoside (**2**)



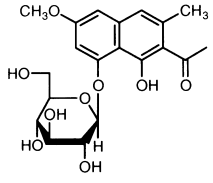
aloe-emodin (**3**) : R=$CH_2OH$
chrysophanol (**4**) : R=$CH_3$



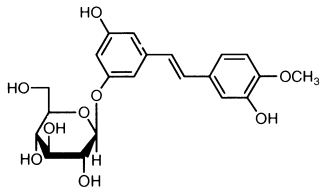
chrysophanol 1-*O*-β-D-glucopyranoside (**5**)



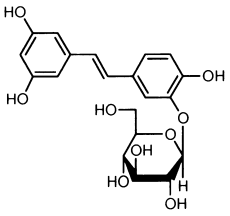
chrysophanol 8-*O*-β-D-glucopyranoside (**6**)



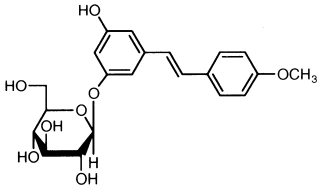
torachrysone 8-*O*-β-D-glucopyranoside (**7**)



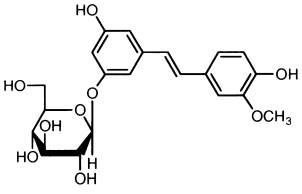
rhaponticin (**8**)



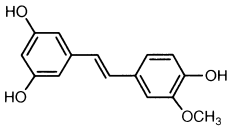
piceatannol 3'-*O*-β-D-glucopyranoside (**9**)



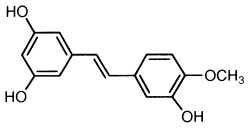
desoxyrhaponticin (**10**)



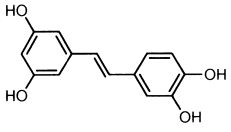
isorhapontin (**11**)



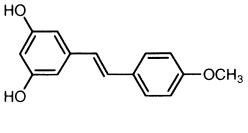
isorhapontigenin (**12**)



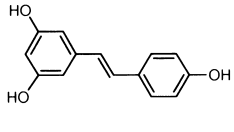
rhapontigenin (**13**)



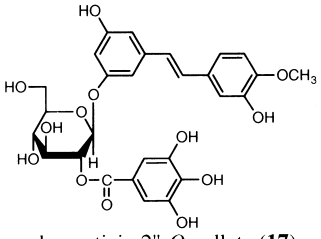
piceatannol (**14**)



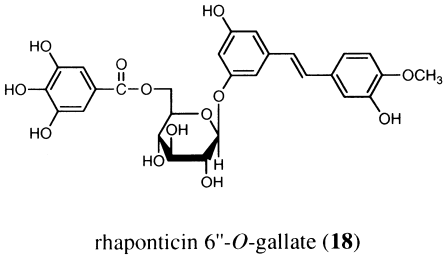
desoxyrhapontigenin (**15**)



resveratrol (**16**)



rhaponticin 2''-*O*-gallate (**17**)



rhaponticin 6''-*O*-gallate (**18**)

**Chart 1.**

glucopyranoside (**5**,[9] 0.25%) and chrysophanol 8-*O*-β-D-glucopyranoside (**6**,[9] 0.16%), a naphthalene glucoside: torachrysone 8-*O*-β-D-glucopyranoside (**7**,[10] 0.12%), and 10 stilbenes: rhaponticin (**8**,[11] 3.5%), piceatannol 3′-*O*-β-D-glucopyranoside (**9**,[11] 2.0%), desoxyrhaponticin (**10**,[11] 0.048%), isorhapontin (**11**,[12] 0.36%), rhapontigenin (**13**,[11] 0.58%), piceatannol (**14**,[11] 0.073%), desoxyrhap-ontigenin (**15**,[13] 0.015%), resveratrol (**16**,[14] 0.048%), rhaponticin 2″-*O*-gallate (**17**,[11] 0.12%), and rhaponticin 6″-*O*-gallate (**18**,[11] 0.087%).

### Structures of chrysophanol 8-*O*-β-D-(6′-galloyl)-glucopyranoside (1) and aloe-emodin 1-*O*-β-D-glucopyranoside (2)

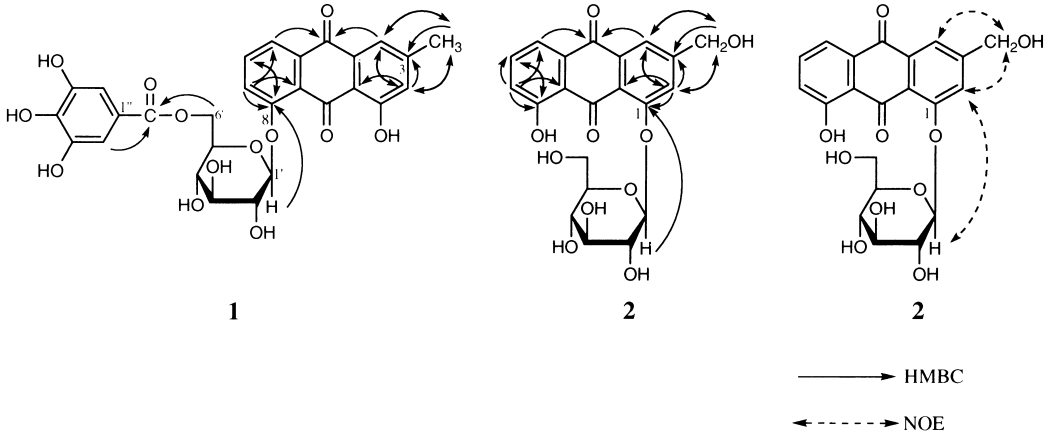
Chrysophanol 8-*O*-β-D-(6′-galloyl)-glucopyranoside (**1**) was isolated as yellow needles of mp 207–210 °C with positive optical rotation ($[\alpha]_D^{25}$ +95.0°). The positive-ion fast atom bonbardment (FAB)–MS of **1** showed a quasi-molecular ion peak at *m*/*z* 591 $(M+Na)^+$, while a quasi-molecular ion peak was observed at *m*/*z* 567 $(M-H)^-$ in the negative-ion FAB–MS. The molecular formula $C_{28}H_{24}O_{13}$ of **1** was determined from the quasimolecular ion peak $(M+Na)^+$ and by high-resolution MS measurement. In the UV spectrum of **1**, absorption maxima were observed at 220 (log ε 4.58), 260 (4.32), 284 (sh, 4.17), and 409 (3.79) nm, suggestive of an anthraquinone structure. The IR spectrum of **1** showed absorption bands ascribable to hydroxyl, free and chelated carbonyl, and aromatic functions at 3410, 1709, 1641, 1637, 1466 and 1075 $cm^{-1}$. The $^1H$ NMR (DMSO-$d_6$) and $^{13}C$ NMR (Table 1) spectra of **1** showed signals assignable to a tertiary methyl [δ 2.40 (s, 3-$CH_3$)], an anomeric [δ 5.24 (d, *J* = 7.7 Hz, Glc-1′-H)], a galloyl group [δ 6.99 (2H, s, galloyl-2″, 6″-H)], aromatic protons [δ 7.16 (1H, br s, 4-H), 7.46 (1H, br s, 2-H), 7.67 (1H, d, *J* = 8.6 Hz, 5-H), 7.72 (1H, dd, *J* = 7.6, 8.6 Hz, 6-H), 7.82 (1H, d, *J* = 7.6 Hz, 7-H)], and a chelated hydroxyl proton [δ 12.82 (br s, 1-OH)]. The proton and carbon signals in the NMR data of **1** were superimposable on those of chrysophanol 8-*O*-β-D-glucopyranoside (**6**), except for the signal due to a galloyl group. Comparison of the $^1H$ NMR and $^{13}C$ NMR (Table 1) spectra for **1** with those for **6** revealed an acylation shift around the 6′-position [δ 4.27, 4.50 (1H each, both m, Glc-6′-$H_2$)]. Alkaline treatment of **1** with 1.0% sodium methoxide (NaOMe)–methanol furnished **6**. Furthermore, in the heteronuclear multiple bond connectivity (HMBC) experiment of **1**, long-range correlations were observed between the following proton and carbon pairs: 1′-H and 8-C; 6′-H and galloyl carbonyl carbon [$\delta_c$ 165.6 (7″-C)], as shown in Figure 1. On the basis of this evidence, **1** was determined to be 6′-galloyl chrysophanol 8-*O*-β-D-glucopyranoside.

**Table 1.** $^{13}C$ NMR Data for chrysophanol 8-*O*-β-D-(6′-galloyl)-glucopyranoside (**1**) and aloe-emodin 1-*O*-β-D-glucopyranoside (**2**)[a]

| | 1 | 2 | | 1 | 2 |
|---|---|---|---|---|---|
| C-1 | 161.5 | 158.3 | Glc-1′ | 100.2 | 100.5 |
| C-2 | 119.3 | 119.2 | Glc-2′ | 73.2 | 73.2 |
| C-3 | 147.5 | 151.8 | Glc-3′ | 76.3 | 76.5 |
| C-4 | 123.9 | 118.0 | Glc-4′ | 69.7 | 69.3 |
| C-5 | 122.2 | 118.3 | Glc-5′ | 74.0 | 77.2 |
| C-6 | 138.5 | 136.1 | Glc-6′ | 63.3 | 60.4 |
| C-7 | 120.5 | 124.2 | | | |
| C-8 | 157.8 | 161.3 | Galloyl-1″ | 119.2 | |
| C-9 | 187.4 | 187.6 | Galloyl-2″,6″ | 108.6 | |
| C-10 | 181.9 | 182.1 | Galloyl-3″,5″ | 145.5 | |
| C-4a | 132.0 | 134.5 | Galloyl-4″ | 135.6 | |
| C-8a | 119.3 | 116.7 | Galloyl-7″ | 165.1 | |
| C-9a | 114.6 | 119.0 | | | |
| C-10a | 134.7 | 132.4 | | | |
| 3-$CH_3$ | 21.4 | | | | |
| 3-$CH_2OH$ | | 62.1 | | | |

[a]Measured in DMSO-$d_6$ at 125 MHz.

Aloe-emodin 1-*O*-β-D-glucopyranoside (**2**) was isolated as yellow needles of mp 174–177 °C with negative optical rotation ($[\alpha]_D^{24}$ −56.4°). The molecular formula $C_{21}H_{20}O_{10}$ of **2** was determined from the positive- and negative-ion FAB–MS [*m*/*z* 455 $(M+Na)^+$, 431 $(M-H)^-$] and by high-resolution MS measurement. The UV spectrum of **2** indicated the presence of the anthraquinone moiety from the characteristic absorption maxima at 222 (log ε 4.55), 257 (4.37), 282 (sh, 4.06), and 407 (3.84) nm. The IR spectrum showed absorption bands at 3410, 1645, 1638, 1603, 1458, and 1072 $cm^{-1}$, suggesting the presence of hydroxyl, free and chelated carbonyl, and




**1** **2** **2**

**Figure 1.** HMBC and NOE correlations of **1** and **2**.

aromatic functions. The $^1$H NMR (DMSO-$d_6$) and $^{13}$C NMR (Table 1) spectra of **2** indicated the presence of a methylene bearing a hydroxyl group [δ 4.65 (2H, s, 3-$CH_2OH$)], a β-D-glucopyranosyl [δ 5.14 (1H, d, $J$ = 7.6 Hz, Glc-1′-H)], and an anthraquinone structure [δ 7.36 (1H, dd, $J$ = 1.2, 8.2 Hz, 7-H), 7.62 (1H, d, $J$ = 1.2 Hz, 2-H), 7.68 (1H, dd, $J$ = 1.2, 7.6 Hz, 5-H), 7.75 (1H, dd, $J$ = 7.6, 8.2 Hz, 6-H), 7.91 (1H, d, $J$ = 1.2 Hz, 4-H)]. Acid hydrolysis of **2** with 5% sulfuric acid furnished D-glucose, which was identified by GLC analysis of the trimethylsilyl (TMS) thiazolidine derivative.[15] Enzymatic hydrolysis of **2** with β-glucosidase liberated aloe-emodin (**3**).[16] The carbon signals of the aglycone part in the $^{13}$C NMR spectrum of **2** were superimposable on those of aloe-emodin 8-*O*-β-D-glucopyranoside[9,17] or aloe-emodin ω-*O*-β-D-glucopyranoside,[9] except for the signals due to a glycoside linked position. In the HMBC experiment of **2**, long-range correlation was observed between 1′-proton and 1-carbon, as shown in Figure 1. In addition, in the nuclear Overhauser effect spectroscopy (NOESY) experiment on **2**, the NOE correlation was observed between the following proton pairs: 1′-H and 2-H; 3-$CH_2OH$ and 2,4-H, as shown in Figure 1. Consequently, **2** was determined to be 1-*O*-β-D-glucopyranosyl-aloe-emodin.

### Preparation of anthraquinone and stilbene derivatives

To clarify the structure–activity relationships in the radicals scavenging activity, the following related compounds were prepared. Aloe-emodin (**3**), chrysophanol (**4**),[17,18] and isorhapontigenin (**12**)[19] were derived by enzymatic hydrolysis of **2**, **6** and **11** with β-glucosidase in 0.2 M acetate buffer (pH 5.0) for 16 h at 37 °C, respectively. Dihydrostilbenes (**8a**, **9a**, **13a**, **14a**,[20] **16a**,[20] and **19a**) were derived by hydrogenation of **8**, **9**, **13**, **14**, **16**, and *trans*-stilbene (**19**) in the presence of palladium–carbon. The completely methylated derivatives (**8b**, **9b**, **13b**,[21] and **16b**[20]) were prepared by $CH_3I$ methylation of **8**, **9**, **13** and **16**. In addition, partially methylated derivatives (**8c**,[20] **9c**[22]) were prepared by methanolysis of **8b** and **9b.**

### DPPH radical and $\cdot O_2^-$ scavenging activities of MeOH extracts from rhubarbs and the fractions from *R. undulatum*

The DPPH radical, which is stable and shows an absorption at 517 nm, has been used as a convenient tool for the radical scavenge assay, and this assay is independent of any enzyme activity.[23,24] When this compound accepts an electron or hydrogen radical to become a more stable compound, the absorption vanishes. The xanthine–xanthine oxidase system was conventionally used for generation of $\cdot O_2^-$, which was detected by reduction of nitroblue tetrazolium (NBT) in the present study.[25,26] The DPPH radical and $\cdot O_2^-$ scavenging activities of the methanolic extracts from five kinds of rhubarb, *R. palmatum*, *R. tanguticum*, *R. officinale*, *R. coreanum*, and *R. undulatum*, were examined. As shown in Table 2, all extracts scavenged both the DPPH radical and $\cdot O_2^-$. However, the methanolic extract of *R. undulatum* did not show the most activity among them.

Next, we isolated the chemical constituents from the rhizome of *R. undulatum*, since neither chemical nor pharmacological studies have been adequately reported. The $H_2O$- and methanol-eluted fractions showed potent scavenging activity for the DPPH radical and $\cdot O_2^-$, while the acetone-eluted fraction showed little activity. A reference compound, α-tocopherol, showed scavenging activity for the DPPH radical, but showed little activity for $\cdot O_2^-$. However, gallic acid and (+)-catechin showed potent scavenging of both radicals. These polyphenols were reported to inhibit the xanthine oxidase activity,[27] but they did not inhibit the enzyme activity in the present conditions at less than 10 μM.

### Scavenging effects of chemical constituents from *R. undulatum* and their derivatives on the DPPH radical and $\cdot O_2^-$

An anthraquinone glucoside (**1**) with a 6′-galloyl moiety showed potent DPPH radical and $\cdot O_2^-$ scavenging

**Table 2.** DPPH radical and $\cdot O_2^-$ scavenging activities and xanthine oxidase inhibitory activity of five rhubarbs

| | DPPH radical | $\cdot O_2^-$ | |
|---|---|---|---|
| | $SC_{50}$[a] | Formazan formation $IC_{50}$ | Xanthine oxidase $IC_{50}$ |
| *R. palmatum* MeOH ext. | 5.2 μg/mL | 5.0 μg/mL | > 100 μg/mL (9%)[d] |
| *R. tanguticum* MeOH ext. | 2.6 μg/mL | 4.1 μg/mL | > 100 μg/mL (3%)[d] |
| *R. officinale* MeOH ext. | 3.3 μg/mL | 3.8 μg/mL | > 100 μg/mL (−4%)[d] |
| *R. coreanum* MeOH ext. | 5.9 μg/mL | 8.5 μg/mL | > 100 μg/mL (11%)[d] |
| *R. undulatum* MeOH ext. | 7.2 μg/mL | 6.3 μg/mL | > 100 μg/mL (−6%)[d] |
| $H_2O$-eluted fraction | 19 μg/mL | 12 μg/mL | – |
| MeOH-eluted fraction | 3.8 μg/mL | 3.5 μg/mL | – |
| Acetone-eluted fraction | 95 μg/mL | > 100 μg/mL (35%)[d] | – |
| α-Tocopherol | 11 μM | > 100 μM (16%)[c] | – |
| Gallic acid | 3.9 μM | 1.8 μM | > 10 μM (0%)[b] |
| (+)-Catechin | 6.0 μM | 5.3 μM | > 10 μM (−7%)[b] |

[a]Concentration required for 50% reduction of 40 μM DPPH radical.
[b]Values in parentheses represent the inhibition (%) at 10 μM.
[c]Value in parentheses represents the inhibition (%) at 100 μM.
[d]Values in parentheses represent the inhibition (%) at 100 μg/mL.

activity, but other anthraquinone glucosides (**2**–**6**) as well as sennosides A and B lacked such an effect (Table 3). A naphthalene glucoside, torachrysone 8-*O*-β-D-glucopyranoside (**7**), also showed DPPH radical scavenging activity (Table 3). Gallic acid also shows potent radical scavenging activity.[28,29] In agreement with these previous studies, gallic acid showed potent radical scavenging activity in the present study (Table 2), while **5** lacked the activity. These findings indicate that the 6′-galloyl moiety is essential to show the radical scavenging activity. Similarly, two stilbenes with a galloyl moiety [rhaponticins 2″-*O*-gallate (**17**) and 6″-*O*-gallate (**18**)] showed equipotent activity, and they were also more potent than rhaponticin (**8**). Among the stilbene constituents lacking a galloyl moiety, piceatannol (**14**), with four hydroxyl groups, showed the most potent activity, and desoxyrhaponticin (**10**) showed the least activity (Table 4).

### Scavenging effects of anthraquinone and stilbene derivatives on DPPH radical and $\cdot O_2^-$

As shown in Table 3, dihydrostilbene derivatives (**8a**, **9a**, **13a**, **14a** and **16a**) maintained the scavenging activity for the DPPH radical, but their $\cdot O_2^-$ scavenging activities were reduced. Since *trans*-stilbene (**19**) and **19a** lacked the oxygen function and methylated stilbenes (**8b**, **9b**, **9c**, **13b** and **16b**) lacked the activity, the phenolic hydroxyl functions of stilbenes are essential for the radical scavenging activity.

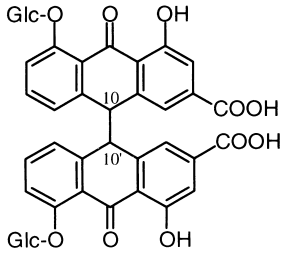
In addition, we examined their inhibitory activities of xanthine oxidase to clarify that their effects were due to the scavenging effect of $\cdot O_2^-$ or the inhibition of xanthine oxidase. As a result, **8c**, **13** and **15** showed the stronger inhibition of xanthine oxidase among them. These findings indicated that inhibition of formazan formation by **8c**, **13** and **15** was due to their inhibitions for xanthine oxidase. With regard to their structural requirements for enzyme inhibition, both 3-hydroxyl and 4′-methoxyl groups are important for the activity.

### Antioxidant effects of the MeOH extract from *R. undulatum* and its stilbene constituents on lipid peroxidation by the erythrocyte membrane ghost system

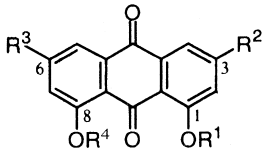
As shown in Table 5, the methanolic extract and the $H_2O$- and methanol-eluted fractions, but not acetone-eluted fraction, inhibited lipid peroxidation of erythrocyte membrane by *t*-BuOOH. Most stilbene constituents (**8**–**18**), except for **10**, showed inhibition of lipid peroxidation of erythrocyte membranes by *t*-BuOOH.

Several stilbenes, including piceatannol (**14**) and resveratrol (**16**), were recently reported to show antioxidant activity (e.g., DPPH radical scavenging activity, $Cu^{2+}$-

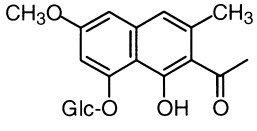
**Table 3.** Radical scavenging activities of sennosides, anthraquinones and a naphthalene




| | 10–10′ | DPPH radical $SC_{50}$ (μM)[a] | $\cdot O_2^-$ Formazan formation $IC_{50}$ (μM) | $\cdot O_2^-$ Xanthine oxidase $IC_{50}$ (μM) |
|---|---|---|---|---|
| Sennoside A | *threo* | >40 (5%)[b] | >100 (0%)[c] | – |
| Sennoside B | *erythro* | >40 (6%)[b] | >100 (−3%)[c] | – |



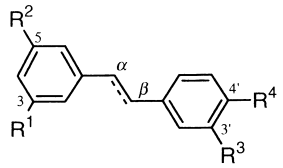

| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | | | |
|---|---|---|---|---|---|---|---|
| Chrysophanol 8-*O*-(6′-galloyl)-Glc (**1**) | H | $CH_3$ | H | Glc(6-gallate) | 13 | 23 | >100 (−6%)[c] |
| Aloe-emodin 1-*O*-Glc (**2**) | Glc | $CH_2OH$ | H | H | >40 (5%)[b] | >100 (8%)[c] | – |
| Aloe-emodin (**3**) | H | $CH_2OH$ | H | H | >40 (5%)[b] | >100 (−3%)[c] | – |
| Chrysophanol (**4**) | H | $CH_3$ | H | H | >40 (19%)[b] | >100 (32%)[c] | >100 (20%)[c] |
| Chrysophanol 1-*O*-Glc (**5**) | Glc | $CH_3$ | H | H | >40 (9%)[b] | >100 (−6%)[c] | – |
| Chrysophanol 8-*O*-Glc (**6**) | H | $CH_3$ | H | Glc | >40 (18%)[b] | >100 (12%)[c] | – |
| Emodin | H | $CH_3$ | OH | H | >40 (40%)[b] | >100 (17%)[c] | – |
| Rhein | H | COOH | H | H | >40 (2%)[b] | >100 (1%)[c] | – |
| Physcion | H | $CH_3$ | $OCH_3$ | H | >40 (2%)[b] | >100 (9%)[c] | – |




| | | | |
|---|---|---|---|
| Torachrysone 8-*O*-Glc (**7**) | 16 | >100 (38%)[c] | >100 (20%)[c] |

[a]Concentration required for 50% reduction of 40 μM DPPH radical.
[b]Values in parentheses represent the reduction or inhibition (%) at 40 μM.
[c]Values in parentheses represent the reduction or inhibition (%) at 100 μM.
[d]Glc, β-D-glucopyranosyl.

**Table 4.** Radical scavenging activities of stilbene constituents and their derivatives




| | | | | | | DPPH radical | $\cdot O_2^-$ | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Formazan formation | Xanthine oxidase |
| | α–β | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $SC_{50}$ (μM)[a] | $IC_{50}$ (μM) | $IC_{50}$ (μM) |
| Rhaponticin (**8**) | C=C | O-Glc[e] | OH | OH | $OCH_3$ | 29 | 38 | >30 (0%)[b] |
| **8a** | C−C | O-Glc | OH | OH | $OCH_3$ | 28 | >100 (38%)[d] | >100 (0%)[d] |
| **8b** | C=C | O-Glc$(CH_3)_4$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | >40 (1%)[c] | >100 (27%)[d] | – |
| **8c** | C=C | OH | $OCH_3$ | $OCH_3$ | $OCH_3$ | >40 (10%)[c] | 24 | 39 |
| Piceatannol 3′-*O*-Glc (**9**) | C=C | OH | OH | O-Glc | OH | 15 | 16 | >30 (−3%)[b] |
| **9a** | C−C | OH | OH | O-Glc | OH | 26 | 58 | >100 (−4%)[d] |
| **9b** | C=C | $OCH_3$ | $OCH_3$ | O-Glc$(CH_3)_4$ | $OCH_3$ | >40 (1%)[c] | >100 (9%)[d] | – |
| **9c** | C=C | $OCH_3$ | $OCH_3$ | OH | $OCH_3$ | >40 (43%)[c] | >100 (36%)[d] | >30 (−1%)[b] |
| Desoxyrhaponticin (**10**) | C=C | O-Glc | OH | H | $OCH_3$ | >40 (9%)[c] | >100 (33%)[d] | – |
| Isorhapontin (**11**) | C=C | O-Glc | OH | $OCH_3$ | OH | >40 (29%)[c] | >100 (49%)[d] | >100 (-6%)[d] |
| **12** | C=C | OH | OH | $OCH_3$ | OH | 32 | 21 | >30 (29%)[b] |
| Rhapontigenin (**13**) | C=C | OH | OH | OH | $OCH_3$ | 24 | 20 | 34 |
| **13a** | C−C | OH | OH | OH | $OCH_3$ | 20 | >100 (49%)[d] | >30 (23%)[b] |
| **13b** | C=C | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | >40 (1%)[c] | >30 (26%)[b] | >30 (32%)[b] |
| Piceatannol (**14**) | C=C | OH | OH | OH | OH | 11 | 6.8 | >30 (34%)[b] |
| **14a** | C-C | OH | OH | OH | OH | 8.6 | 34 | >100 (17%)[d] |
| Desoxyrhapontigenin (**15**) | C=C | OH | OH | H | $OCH_3$ | >40 (45%)[c] | 11 | 24 |
| Resveratrol (**16**) | C=C | OH | OH | H | OH | 24 | >100 (40%)[d] | >30 (9%)[b] |
| **16a** | C−C | OH | OH | H | OH | >40 (31%)[c] | >100 (11%)[d] | – |
| **16b** | C=C | $OCH_3$ | $OCH_3$ | H | $OCH_3$ | >40 (2%)[c] | >100 (−5%)[d] | – |
| Rhaponticin 2″-*O*-gallate (**17**) | C=C | O-Glc(2-gallate) | OH | OH | $OCH_3$ | 4.8 | 11 | >30 (−8%)[b] |
| Rhaponticin 6″-*O*-gallate (**18**) | C=C | O-Glc(6-gallate) | OH | OH | $OCH_3$ | 4.8 | 13 | >30 (−3%)[b] |
| *Trans*-stilbene (**19**) | C=C | H | H | H | H | >40 (1%)[c] | >100 (2%)[d] | – |
| **19a** | C−C | H | H | H | H | >40 (0%)[c] | >100 (−4%)[d] | – |

[a]Concentration required for 50% reduction of 40 μM DPPH radical.
[b]Values in parentheses represent the reduction or inhibition (%) at 30 μM.
[c]Values in parentheses represent the reduction or inhibition (%) at 40 μM.
[d]Values in parentheses represent the reduction or inhibition (%) at 100 μM.
[e]Glc, β-D-glucopyranosyl.

induced lipid peroxidation of LDL, TPA-induced free radical formation in HL-60 cells).[30,31] Since most stilbenes from *R. undulatum* showed antioxidant activities, the findings in this study present significant evidence in support of the previous study.[30,31]

Stilbenes from the rhizome of *R. undulatum* were reported to inhibit NO production in lipopolysaccharide-activated macrophages.[3] Recently, it was revealed that peroxynitrite ($ONOO^-$), which is easily produced by the reaction of NO with $\cdot O_2^-$, is a highly reactive oxidant and induces various oxidative damage.[32] This rhubarb and its stilbene constituents with the antioxidant and NO production inhibitory activities may be important evidence substantiating these traditional effects.

In conclusion, the methanolic extracts from five kinds of rhubarb were found to show scavenging activity for the DPPH radical and $\cdot O_2^-$. In screening tests for DPPH radical and $\cdot O_2^-$ scavenging activity, most stilbenes and a naphthalene glucoside, torachrysone 8-*O*-glucoside, isolated from *R. undulatum* showed the activity. However,

**Table 5.** Antioxidative activity of the extracts from *R. undulatum* and its stilbene constituents for lipid peroxidation by the erythrocyte membrane ghost system

| Sample | $IC_{50}$ |
|---|---|
| MeOH Ext. | 5.3 μg/mL |
| $H_2O$-eluted fraction | 8.3 μg/mL |
| MeOH-eluted fraction | 3.3 μg/mL |
| Acetone-eluted fraction | 55 μg/mL |
| Rhaponticin (**8**) | 9.5 μM |
| Piceatannol 3′-*O*-β-D-glucopyranoside (**9**) | 40 μM |
| Desoxyrhaponticin (**10**) | >100 μM (9%)[a] |
| Isorhapontin (**11**) | 33 μM |
| Rhapontigenin (**13**) | 18 μM |
| Piceatannol (**14**) | 6.0 μM |
| Desoxyrhapontigenin (**15**) | 49 μM |
| Resveratrol (**16**) | 47 μM |
| Rhaponticin 2″-*O*-gallate (**17**) | 11 μM |
| Rhaponticin 6″-*O*-gallate (**18**) | 12 μM |
| α-Tocopherol | 6.9 μM |
| (+)-Catechin | 2.4 μM |

[a]Value in parentheses represents inhibition (%) at 100 μM.

anthraquinone glucosides (chrysophanol 1-*O*-β-D-glucopyranoside and chrysophanol 8-*O*-β-D-glucopyranoside) and sennosides A and B did not. In addition, most stilbenes, except for desoxyrhaponticin (**10**), showed lipid peroxidation of erythrocyte membranes by *t*-BuOOH. To elucidate the structure–activity relationships in anthraquinones and stilbenes, we examined various related compounds. The findings of these experiments suggested the following structural requirements: 1) phenolic hydroxyl groups of stilbene are essential to show the activity; 2) the galloyl moiety enhanced the activity; 3) the glucoside moiety of stilbene reduced the activity; 4) dihydrostilbene derivatives maintained the scavenging activity for DPPH radical, but they showed weak activity for $\cdot O_2^-$. In addition, several stilbenes with both the 3-hydroxyl and 4′-methoxyl groups inhibited xanthine oxidase.

## Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l = 5$ cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI–MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB–MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; $^1$H NMR spectra, JNM-LA500 (500 MHz) spectrometer and JEOL EX-270 (270 MHz); $^{13}$C NMR spectra, JNM-LA500 (125 MHz) and JEOL EX-270 (68 MHz) spectrometers with tetramethylsilane as an internal standard.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, pre-coated TLC plates with Silica gel $60F_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 $60F_{254}$ (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, pre-coated TLC plates with Silica gel RP-18 $60WF_{254S}$ (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous $H_2SO_4$ and heating.

### Extraction and isolation

The dried rhizome of *R. palmatum* L. [500 g, cultivated in China (Kansu province) and purchased from MAE CHU Co., Ltd., Nara, Japan], *R. tanguticum* Maxim. [500 g, cultivated in China (Chinghai province) and purchased from MAE CHU Co., Ltd.], *R. officinale* Baill. [500 g, cultivated in China (Szechwan province) and purchased from MAE CHU Co., Ltd.], *R. coreanum* Nakai [500 g, cultivated in Korea and purchased from MAE CHU Co., Ltd.], and *R. undulatum* (5.8 kg, cultivated in Korea), which were botanically identified by comparison of their taxonomical features with authentic rhubarb samples, were crushed and then extracted three times with methanol at room temperature for 24 h. Evaporation of the solvent under reduced pressure gave the MeOH extracts [110 g (22.1%) from *R. palmatum*, 147 g (29.4%) from *R. tanguticum*, 162 g (32.4%) from *R. officinale*, 141 g (28.1%) from *R. coreanum*, and 1960 g (33.8%) from *R. undulatum*].

The MeOH extract (850 g) from *R. undulatum* was subjected to Diaion HP-20 column chromatography (3.0 kg, $H_2O \rightarrow$MeOH$\rightarrow$acetone) to afford the $H_2O$-eluted fraction (335 g, 13.3%), MeOH-eluted fraction (470 g, 18.7%), and acetone-eluted fraction (45 g, 1.8%).

The MeOH-eluted fraction (150 g) was separated by ordinary-phase silica gel column chromatography [3.0 kg, $CHCl_3$–MeOH (10:1, v/v)$\rightarrow$(4:1)$\rightarrow$$CHCl_3$–MeOH–$H_2O$ (10:3:1, lower layer)$\rightarrow$MeOH] to give five fractions [Fr. 1 (4.4 g), Fr. 2 (16.5 g), Fr. 3 (14.8 g), Fr. 4 (71.5 g), Fr. 5 (42.8 g)]. Fraction 2 (12.0 g) was further subjected to reversed-phase silica gel column chromatography [360 g, MeOH–$H_2O$ (50:50 v/v)$\rightarrow$(80:20)$\rightarrow$MeOH] to afford five fractions [Fr. 2-1 (5.3 g), Fr. 2-2 (0.6 g), Fr. 2-3 (3.2 g), Fr. 2-4 (1.2 g), Fr. 2-5 (1.7 g)]. Finally, fraction 2-1 (500 mg) was subjected to HPLC [YMC-pack R&D ODS-5-A, 250×20 mm id, MeOH–$H_2O$ (50:50 v/v)] to give rhapontigenin (**13**, 292 mg, 0.58%), piceatannol (**14**, 40 mg, 0.073%), and resveratrol (**16**, 26 mg, 0.048%). Through a similar procedure, fraction 2-2 (300 mg) was subjected to HPLC [MeOH–$H_2O$ (55:45 v/v)] to furnish desoxyrhapontigenin (**15**, 41 mg, 0.015%), fraction 2-3 (300 mg) was subjected to HPLC [$CH_3CN$–$H_2O$ (40:60 v/v)] to give chrysophanol 1-*O*-β-D-glucopyranoside (**5**, 136 mg, 0.25%) and chrysophanol 8-*O*-β-D-glucopyranoside (**6**, 87 mg, 0.16%). Fraction 3 (12.0 g) was further separated by reversed-phase silica gel column chromatography [360 g, MeOH–$H_2O$ (60:40 v/v)$\rightarrow$MeOH] to yield five fractions [Fr. 3-1 (3.3 g), Fr. 3-2 (3.6 g), Fr. 3-3 (3.3 g), Fr. 3-4 (1.4 g), Fr. 3-5 (0.4 g)]. Fraction 3-2 (300 mg) and fraction 3-4 (300 mg) were further subjected to HPLC [MeOH–$H_2O$ (55:45 v/v)] to furnish chrysophanol 8-*O*-β-D-(6′-galloyl)-glucopyranoside (**1**, 129 mg, 0.092%) and torachrysone 8-*O*-β-D-glucopyranoside (**7**, 65 mg, 0.12%), respectively. Fraction 4 (28.0 g) was subjected to reversed-phase silica gel column chromatography [1.0 kg, MeOH–$H_2O$ (30:70 v/v) $\rightarrow$(40:60)$\rightarrow$MeOH] to afford seven fractions [Fr. 4-1 (1.5 g), Fr. 4-2 (6.3 g), Fr. 4-3 (11.0 g), Fr. 4-4 (2.1 g), Fr. 4-5 (0.7 g), Fr. 4-6 (3.2 g), Fr. 4-7 (3.2 g)]. Fractions 4-2 and 4-3 were identified as piceatannol 3′-*O*-β-D-glucopyranoside (**9**, 2.0%) and rhaponticin (**8**, 3.5%), respectively. Fraction 4-4 (1.45 g) was subjected to HPLC [MeOH–$H_2O$ (50:50 v/v) and (40:60)] to give aloe-emodin 1-*O*-β-D-glucopyranoside (**2**, 14 mg, 0.0065%), desoxyrhaponticin (**10**, 104 mg, 0.048%), isorhapontin (**11**, 782 mg, 0.36%), rhaponticin 2″-*O*-gallate (**17**, 261 mg, 0.12%), and rhaponticin 6″-*O*-gallate (**18**,189 mg, 0.087%). These constituents were identified by comparison of their physical data with those of authentic samples (**14**, **16**) or with reported values.[9–14]

Chrysophanol 8-*O*-β-D-(6′-galloyl)-glucopyranoside (**1**): Yellow needles from MeOH, mp 207–210 °C, $[\alpha]_D^{25}$ +95.0° ($c = 0.1$, MeOH). High-resolution positive-ion FAB–MS: calcd for $C_{28}H_{24}O_{13}Na$ $(M + Na)^+$: 591.1114. Found: 591.1168. UV [MeOH, nm (log ε)]: 220 (4.58),

260 (4.32), 284 (sh, 4.17), 409 (3.79). IR (KBr): 3410, 2923, 1709, 1641, 1637, 1466, 1075 $cm^{-1}$. $^1H$ NMR (500 MHz, DMSO-$d_6$) δ: 2.40 (3H, s, 3-$CH_3$), 4.27, 4.50 (1H each, both m, Glc-6′-$H_2$), 5.24 (1H, d, $J$ = 7.7, Glc-1′-H), 6.99 (2H, s, galloyl-2″,6″-H), 7.16 (1H, br s, 4-H), 7.46 (1H, br s, 2-H), 7.67 (1H, d, $J$ = 8.6 Hz, 5-H), 7.72 (1H, dd, $J$ = 7.6, 8.6 Hz, 6-H), 7.82 (1H, d, $J$ = 7.6 Hz, 7-H), 12.82 (1H, br s, 1-OH). $^{13}C$ NMR (125 MHz, DMSO-$d_6$) $\delta_C$: given in Table 1. Positive-ion FAB–MS $m/z$: 591 $(M+Na)^+$. Negative-ion FAB–MS $m/z$: 567 $(M-H)^-$.

Aloe-emodin 1-*O*-β-D-glucopyranoside (**2**): Yellow needles from MeOH, mp 174–177 °C, $[\alpha]_D^{24}$ −56.4° ($c$ = 0.1, MeOH). High-resolution positive-ion FAB–MS: calcd for $C_{21}H_{20}O_{10}Na$ $(M+Na)^+$: 455.0954. Found: 455.0952. UV [MeOH, nm (log ε)]: 222 (4.55), 257 (4.37), 282 (sh, 4.06), 407 (3.84). IR (KBr): 3410, 2924, 1645, 1638, 1603, 1458, 1072 $cm^{-1}$. $^1H$ NMR (500 MHz, DMSO-$d_6$) δ: 4.65 (2H, s, 3-$CH_2OH$), 5.14 (1H, d, $J$ = 7.6 Hz, Glc-1′-H), 7.36 (1H, dd, $J$ = 1.2, 8.2 Hz, 7-H), 7.62 (1H, d, $J$ = 1.2 Hz, 2-H), 7.68 (1H, dd, $J$ = 1.2, 7.6 Hz, 5-H), 7.75 (1H, dd, $J$ = 7.6, 8.2 Hz, 6-H), 7.91 (1H, d, $J$ = 1.2 Hz, 4-H), 12.90 (1H, br s, 8-OH). $^{13}C$ NMR (125 MHz, DMSO-$d_6$) $\delta_C$: given in Table 1. Positive-ion FAB–MS $m/z$: 455 $(M+Na)^+$. Negative-ion FAB–MS $m/z$: 431 $(M-H)^-$, 269 $(M^+-C_6H_{11}O_5)^-$.

**Alkaline hydrolysis of chrysophanol 8-*O*-β-D-(6′-galloyl)-glucopyranoside (1).** A solution of **1** (10 mg, 0.018 mmol) in 1.0% NaOMe–MeOH (2.0 mL) was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex HCR-W2 ($H^+$ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure gave a residue, which was purified by silica gel column chromatography [1.0 g ($CHCl_3$–MeOH–$H_2O$ = 10:3:1, lower layer)] to furnish **6** (7.2 mg, 98%), which was identified by comparison of the physical data with reported values.[9]

**Acid hydrolysis of aloe-emodin 1-*O*-β-D-glucopyranoside (2).** A solution of aloe-emodin 1-*O*-β-D-glucopyranoside (**2**, 2 mg) in 5% aq $H_2SO_4$–1,4-dioxane (1:1, v/v, 1 mL) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 ($OH^-$ form) and the insoluble portion was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was separated on a Sep-Pack C18 cartridge column ($H_2O$, MeOH). The $H_2O$ eluate was concentrated under reduced pressure to give a residue, which was treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (0.02 mL) and the mixture was left standing at 60 °C for 1 h. The reaction solution was then treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.01 mL) and the whole mixture was left standing at 60 °C for 1 h. The supernatant of the reaction mixture was subjected to GLC analysis to identify the thiazolidine derivatives of D-glucose. GLC conditions: column, Supelco SPB™-1, 0.25 mm id × 30 m; injection temperature, 230 °C; detection temperature, 230 °C; column temperature, 230 °C; He flow rate 15 mL/min. $t_R$: 24.2 min.

**Enzymatic hydrolysis of aloe-emodin 1-*O*-β-D-glucopyranoside (2), 6 and 11.** A solution of **2** (10 mg, 0.023 mmol), **6** (10 mg, 0.024 mmol), or **11** (10 mg, 0.024 mmol) in 0.2 M acetate buffer (pH 5.0, 2.0 mL) was treated with β-glucosidase (10 mg, SIGMA G-0395 from almonds) and the solution was stirred at 37 °C for 16 h. The reaction mixture was extracted with AcOEt. The AcOEt extract was washed with brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [200 mg (*n*-hexane–acetone = 1:1)] to give **3** (5.8 mg, 95%), **4** (6.0 mg, 98%), or **12** (5.9 mg, 96%), respectively. Compounds **3**, **4** and **12** were identified by comparison of the physical data with reported values.[16,18,19]

**Hydrogenation of 8, 9, 13, 14, 16, and *trans*-stilbene (19).** A solution of **8**, **9**, **13**, **14**, **16**, or *trans*-stilbene (**19**) (20 mg each) in EtOH (2.0 mL) was treated with 10% palladium carbon (Pd–C, 10 mg) and the whole mixture was stirred at room temperature under an $H_2$ atmosphere for 2 h. The reaction mixture was filtered and then evaporation of the solvent under reduced pressure furnished dihydrostilbene derivatives (**8a**, **9a**, **13a**, **14a**, **16a**, or **19a**) quantitatively. Compounds **14a**, **16a** and **19a** were identified by comparison of the physical data with reported values.[20]

**8a**: Light yellow powder, $[\alpha]_D^{25}$ −37.2° ($c$ = 0.1, MeOH). High-resolution EI–MS: calcd for $C_{21}H_{26}O_9$ $(M^+)$: 422.1576. Found: 422.1588. UV [MeOH, nm (log ε (4.30), 282 (3.76), 322 (3.60). IR (KBr): 3480, 1636, 1559, 1509, 1075 $cm^{-1}$. $^1H$ NMR (270 MHz, $CD_3OD$) δ: 2.74 (4H, br s, α- and β-$H_2$), 3.85 (3H, s, -OMe), 4.77 (1H, d, $J$ = 7.3 Hz, Glc-1″-H), 6.30 (1H, t, $J$ = 2.0 Hz, 4-H), 6.37, 6.38 (1H each, both d, $J$ = 2.0 Hz, 2, and 6-H), 6.57 (1H, dd, $J$ = 2.3, 8.2 Hz, 6′-H), 6.64 (1H, d, $J$ = 2.3 Hz, 2′-H), 6.78 (1H, d, $J$ = 8.2 Hz, 5′-H). EI–MS $m/z$: 422 ($M^+$, 2), 260 (64), 137 (100).

**9a**: Light yellow powder, $[\alpha]_D^{23}$ −33.3° ($c$ = 0.1, MeOH). High-resolution positive-ion FAB–MS: calcd for $C_{20}H_{24}O_9Na$ $(M+Na)^+$: 431.1319. Found: 431.1310. UV [MeOH, nm, (log ε)]: 213 (4.31), 282 (3.75), 301 (3.55). IR (KBr): 3480, 1636, 1559, 1509, 1081 $cm^{-1}$. $^1H$ NMR (270 MHz, $CD_3OD$) δ: 2.75 (4H, m, α- and β-$H_2$), 4.63 (1H, d, $J$ = 7.6 Hz, Glc-1″-H), 6.09 (3H, br s, 2, 4, and 6-H), [6.72 (2H, br d), 6.93 (1H, br s), 2′, 5′, and 6′-H]. Positive-ion FAB–MS $m/z$: 431 $(M+Na)^+$.

**13a**: Light yellow powder. High-resolution EI–MS: calcd for $C_{15}H_{16}O_4$ $(M^+)$: 260.1048. Found: 260.1041. UV [MeOH, nm (log ε)]: 223 (4.25), 295 (4.05), 305 (4.10). IR (KBr): 3478, 1614, 1559, 1509 $cm^{-1}$. $^1H$ NMR (270 MHz, $CD_3OD$) δ: 2.69 (4H, m, α- and β-$H_2$), 3.85 (3H, s, -OMe), 6.16 (1H, t, $J$ = 2.0 Hz, 4-H), 6.43 (2H, d, $J$ = 2.0 Hz, 2 and 6-H), 6.88 (1H, br d, 5′-H), 6.88 (1H, dd-like, 6′-H), 7.00 (1H, d, $J$ = 1.7 Hz, 2′-H). EI–MS $m/z$: 260 ($M^+$, 25), 137 (100).

**Complete methylation of 8, 9, 13 and 16.** A solution of **8** or **9** (150 mg each, 0.36 mmol) in *N,N*-dimethylformamide

(DMF, 5.0 mL) was treated with methyl iodide ($CH_3I$, 0.5 mL) in the presence of sodium hydride (NaH, 58 mg) and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [5.0 g (*n*-hexane–AcOEt = 1:1)] to give **8b** (115 mg, 64%) or **9b** (125 mg, 68%). Through a similar procedure, a solution of **13** (30 mg, 0.12 mmol) or **16** (30 mg, 0.13 mmol) in DMF (3.0 mL) using $CH_3I$ (0.3 mL) and NaH (20 mg) was stirred at room temperature for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was treated in the usual manner to give a residue, which was purified by silica gel column chromatography [500 mg, (*n*-hexane–AcOEt = 5:1)] to furnish **13b** (33 mg, 86%) or **16b** (36 mg, quant.). Compounds **13b** and **16b** were identified by comparison of the physical data with reported values.[20,21]

**8b**: Yellow powder, $[\alpha]_D^{25}$ −68.0° ($c = 0.3$, MeOH). High-resolution EI–MS: calcd for $C_{27}H_{36}O_9$ ($M^+$): 504.2359. Found: 504.2373. UV [MeOH, nm, (log ε)]: 220 (4.38), 305 (4.35), 323 (4.44). IR (KBr): 1610, 1592, 1516, 1065 $cm^{-1}$. $^1H$ NMR (270 MHz, $CDCl_3$) δ: 3.39, 3.56, 3.66, 3.68, 3.82, 3.89, 3.94 (3H each, both s, -OMe), 4.86 (1H, d, $J = 7.3$ Hz, Glc-1″-H), 6.53 (1H, t, $J = 2.0$ Hz, 4-H), 6.72, 6.82 (1H each, both br s, 2, and 6-H), 6.85 (1H, d, $J = 8.2$ Hz, 5′-H), 6.88, 7.03 (1H, each, both d, $J = 16.2$ Hz, α, and β-H), 7.03 (1H, dd, $J = 2.0$, 8.2 Hz, 6′-H), 7.06 (1H, br s, 2′-H). EI–MS *m*/*z*: 504 ($M^+$, 23), 286 (100).

**9b**: Yellow powder, $[\alpha]_D^{26}$ −33.2° ($c = 0.1$, MeOH). High-resolution EI–MS: calcd for $C_{27}H_{36}O_9$ ($M^+$): 504.2359. Found: 504.2357. UV [MeOH, nm (log ε)]: 213 (3.68), 301 (3.03), 325 (2.98). IR (KBr): 1630, 1582, 1516, 1060 $cm^{-1}$. $^1H$ NMR (270 MHz, $CDCl_3$) δ: 3.70, 3.78, 3.79, 3.85, 3.86 (3H each, both s, -OMe), 3.82 (6H, s, -OMe), 4.82 (1H, d, $J = 7.6$ Hz, Glc-1″-H), 6.38 (1H, t, $J = 2.2$ Hz, 4-H), 6.64 (2H, d, $J = 2.2$ Hz, 2- and 6-H), 6.81, 6.83 (1H, each, both d, $J = 17.3$ Hz, α- and β-H), [7.02 (1H, br s), 7.17 (2H, br s), 2′, 5′, and 6′-H]. EI–MS *m*/*z*: 504 ($M^+$,1), 286 (100).

### Methanolysis of 8b and 9b

A solution of **8b** or **9b** (50 mg each, 0.10 mmol) in 9% HCl–dry MeOH (4.0 mL) was heated under reflux for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [500 mg (*n*-hexane–AcOEt = 3:1)] to give **8c** (27 mg, 98%) and **9c** (22 mg, 81%), respectively. **8c** and **9c** were identified by comparison of the physical data with reported values.[20,22]

## Bioassay Methods

### DPPH radical scavenging activity

The free radical scavenging activity of the constituents of rhubarb was assessed using the DPPH radical.[23,24] An ethanol solution of DPPH (100 μM, 1.0 mL) was mixed with different concentrations of each test compound (0–200 μM, 0.5 mL) and 0.1 M acetate buffer (pH 5.5, 1.0 mL), and the absorbance change at 517 nm was measured 30 min later. The reaction solution without DPPH was used as a blank test. Measurements were performed in duplicate, and the concentration required for a 50% reduction (50% scavenging concentration, $SC_{50}$) of 40 μM DPPH radical solution was determined graphically.

### $\cdot O_2^-$ scavenging activity

The improved assay method for superoxide dismutase described by Imanari et al. was used.[25] Briefly, a reaction mixture containing 100 μM xanthine, 100 μM EDTA, 25 μM NBT, 0.005% bovine serum albumin, and ca. 1.8 mU/mL xanthine oxidase in 33.3 mM sodium carbonate buffer (pH 10.2) was incubated with or without each test sample for 20 min at 25 °C (total volume: 3.0 mL). After incubation, the solution was mixed with 0.1 mL of 6 mM $CuCl_2$ to stop the reaction. The formazan formation was monitored at 560 nm. In addition, inhibitory effects of test compounds on xanthine oxidase activity were examined to clarify whether the inhibition of formazan formation was due to inhibition of xanthine oxidase. The reaction mixture without NBT was incubated in similar conditions described above and 0.1 mL of 2 M HCl was added to stop the reaction. Uric acid formation was monitored at 290 nm. Several stilbenes (**8**, **8c**, **9**, **9c**, **12**, **13**, **13a**, **13b**, **14**, **15**, **16**, **17**, **18**) at 100 μM showed high optical density in the blank test, therefore 30 μM or 50 μM was chosen as the maximum concentration.

### Antioxidant activity for lipid peroxidation by the erythrocyte membrane ghost system

The method described by Osawa et al. was used.[33] Briefly, rabbit blood was washed with isotonic buffer solution (10 mM phosphate/152 mM NaCl, pH 7.4) three times, and lysed in hypotonic buffer solution (10 mM phosphate buffer, pH 7.4). Erythrocyte membrane ghosts were pelleted by centrifugation (20,000×g, 40 min), and the precipitate was diluted to give a suspension (2.5 mg protein/mL) as determined by the Lowry method. The reaction mixture containing erythrocyte membrane ghost suspension (0.85 mL), *t*-BuOOH (24 mM, 0.05 mL) and different concentrations of each test compound in ethanol (0.1 mL) was incubated for 30 min at 37 °C. The solution was cooled in an ice-cold water bath, and 10 μL of 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT, 50 mM), 1.5 mL of thiobarbituric acid (TBA) reagent composed of 0.375% TBA, 15% trichloroacetic acid, and 8.8 mg/mL BHT in 0.25 M HCl were added to the solution. After heating in boiling water for 15 min, the solution was cooled and centrifuged (4 °C, 1000×g, 15 min) and the quantity of

TBA-reacting substance in supernatant was determined at 535 nm.

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