Biosci. Biotechnol. Biochem., **70** (8), 1934–1940, 2006

Radical and Superoxide Scavenging Activities of Matairesinol and Oxidized Matairesinol

Satoshi YAMAUCHI,[1,†] Takuya SUGAHARA,[1] Yuki NAKASHIMA,[1] Akihiro OKADA,[1] Koichi AKIYAMA,[2] Taro KISHIDA,[1] Masashi MARUYAMA,[1] and Toshiya MASUDA[3]

[1]*Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan*
[2]*Integrated Center for Sciences, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan*
[3]*Faculty of Integrated Arts and Sciences, University of Tokushima, Tokushima 770-8502, Japan*

Received February 20, 2006; Accepted April 6, 2006; Online Publication, August 23, 2006
[doi:10.1271/bbb.60096]

The radical and superoxide scavenging activities of oxidized matairesinols were examined. It could be assumed that the free benzylic position was important for higher radical scavenging activity. The different level of activity was observed between 7′-oxomatairesinol (Mat 2) and 7-oxomatairesinol (Mat 3). The activity of 8-hydroxymatairesinol was lower than that of matairesinol (Mat 1). The superoxide scavenging activity of the oxidized matairesinols was also demonstrated for the first time. It is assumed that the p*K*a value of phenol in the oxidized matairesinols affected this activity.

Key words: matairesinol; radical scavenging activity; superoxide scavenging activity

Lignans are widely distributed among plant bioresources containing dietary plants and in the waste of the forestry and pulp industry. Our efforts are continuing to clarify the effect of dietary plants containing many kinds of lignan and to discover new worth in waste from plant bioresources. Research into antioxidant activity is important to evaluate the effect of plant materials containing lignans on health.[1)] Except for the aromatic portion, the structure of lignans had not been paid attention to in respect of antioxidant activity. The relationship between the structure of lignans and their antioxidant activities has recently been reported.[2–4)] The fact that the structure, apart from the aromatic portion, of lignans affected the degree of antioxidant activity was clarified by these reports. In particular, in the case of the 3,4-dibenzyltetrahydrofuran type of lignan, a higher oxidative degree of the benzylic position decreased the antioxidant activity.[4)] This present report describes the radical and superoxide scavenging activity by employing matairesinol and oxidized matairesinol. **Mat 1**–**Mat 6**, and **Lio 7** were prepared to examine the effect of the chemically active 7, 7′, and 8′ positions on the antioxidant activity (Fig. 1). **Mat 2** and **Mat 4** were first prepared. P. C. Eklund and co-workers have isolated **Mat 3** from the reaction of matairesinol (**Mat 1**) with 2,2-diphenyl-1-picrylhydrazyl (DPPH).[5)] It could be assumed from this result that the 7 position of matairesinol played a more important role in the antioxidant activity than the 7′ position. Many oxidized lignans are biosynthesized by plants and the preparation of oxidized lignan has been reported.[6)] Evaluating the antioxidant activity of oxidized lignan is important for the utilization of lignans. Our experiment compared the radical scavenging activity of oxidized matairesinol. This is the first report about the relationship between the lignan structure and superoxide scavenging activity by employing **Mat 1**–**Mat 6** and **Lio 7**[4)] (Fig. 1).

Results and Discussion

Preparation of the compounds

Matairesinol (**Mat 1**) and **Lio 7** were prepared from L-glutamic acid by employing previously reported methods.[4)] As shown in scheme 1, **Mat 2**, **Mat 3**, and **Mat 4** were respectively prepared from **6**, **8**, and **11** and **Mat 5** and **Mat 6** were prepared from **13**. Compounds **6**, **8**, **11**, and **13** were synthesized from L-glutamic acid.[4)] Aldol product **6** was oxidized by pyridinium chlorochromate (PCC) to ketone **7** in 95% yield. Hydrogenolysis of **7** gave **Mat 2** in 86% yield. To obtain **Mat 3**, compound **8** was selected as the starting material. After α-benzylation of **8** by using potassium hexamethyldisilazane (KHMDS) and 4-benzyloxy-3-methoxybenzyl bromide (58%), cleavage of the silyl ether by using *n*-Bu_4NF and PCC oxidation gave **10** in 42% yield (2 steps). Hydrogenolysis of **10** gave **Mat 3** in 45% yield. The value of specific rotation was higher than that in literature,[6)] in which **Mat 3** had been prepared from naturally occurring material. There is a possibility that the naturally occurring lignan was a mixture of enantiomers.[7)] **Mat 4** was prepared from **11**[4)] *via* **12** by desilylation and PCC oxidation (58% yield in 2 steps) followed by hydrogenolysis (90% yield). To prepare 8′-

† To whom correspondence should be addressed. Fax: +81-89-977-4364; E-mail: syamauch@agr.ehime-u.ac.jp

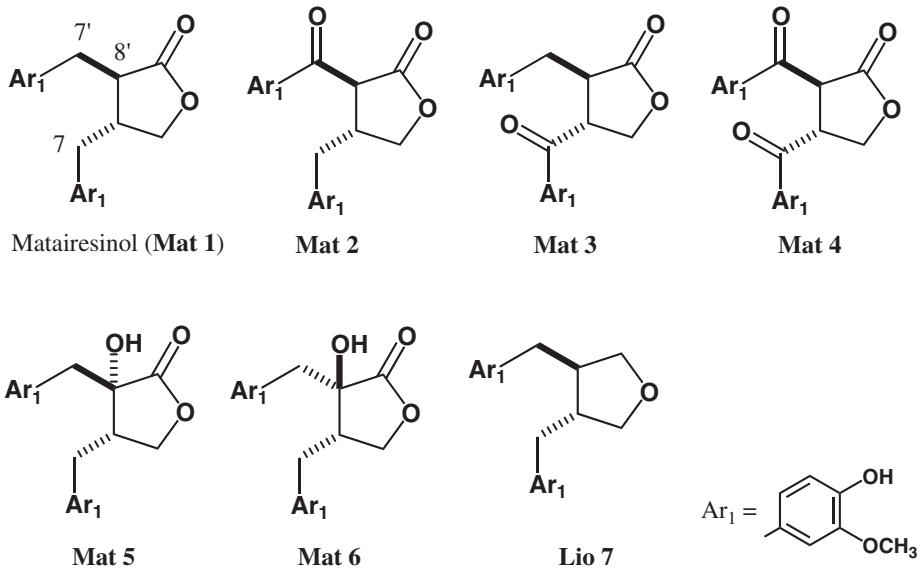


Fig. 1. Matairesinol and Oxidized Matairesinols.

hydroxymatairesinol (**Mat 5** and **Mat 6**), benzyl lactone **13** was treated with KHMDS and 4-benzyloxy-3-methoxybenzyl bromide, giving dibenzyllactone **14** in 47% yield. α-Hydroxylation of **14** was achieved by using oxodiperoxymolybdenum(pyridine)(hexamethylphosphoric triamide) (MoOPH) and KHMDS[8] to give **15** (13%) and **16** (10%). Lactone **14** was recovered (43%). Finally, hydrogenolysis of **15** and **16** respectively gave **Mat 5** (86%) and **Mat 6** (73%). The stereochemistry of **Mat 5** and **Mat 6** was confirmed by comparing with ^{1}H-NMR reference data.[8] Although optically active compounds are not necessary to achieve this result, all compounds were obtained from L-glutamic acid. Oxidized matairesinols **Mat 2** and **Mat 4** were synthesized for the first time.

Antioxidant activity of Mat 1–Mat 6

The radical scavenging activity was measured by employing DPPH in methanol (Fig. 2). In this study, matairesinol (**Mat 1**) showed the strongest activity, this activity being stronger than that of sesamol which is a well known natural antioxidant. The activities of 7′- and 7-oxomatairesinol **Mat 2** and **Mat 3** were weaker than that of matairesinol (Mat 1). Oxidation at the benzylic position of matairesinol decreased the activity, the tendency also being observed in previous research about tetrahydrofuran-type lignans.[4] These results mean that a free benzylic position was necessary for higher activity. To show antioxidant activity, the presence of a benzyl radical is necessary to give oxidized marairesinol.[5] In the case of the symmetric lactone type of lignan, a different level of activity was observed between **Mat 2** and **Mat 3**, the activity of **Mat 3** being a little weaker than that of **Mat 2**. One reason for this difference would be due to the production of an α,β-unsaturated compound between the 8′-position and 7′-benzylic position

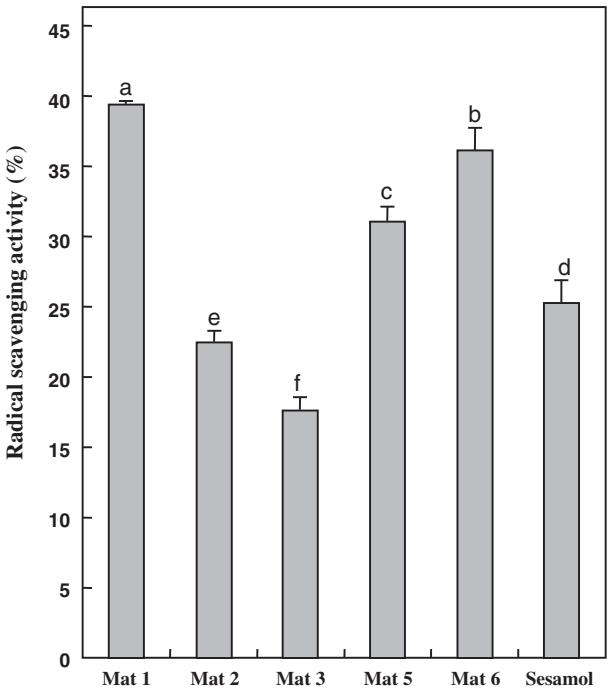
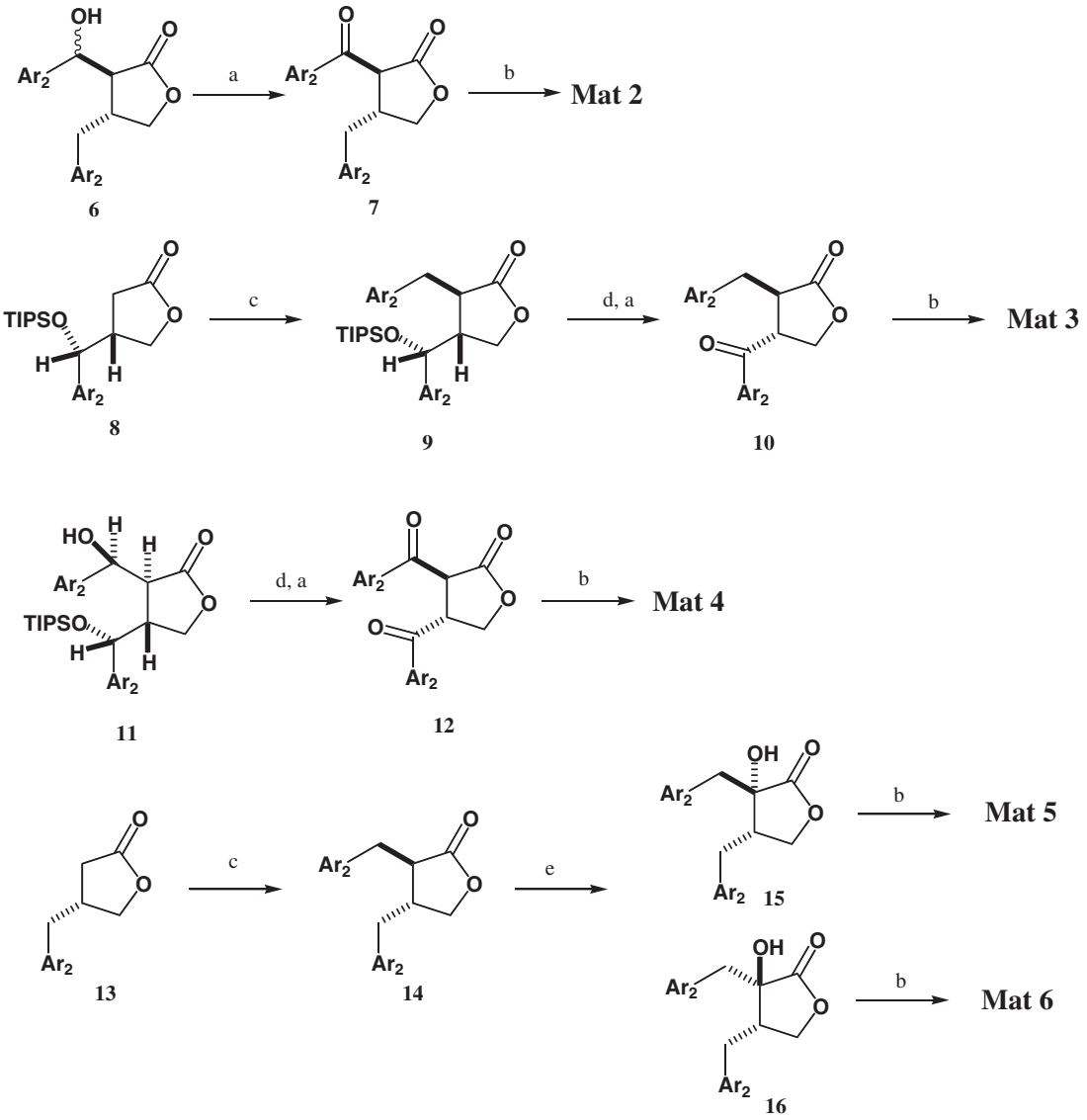


Fig. 2. Radical Scavenging Activity of Compounds **Mat 1**–**Mat 3**, **Mat 5**, and **Mat 6**.

Conditions: final concentration of a test sample, 20 μM; DPPH, 0.1 mM; detected at 517 nm. Each data value shows the mean ± SD (n = 3). Different letters denote significant difference at $p < 0.01$ (Student's *t*-test).

of **Mat 3**[5] resulting from the presence of a 7′-benzyl radical and α-radical. Since the 7′-benzyl radical formed an α,β-unsaturated compound without any reaction with oxygen, the conversion of **Mat 3** to an α,β-unsaturated compound reduced the activity. Another reason could have been the production of the enol form from the β-diketone of **Mat 2**. The antioxidant activity of the β-



Scheme 1. Preparation of **Mat 2**–**Mat 6**.

(a) PCC, MS 4A, CH_2Cl_2, r.t., 16 h (**7**, 95%; **10**, 42%, 2 steps; **12**, 58%, 2 steps); (b) H_2, 5% Pd/C, 2 h (**Mat 2**, 86%; **Mat 3**, 67%; **Mat 4**, 90%; **Mat 5**, 86%; **Mat 6**, 73%); (c) KHMDS, 4-BnO-3-$MeOC_6H_3CH_2Br$, THF, −70 °C, 30 min (**9**, 58%; **14**, 47%); (d) *n*-Bu_4NF, THF, 0 °C; (e) KHMDS, MoOPH, THF, −70 °C, 30 min (**15**, 13%; **16**, 10%; recovered **14**, 43%).

diketone has been reported.[9] The enol form from **Mat 2** increased the activity. Considering the resonance effect of the phenoxy radical with the benzylic radical, the former factor would be important. The activities of 8′-OH derivatives **Mat 5** and **Mat 6** were higher than those of **Mat 2** and **Mat 3**. These results indicate that two free benzylic positions were more important for higher activity than the α-position. The activities of **Mat 5** and **Mat 6** were a little weaker than that of **Mat 1**. It could be assumed that the 8′-position of matairesinol also contributed to the radical scavenging activity due to the presence of the α-radical. Unexpectedly, **Mat 6** showed higher activity than that of diastereomer **Mat 5**. It could be assumed that the *cis*-benzylic radical was a little more stable than the *trans*-benzylic radical.

The superoxide scavenging activities of **Mat 1**–**Mat 6** and **Lio 7** are shown in Fig. 3. It can be assumed that the p*K*a value for phenol affected the activity. In the presence of a benzyl ketone, which is an electron-withdrawing group, the p*K*a value of phenol was decreased. When this benzyl ketone was one of the carbonyl group of the β-diketone, the p*K*a value for phenol was increased because of formation of the enol form which decreased the electron-withdrawing effect of the benzyl ketone. The activity of **Mat 3** was highest due to the electron-withdrawing effect by the 7-benzyl ketone group. On the other hand, due to the formation of the enol form, the activity of **Mat 2** was weaker than that of **Mat 3**, showing the same level of activity as that of matairesinol (**Mat 1**). In the case of **Mat 4**, double enol forms of two benzyl ketones which is a conjugated diene caused the lowest activity. The activity of one

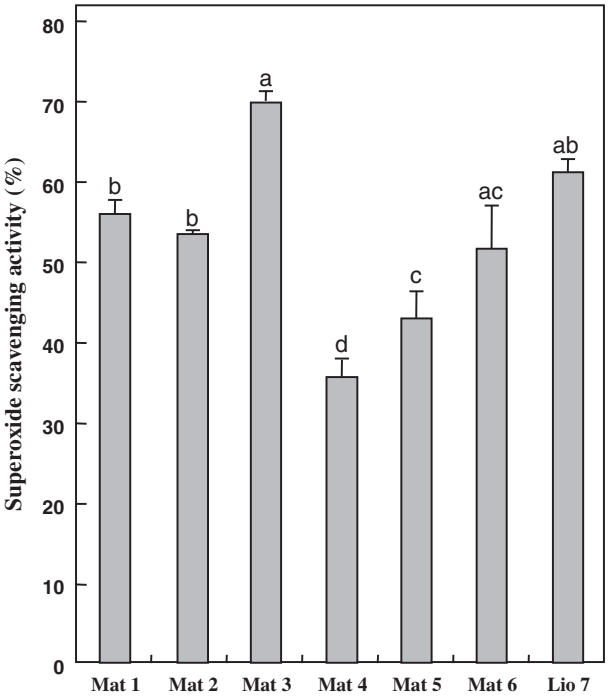


Fig. 3. Relative Superoxide Scavenging Activity of **Mat 1**–**Mat 6** and **Lio 7**.

Final concentration of a test sample, 160 μM. Conditions: final concentration of a test sample, 20 μM; DPPH, 0.1 mM; detected at 517 nm. Each data value is the mean ± SD (n = 3). Different letters denote significant difference at $p < 0.01$ (Student's *t*-test).

of the diastereomers **Mat 5** was weaker than that of matairesinol (**Mat 1**). It was found that a proton from the α position would have little effect on the activity. The p*K*a value of the α-hydroxy group is low because of a hydrogen bond with the carbonyl group of lactone. The activity of **Lio 7** was almost the same as that of matairesinol (**Mat 1**), **Mat 2**, and **Mat 3**.

This study compared the radical scavenging activity of 7′-oxomatairesinol, 7-oxomatairesinol, and 8′-hydroxymatairesinol for the first time. The relationship between the structure of oxidized matairesinol and the superoxide scavenging activity was also examined for the first time. These results will contribute to the practical utilization of lignans.

Experimental

Melting point (mp) data are uncorrected. NMR data were measured by a JNM-EX400 spectrometer using TMS as standard (0 ppm), and optical rotation values were evaluated with a HORIBA SEPA-200 instrument. The silica gel used was Wakogel C-300 (Wako, 200–300 mesh). The numbering of compounds follows IUPAC nomenclatural rules.

*(2S,3R)-2-(4-Benzyloxy-3-methoxybenzoyl)-3-(4-benzyloxy-3-methoxybenzyl)-4-butanolide (**7**).* A reaction mixture of benzyl alcohol **6** (0.42 g, 0.76 mmol), PCC (0.18 g, 0.84 mmol), and MS 4A (0.1 g) in CH_2Cl_2 (20 ml) was stirred at room temperature for 16 h before addition of ether. After the mixture was filtered, the filtrate was concentrated. The residue was applied to silica gel column chromatography (EtOAc/hexane = 1/2) to give α-benzoyl lactone **7** (0.40 g, 0.72 mmol, 95%) as a colorless oil, $[\alpha]^{20}_D = +31$ (*c* 1.1, $CHCl_3$). NMR δ_H ($CDCl_3$): 2.75 (1H, dd, *J* 13.7, 8.3 Hz, ArC*H*H), 2.83 (1H, dd, *J* 13.7, 8.3 Hz, ArCH*H*), 3.40 (1H, m, 3-H), 3.79 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.13 (1H, dd, *J* 8.8, 5.9 Hz, 4-*H*H), 4.26 (1H, d, *J* 6.3 Hz, 2-H), 4.52 (1H, dd, *J* 8.8, 7.3 Hz, 4-H*H*), 5.11 (2H, s, OCH_2Ar), 5.23 (2H, s, OCH_2Ar), 6.62 (1H, d, *J* 7.8 Hz, ArH), 6.65 (1H, s, ArH), 6.79 (1H, d, *J* 7.8 Hz, ArH), 6.86 (1H, d, *J* 8.8 Hz, ArH), 7.31–7.50 (12H, m, ArH). NMR δ_C ($CDCl_3$): 37.9, 41.3, 53.5, 55.9, 56.0, 70.8, 71.1, 71.9, 111.2, 112.0, 112.5, 114.3, 121.0, 124.3, 127.20, 127.24, 127.9, 128.2, 128.5, 128.7, 128.9, 130.6, 136.0, 137.0, 147.2, 149.6, 149.9, 153.3, 172.9, 191.5. FABMS *m/z* (%): 552 (M^+, 31), 461 (M^+–$C_6H_5CH_2$, 10), 325 (M^+–$(C_6H_5CH_2O)(CH_3O)PhCH_2$, 28), 253 ($(C_6H_5CH_2O)(CH_3O)C_6H_3C(=O)C$, 35), 241 ($(C_6H_5CH_2O)(CH_3O)C_6H_3C(=O)$, 34), 163 ($(CH_3O)(HO)C_6H_3CH_2(CH)_2$, 29), 137 ($(CH_3O)(HO)C_6H_3CH_2$, 12), 91 ($C_6H_5CH_2$, 100). HRFABMS ($M^+$): Calcd. for $C_{34}H_{32}O_7$, 552.2148. Found, 552.2148.

*(2S,3R)-2-(4-Hydroxy-3-methoxybenzoyl)-3-(4-hydroxy-3-methoxybenzyl)-4-butanolide (**Mat 2**).* A reaction mixture of benzyl ether **7** (0.40 g, 0.72 mmol) and 5% Pd–C (0.32 g) in EtOAc (10 ml) was stirred at the ambient temperature for 2 h under H_2 gas before filtration. The filtrate was concentrated, and then the residue was applied to silica gel column chromatography (EtOAc/hexane = 1/1) to give **Mat 2** (0.23 g, 0.62 mmol, 86%) as a colorless oil, $[\alpha]^{20}_D = +58$ (*c* 1.1, $CHCl_3$). NMR δ_H ($CDCl_3$): 2.75 (1H, dd, *J* 13.9, 8.1 Hz, ArC*H*H), 2.84 (1H, dd, *J* 13.9, 7.6 Hz, ArCH*H*), 3.36 (1H, m, 3-H), 3.80 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.15 (1H, dd, *J* 8.8, 5.9 Hz, 4-*H*H), 4.27 (1H, d, *J* 6.4 Hz, 2-H), 4.53 (1H, dd, *J* 8.8, 6.8 Hz, 4-H*H*), 5.61 (1H, s, ArOH), 6.28 (1H, s, ArOH), 6.62 (1H, s, ArH), 6.64 (1H, d, *J* 7.8 Hz, ArH), 6.83 (1H, d, *J* 7.8 Hz, ArH), 6.89 (1H, d, *J* 8.3 Hz, ArH), 7.30 (1H, dd, *J* 8.3, 2.0 Hz, ArH), 7.44 (1H, d, *J* 2.0 Hz, ArH). NMR δ_C ($CDCl_3$): 38.0, 41.6, 53.5, 55.8, 56.1, 71.9, 110.6, 111.3, 114.0, 114.6, 121.7, 125.0, 128.4, 129.3, 144.6, 146.7, 151.3, 173.1, 191.5. EIMS *m/z* (%): 372 (M^+, 20), 163 ($(HO)(CH_3O)C_6H_3CH_2(CH)_2$, 100), 151 ($(HO)(CH_3O)C_6H_3C(=O)$, 98), 137 ($(HO)(CH_3O)C_6H_3CH_2$, 33). HREIMS ($M^+$): Calcd. for $C_{20}H_{20}O_7$, 372.1209. Found, 372.1208.

*(2R,3R)-2-(4-Benzyloxy-3-methoxybenzyl)-3-[(R)-(4-benzyloxy-3-methoxyphenyl)(triisopropylsilyloxy)methyl]-4-butanolide (**9**).* To a solution of KHMDS (7.42 ml, 0.5 M in toluene, 3.71 mmol) in THF (60 ml) was added a solution of lactone **8** (1.50 g, 3.09 mmol) in THF (10 ml) at −70 °C. After 15 min at −70 °C, a solution of

4-benzyloxy-3-methoxybenzyl bromide (0.95 g, 3.09 mmol) in THF (10 ml) was added. After stirring at −70 °C for 30 min, sat. aq. NH_4Cl solution was added. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/toluene = 1/4) gave benzyl lactone **9** (1.27 g, 1.79 mmol, 58%) as a colorless oil, $[\alpha]^{20}_D = +37$ (*c* 1.8, $CHCl_3$). NMR δ_H ($CDCl_3$): 0.92–0.98 (21H, m, *iso*-Pr), 2.49 (1H, m, 2-H), 2.61–2.68 (2H, m, $ArCH_2$), 2.80 (1H, m, 3-H), 3.79 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 4.12 (1H, dd, *J* 9.3, 7.8 Hz, 4-*H*H), 4.43 (1H, dd, *J* 9.3, 6.4 Hz, 4-H*H*), 4.52 (1H, d, *J* 6.8 Hz, ArCHOTIPS), 5.12 (2H, s, OCH_2Ar), 5.14 (2H, s, OCH_2Ar), 6.42 (1H, dd, *J* 8.3, 2.0 Hz, ArH), 6.56–6.59 (2H, m, ArH), 6.67 (1H, d, *J* 2.0 Hz, ArH), 6.73 (1H, d, *J* 8.3 Hz, ArH), 6.77 (1H, d, *J* 8.3 Hz, ArH), 7.18 (1H, m, ArH), 7.24–7.37 (7H, m, ArH), 7.42–7.44 (2H, m, ArH). NMR δ_C ($CDCl_3$): 12.5, 17.9, 18.0, 34.7, 43.6, 47.7, 55.8, 55.9, 68.2, 71.0, 75.6, 109.9, 112.9, 113.6, 113.9, 118.7, 121.4, 127.3, 127.4, 127.8, 127.9, 128.2, 128.5, 129.0, 130.6, 135.4, 136.9, 137.2, 147.1, 147.8, 149.7, 179.0. *Anal.* Found: C, 73.02; H, 7.61. Calcd. for $C_{43}H_{54}O_7Si$: C, 72.64; H, 7.66%.

*(2R,3R)-3-(4-Benzyloxy-3-methoxybenzoyl)-2-(4-benzyloxy-3-methoxybenzyl)-4-butanolide (**10**).* A reaction solution of silyl ether **9** (0.88 g, 1.24 mmol) and *n*-Bu_4NF (1.36 ml, 1 M in THF, 1.36 mmol) in THF (15 ml) was stirred at 0 °C for 1 h before addition of sat. aq. NH_4Cl solution. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration gave crude benzyl alcohol. This crude benzyl alcohol was converted to **10** by PCC oxidation by the same method as that described for compound **7**. Colorless crystals, mp 105–107 °C (EtOAc), 42% yield (2 steps), $[\alpha]^{20}_D = +13$ (*c* 1.2, $CHCl_3$). NMR δ_H ($CDCl_3$): 2.97 (1H, dd, *J* 14.2, 7.3 Hz, ArC*H*H), 3.03 (1H, dd, *J* 14.2, 5.4 Hz, ArCH*H*), 3.53 (1H, m, 2-H), 3.70 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.01–4.14 (2H, m, 3-H, 4-*H*H), 4.35 (1H, dd, *J* 7.8, 7.3 Hz, 4-H*H*), 5.03 (2H, s, OCH_2Ar), 5.21 (2H, s, OCH_2Ar), 6.54 (1H, dd, *J* 8.3, 2.0 Hz, ArH), 6.63 (1H, d, *J* 2.0 Hz, ArH), 6.66 (1H, d, *J* 8.3 Hz, ArH), 6.81 (1H, d, *J* 8.3 Hz, ArH), 7.15 (1H, dd, *J* 8.3, 2.0 Hz, ArH), 7.29–7.41 (11H, m, ArH). NMR δ_C ($CDCl_3$): 34.2, 44.5, 46.4, 55.6, 55.9, 68.2, 70.7, 70.9, 110.6, 111.8, 112.8, 113.9, 121.4, 122.6, 127.1, 127.2, 127.7, 128.1, 128.4, 128.6, 128.9, 130.1, 135.8, 137.0, 147.1, 149.6, 149.8, 153.2, 177.2, 194.9. *Anal.* Found: C, 73.54; H, 5.81. Calcd. for $C_{34}H_{32}O_7$: C, 73.89; H, 5.84%.

*(2R,3R)-3-(4-Hydroxy-3-methoxybenzoyl)-2-(4-hydroxy-3-methoxybenzyl)-4-butanolide (**Mat 3**).* The same synthetic method as that described for **Mat 2** gave **Mat 3** from **10** as a colorless oil in 67% yield, $[\alpha]^{20}_D = +75$ (*c* 0.2, THF), $[\alpha]^{20}_D = +44.3$ (*c* 1.0, THF) in the literature.[6] NMR δ_H ($CDCl_3$): 2.99 (1H, dd, *J* 13.6, 6.8 Hz, ArC*H*H), 3.05 (1H, dd, *J* 13.6, 6.8 Hz, ArCH*H*), 3.52 (1H, m, 2-H), 3.76 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 4.06–4.18 (2H, m, 3-H, 4-*H*H), 4.39 (1H, dd, *J* 7.3, 7.3 Hz, 4-H*H*), 5.52 (1H, br. s, ArOH), 6.21 (1H, br. s, ArOH), 6.57 (1H, d, *J* 7.8 Hz, ArH), 6.62 (1H, s, ArH), 6.74 (1H, d, *J* 7.8 Hz, ArH), 6.90 (1H, d, *J* 8.3 Hz, ArH), 7.21 (1H, d, *J* 8.3 Hz, ArH), 7.35 (1H, s, ArH). NMR δ_C ($CDCl_3$): 34.3, 44.8, 46.3, 55.6, 56.0, 68.2, 109.9, 111.6, 113.7, 114.2, 119.7, 122.1, 123.4, 128.5, 128.8, 144.5, 146.5, 146.9, 151.3, 177.2, 194.8. EIMS *m/z* (%): 372 (M^+, 63), 194 (M^+–(HO)$(CH_3O)C_6H_3CH_2CHC$=O, 100), 151 ((HO)(CH_3O)-C_6H_3C=O, 74), 137 ((HO)$(CH_3O)C_6H_3CH_2$, 63). HREIMS (M^+): Calcd. for $C_{20}H_{20}O_7$, 372.1209. Found, 372.1210.

*(2S,3R)-2,3-Bis(4-benzyloxy-3-methoxybenzoyl)-4-butanolide (**12**).* The same synthetic method as that described for compound **10** gave **12** from **11** as colorless crystals, mp 147–149 °C (EtOAc), in 58% yield (2 steps), $[\alpha]^{20}_D$ +6 (*c* 1.1, $CHCl_3$). NMR δ_H ($CDCl_3$): 3.90 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.39 (1H, dd, *J* 8.8, 7.3 Hz, 4-*H*H), 4.72 (1H, dd, *J* 8.8, 8.8 Hz, 4-H*H*), 5.03 (1H, ddd, *J* 8.8, 7.3, 7.3 Hz, 3-H), 5.11 (1H, d, *J* 7.3 Hz, 2-H), 5.21 (4H, s, OCH_2Ar), 6.90 (1H, d, *J* 7.3 Hz, ArH), 6.93 (1H, d, *J* 8.8 Hz, ArH), 7.30–7.42 (10H, m, ArH), 7.48 (1H, d, *J* 8.3 Hz, ArH), 7.51 (1H, s, ArH), 7.61 (1H, d, *J* 1.5 Hz, ArH), 7.71 (1H, dd, *J* 8.3, 2.0 Hz, ArH). NMR δ_C ($CDCl_3$): 45.2, 50.4, 56.0, 68.0, 70.7, 70.8, 110.9, 111.6, 112.1, 112.2, 123.3, 125.2, 127.1, 125.2, 127.1, 128.0, 128.1, 128.2, 128.4, 128.61, 128.63, 135.8, 135.9, 149.5, 149.9, 153.5, 153.6, 171.3, 190.2, 194.4. *Anal.* Found: C 71.81%, H 5.33%. Calcd for $C_{34}H_{30}O_8$: C 72.07%, H 5.34%.

*(2S,3R)-2.3-Bis(4-hydroxy-3-methoxybenzoyl)-4-butanolide (**Mat 4**).* The same synthetic method as that described for **Mat 2** gave **Mat 4** from compound **12** as a colorless oil in 90% yield, $[\alpha]^{20}_D$ +43 (*c* 1.1, $CHCl_3$). NMR δ_H ($CDCl_3$): 3.83 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.37 (1H, dd, *J* 8.8, 6.8 Hz, 4-*H*H), 4.73 (1H, dd, *J* 8.8, 8.8 Hz, 4-H*H*), 5.02 (1H, ddd, *J* 8.8, 6.8, 6.8 Hz, 3-H), 5.14 (1H, d, *J* 6.8 Hz, 2-H), 6.56 (2H, s, ArOH), 6.90 (2H, d, *J* 8.8 Hz, ArH), 7.46–7.48 (2H, m, ArH), 7.54 (1H, d, *J* 2.0 Hz, ArH), 7.68 (1H, dd, *J* 8.3, 2.0 Hz, ArH). NMR δ_C ($CDCl_3$): 45.3, 50.3, 55.9, 56.0, 68.8, 110.4, 111.1, 114.2, 114.3, 124.1, 125.8, 127.4, 127.9, 146.6, 147.1, 151.6, 151.7, 171.7, 190.3, 194.4; EIMS *m/z* (%): 386 (M^+, 13), 342 (M^+–CO_2, 14), 235 ([M^+–C(=O)C_6H_3(OH)(OCH_3), 100], 151 [M^+–C(=O)-$C_6H_3(OCH_3)$(OH), 99], 123 [(C_6H_3(OH)(OCH_3), 47); HREIMS (M^+): calcd. for $C_{20}H_{18}O_8$, 386.1002. Found: 386.1002.

*(2R,3R)-2,3-Bis(4-benzyloxy-3-methoxybenzyl)-4-butanolide (**14**).* The same synthetic method as that described for **9** gave **14** from **13** as a colorless oil in 47% yield, $[\alpha]^{20}_D = 11$ (*c* 5.7, $CHCl_3$). NMR δ_H ($CDCl_3$): 2.41–2.51 (2H, m, 2-H, 2-ArC*H*H), 2.51–2.62 (2H, m, 3-H, 2-ArCH*H*), 2.88 (1H, dd, *J* 9.8, 5.9

Hz, 3-ArC*H*H), 2.92 (1H, dd, *J* 9.8, 5.9 Hz, 3-ArCH*H*), 3.79 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.83–3.88 (1H, m, 4-*H*H), 4.10 (1H, dd, *J* 8.3, 7.3 Hz, 4-H*H*), 5.10 (4H, s, OCH_2Ar), 6.45 (1H, d, *J* 8.3 Hz, ArH), 6.48 (1H, s, ArH), 6.56 (1H, d, *J* 7.8 Hz, ArH), 6.69 (1H, s, ArH), 6.73–6.77 (2H, m, ArH), 7.24–7.36 (6H, m, ArH), 7.40–7.42 (4H, m, ArH). NMR δ_C ($CDCl_3$): 34.5, 38.2, 41.0, 46.5, 56.0, 71.1, 71.2, 112.5, 112.9, 114.1, 114.3, 120.5, 121.3, 127.2, 127.3, 127.7, 127.80, 127.83, 128.49, 128.52, 130.8, 131.1, 137.1, 147.0, 147.1, 149.7, 149.8, 178.7. *Anal.* Found: C, 75.74; H, 6.50. Calcd. for $C_{34}H_{34}O_6$: C, 75.82; H, 6.36%.

*(2S,3S)-2,3-Bis(4-benzyloxy-3-methoxybenzyl)-2-hydroxy-4-butanolide (**15**) and (2R,3S)-2,3-bis(4-benzyloxy-3-methoxybenzyl)-2-hydroxy-4-butanolide (**16**).* To a solution of KHMDS (4.24 ml, 0.5 M toluene solution, 2.12 mmol) in THF (8.5 ml) was added a solution of lactone **14** (0.57 g, 1.06 mmol) in THF (8.5 ml) at −70 °C. After the mixture was stirred at −70 °C for 30 min, MoOPH (0.56 g, 1.29 mmol) was added. The resulting reaction mixture was stirred at −70 °C for 2 h before additions of sat. aq. Na_2SO_3 solution and EtOAc. The organic solution was separated, washed with 2 M aq. HCl solution, sat. aq. $NaHCO_3$ solution, and brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/4) gave (2*S*)-**15** (80 mg, 0.14 mmol, 13%) as a colorless oil and (2*R*)-**16** (60 mg, 0.11 mmol, 10%) as a colorless oil. Lactone **14** (0.25 g, 0.46 mmol, 43%) was recovered. (2*S*)-**15**, $[\alpha]^{20}_D = -25$ (*c* 0.5, $CHCl_3$). NMR δ_H ($CDCl_3$): 2.49–2.53 (2H, m, 3-H, 3-ArC*H*H), 2.88–2.95 (1H, m, 3-ArCH*H*), 2.91 (1H, d, *J* 13.7 Hz, 2-ArC*H*H), 3.08 (1H, d, *J* 13.7 Hz, 2-ArCH*H*), 3.83 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.96–4.05 (2H, m, 4-H_2), 5.119 (2H, s, $ArCH_2O$), 5.124 (2H, s, $ArCH_2O$), 6.58–6.60 (2H, m, ArH), 6.64 (1H, s, ArH), 6.74 (1H, s, ArH), 6.78–6.80 (2H, m, ArH), 7.27–7.29 (2H, m, ArH), 7.31–7.37 (4H, m, ArH), 7.41–7.43 (4H, m, ArH). NMR δ_C ($CDCl_3$): 31.6, 42.1, 43.8, 56.0, 70.0, 71.0, 71.1, 76.3, 112.8, 113.9, 114.0, 114.4, 120.8, 122.4, 127.21, 127.22, 127.82, 127.84, 128.5, 131.6, 137.0, 137.1, 146.9, 147.6, 149.6, 149.8, 178.3. *Anal.* Found: C, 73.46; H, 6.36. Calcd. for $C_{34}H_{34}O_7$: C, 73.63; H, 6.18%. (2*R*)-**16**, $[\alpha]^{20}_D = -11$ (*c* 0.9, $CHCl_3$). NMR δ_H ($CDCl_3$): 2.64 (1H, dd, *J* 13.7, 11.7 Hz, 3-ArC*H*H), 2.82 (1H, s, OH), 2.88–2.98 (1H, m, 3-H), 2.96 (2H, s, 2-$ArCH_2$), 3.12 (1H, dd, *J* 13.7, 3.9 Hz, 3-ArCH*H*), 3.82–3.84 (1H, m, 4-*H*H), 3.88 (6H, s, OCH_3), 4.17 (1H, dd, *J* 8.8, 7.3 Hz, 4-H*H*), 5.14 (4H, s, OCH_2Ar), 6.62 (1H, d, *J* 8.3 Hz, ArH), 6.71–6.72 (2H, m, ArH), 6.77 (1H, d, *J* 2.0 Hz, ArH), 6.81–6.85 (2H, m, ArH), 7.28–7.30 (2H, m, ArH), 7.32–7.39 (4H, m, ArH), 7.43–7.45 (4H, m, ArH). NMR δ_C ($CDCl_3$): 32.0, 38.2, 48.0, 56.0, 69.3, 71.0, 71.1, 75.8, 112.1, 113.9, 114.1, 114.4, 120.4, 122.6, 126.0, 127.2, 127.3, 127.8, 128.5, 130.9, 137.0, 137.1, 147.1, 147.8, 149.5, 150.0, 177.8. *Anal.* Found: C, 73.23; H, 6.47. Calcd. for $C_{34}H_{34}O_7$: C, 73.63; H, 6.18%.

*(2S,3S)-2-Hydroxy-2,3-bis(4-hydroxy-3-methoxybenzyl)-4-butanolide (**Mat 5**).* The same synthetic method as that described for **Mat 2** gave **Mat 5** from compound **15** as a colorless oil in 86% yield, $[\alpha]^{20}_D = -30$ (*c* 1.1, $CHCl_3$). NMR δ_H ($CDCl_3$): 2.46–2.53 (2H, m, 3-H, 3-ArC*H*H), 2.73 (1H, s, OH), 2.88–2.96 (1H, m, 3-ArCH*H*), 2.91 (1H, d, *J* 13.7 Hz, 2-ArC*H*H), 3.08 (1H, d, *J* 13.7 Hz, 2-ArCH*H*), 3.82 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.97–4.04 (2H, m, 4-H_2), 5.56 (1H, s, ArOH), 5.58 (1H, s, ArOH), 6.59–6.62 (3H, m, ArH), 6.69 (1H, s, ArH), 6.81–6.83 (2H, m, ArH). NMR δ_C ($CDCl_3$): 31.6, 42.1, 43.8, 55.89, 55.94, 70.1, 76.4, 111.5, 112.7, 114.3, 114.5, 121.3, 121.4, 126.1, 130.3, 144.3, 145.0, 146.56, 146.60, 178.5. EIMS *m/z* (%): 374 (M^+, 55), 358 (M^+–OH + 1, 23), 137 ((HO)(CH_3O)$C_6H_3CH_2$, 100). HREIMS (M^+): Calcd. for $C_{20}H_{22}O_7$, 374.1366. Found, 374.1365.

*(2R,3S)-2-Hydroxy-2,3-bis(4-hydroxy-3-methoxybenzyl)-4-butanolide (**Mat 6**).* The same synthetic method as that described for **Mat 2** gave **Mat 6** from compound **16** as colorless powder, mp 170–172 °C, in 73% yield, $[\alpha]^{20}_D = -4.9$ (*c* 1.2, $CHCl_3$). NMR δ_H ($CDCl_3$): 2.64 (1H, dd, *J* 13.4, 11.7 Hz, 3-ArC*H*H), 2.88–2.99 (2H, m, OH, 3-H), 2.95 (2H, s, 2-$ArCH_2$), 3.12 (1H, dd, *J* 13.4, 1.3 Hz, 3-ArCH*H*), 3.83–3.88 (1H, m, 4-*H*H), 3.86 (3H, s,OCH_3), 3.87 (3H, s, OCH_3), 4.17 (1H, dd, *J* 8.8, 8.6 Hz, 4-H*H*), 5.62 (1H, s, ArOH), 5.70 (1H, s, ArOH), 6.63–6.68 (2H, m, ArH), 6.72–6.74 (2H, m, ArH), 6.83–6.86 (2H, m, ArH). NMR δ_C ($CDCl_3$): 32.0, 38.2, 48.1, 55.9, 56.0, 69.4, 75.8, 110.8, 112.9, 114.4, 114.6, 121.1, 123.3, 124.7, 129.6, 144.5, 145.2, 146.5, 146.8, 178.0. EIMS *m/z* (%): 374 (M^+, 38), 137 ((HO)(CH_3O)$C_6H_3CH_2$, 100). HREIMS (M^+): Calcd. for $C_{20}H_{22}O_7$, 374.1366. Found, 374.1366.

*Measurement of the radical scavenging activities of **Mat 1–Mat 3**, **Mat 5** and **Mat 6**.* To an appropriate amount of a sample in a methanol solution (4.9 ml) was added 100 µl of 5 mM DPPH in a methanol solution. After the solution had stood at 25 °C for 0.5 h, the absorbance at 517 nm was measured. The antiradical activity was evaluated from the decreased value of the 51-nm absorption, which was calculated by the following equation: %scavenging effect = $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorption of the control (without a sample) and A_1 is the absorption of the mixture containing a sample.

*Measurement of the superoxide scavenging activities of **Mat 1–Mat 6** and **Lio 7**.* The superoxide scavenging activity was determined by using a WST superoxide dismutase assay kit (Dojindo Molecular Technology, Kumamoto, Japan). Briefly, superoxide radicals were generated by the xanthine/xanthine oxidase system, and reduced WST-8 to water-soluble formazane which exhibited an absorption maximum at 450 nm. Decreased absorption of the reaction mixture indicated increased

superoxide scavenging activity. The reaction mixture was incubated at 37 °C for 20 min, and the absorption was read at 450 nm by a Bio-Rad 680 microplate eader. The capability of scavenging the superoxide radicals was calculated by using the following equation: % scavenging effect = $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorption of the control (without a sample) and A_1 is the absorption of the mixture containing a sample.

Statistical analysis. Each results is expressed as the means ± standard deviation (SD: n = 3). Student's *t*-test was used to assess the statistical significance of a difference which was considered to be significant at $p < 0.01$.

Acknowledgments

We measured the 400 MHz NMR data at INCS of Ehime University. Our thanks to the president of Ehime University for supporting this project. We are also grateful to Marutomo Co. for financial support.

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