近畿大学工学部研究報告　No.49，2015年，pp.7−13
Research Reports of the Faculty of Engineering,
Kinki University No.49 2015, pp.7−13

Convenient Synthesis and Physiological Activities of 4-(Hydroxyphenyl)-2-butanols

Masato NAKAMOTO[2)], Mayu OKAMURA[1)], Yoshiharu OKADA[*1)], Chiaki OISHI[1)], Riho URUSHIHARA[1)], Hatsune SUNAMI[1)], Daisuke NAKABO[1)], Kensuke NAKAMURA[1)], Ayane YOKOUCHI[1)], Michiko MOTOYA[2)], and Masato NOMURA[1)]

ABSTRACT

4-(Hydroxyphenyl)-2-butanols were conveniently synthesized from the corresponding aryl aldehydes and dimethyl 2-oxopropylphophonate in moderate yields. The Physiological activities of these compounds were assessed on the basis of DPPH free radical scavenging assay and tyrosinase inhibition activity assay. When the tyrosinase activity inhibition rate of these compounds using *L*-DOPA as a substrate was compared with arbutin as reference compound, the activity of 4-(4-hydroxypheny)-2-butanol (rhododenol), 4-(4-hydroxy-3-methoxyphenyl)-2-butanol, 4-(3-hydroxy-4-methoxyphenyl)-2-butanol, 4-(2,4-dihydroxyphenyl)-2-butanol, and 4-(3,5-dihydroxyphenyl)-2-butanol were superior to that of arbutin.

Keywords: Rhododenol, Antioxidant, Tyrosinase

INTRODUCTION

Polyphenols such as *trans*-resveratrol were widely known as antioxidant compounds. Especially vitamin E (α-tocopherol) and vitamin C (ascorbic acid) were popular antioxidant compounds to use foods and cosmetics. On the other hand, 4-(4-hydroxyphenyl)-2-butanol (rhododenol) was isolated from *Rhododendron brachycarpum*, bark of *Acer nikoence*, and bark of *Betula platyphylla* and has high whitening effect[1)]. But, this compound was reported to damage the skin in 2013. We have interested in this natural occurring compound and

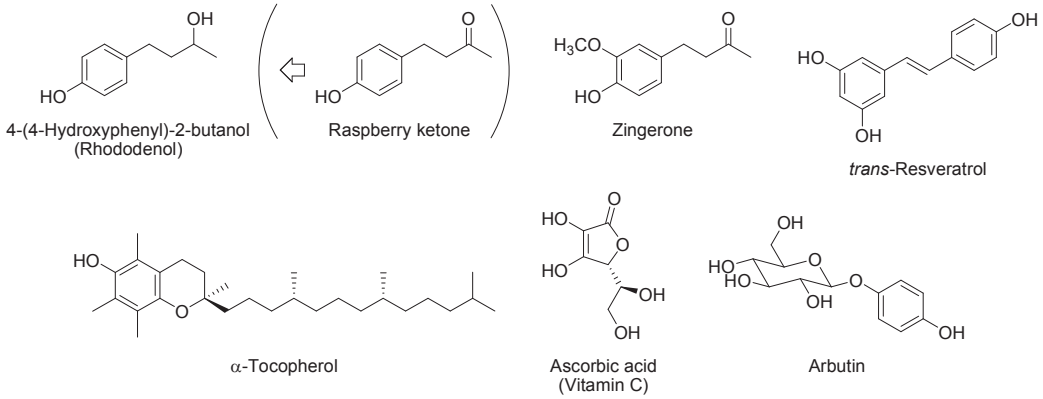

Fig. 1. Structures of Popular Antioxidative and Whitening Compounds.

1) 近畿大学工学部化学生命工学科　Department of Biotechnology and Chemistry, Faculty of Engineering, Kinki University
2) 近畿大学大学院システム工学研究科　Graduate School of Systems Engineering, Kinki University

Scheme 1. Synthesis of 4-(Hydroxyphenyl)-2-butanols **3a-k**

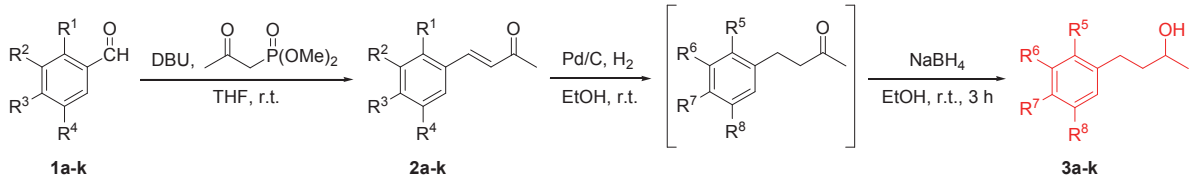

Table 1. Synthesis of **2a-k** and **3a-k**.

entry	Product						Product					
	2	R^1	R^2	R^3	R^4	Yield (%)	**3**	R^5	R^6	R^7	R^8	Yield (%)
1	**2a**	OBn	H	H	H	79	**3a**	OH	H	H	H	49
2	**2b**	H	OBn	H	H	77	**3b**	H	OH	H	H	67
3							**3c**	H	H	OH	H	88
4	**2d**	H	OCH_3	OBn	H	93	**3d**	H	OCH_3	OH	H	77
5	**2e**	H	OBn	OCH_3	H	85	**3e**	H	OH	OCH_3	H	57
6	**2f**	OBn	OBn	H	H	93	**3f**	OH	OH	H	H	47
7	**2g**	OBn	H	OBn	H	91	**3g**	OH	H	OH	H	40
8	**2h**	OBn	H	H	OBn	71	**3h**	OH	H	H	OH	58
9	**2i**	H	OBn	OBn	H	94	**3i**	H	OH	OH	H	35
10	**2j**	H	OBn	H	OBn	99	**3j**	H	OH	H	OH	51
11	**2k**	H	OCH_3	OBn	OCH_3	89	**3k**	H	OCH_3	OH	OCH_3	38

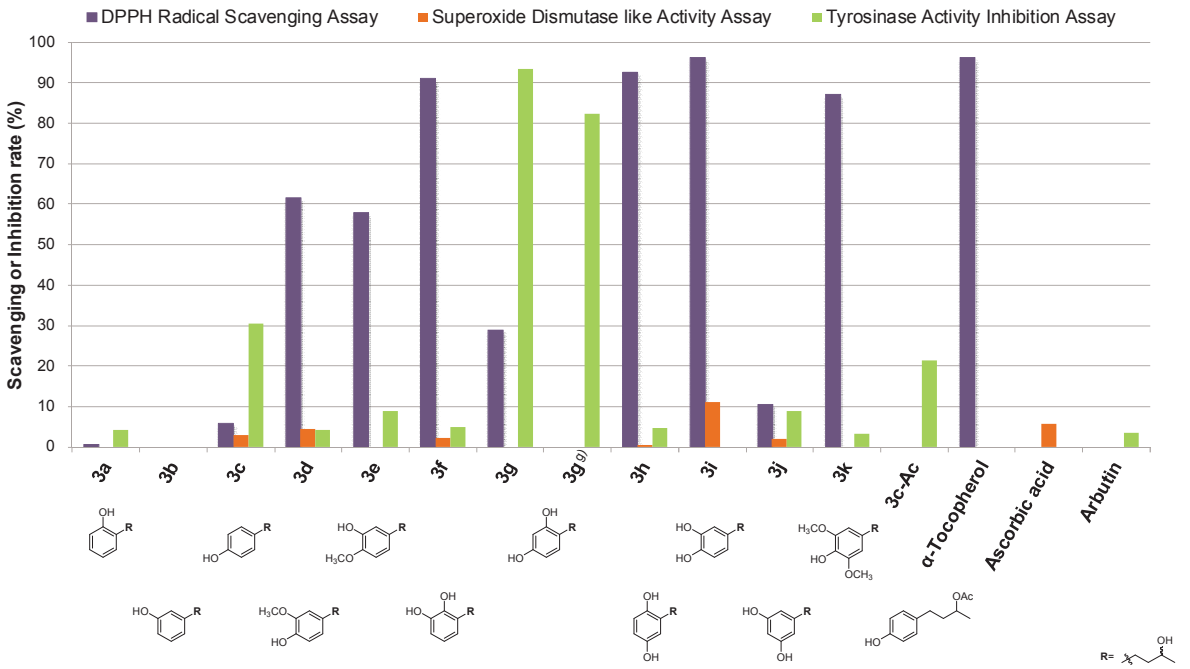

Fig. 2.　Physiological Activities of **3a-k**.

investigated the activity of its analogues. So, we report here the convenient synthesis of 4-(hydroxyphenyl)-2-butanols and their physiological activities.

RESULTS and DISCUSSION

Protection of salicylaldehyde, 3-hydroxybenzaldehyde, vanillin, isovanillin, 2,3-dihydroxybenzaldehyde, 2,4-dihydroxybenzaldehyde, 2,5-dihydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, 3,5-dihydroxybenzaldehyde, 2,3,4-trihydroxybenaldehyde, 2,4,5-trihydroxybenzaldehyde, and silingaldehyde with benzyl chloride in the presence of potassium carbonate in ethanol at reflux afforded the corresponding benzyloxybenzaldehydes **1a-k** in good yields. Horner-Wadsworth-Emmons reaction of **1a-k** with dimethyl 2-oxopropylphosphonate in the presence of 1,7-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded the α,β-unsaturated ketones **2a-k** in good yields. Hydrogenation and deprotection of **2a-k** in ethanol in hydrogen atmosphere at room temperature gave the 4-(hydroxylphenyl)-2-butanones and subsequent reduction of the crude 2-butanones with sodium borohydride in ethanol afforded the corresponding 2-butanols **3a-k** in moderate yields (Scheme 1, Table 1). Also similar reduction of raspberry ketone afforded rhododenol **3c** in good yield. The physiological activities of **3a-k** were assessed in the basis of DPPH-radical scavenging assay[2] and superoxide dismutase (SOD)-like activity assay[3] as an antioxidant activity, and tyrosinase activity inhibition assay[3] as a whitening effect. The results were summarized in Table 2 and Fig. 2. 4-(2,3-Dihydroxyphenyl)-2-butanol **3f**, 4-(2,5-dihydroxyphenyl)-2-butanol **3h**, 4-(3,4-dihydroxyphenyl)-2-butanol **3i**, and 4-(4-hydroxy-3,5-dimethoxyphenyl)-2-butanol **3k** showed high radical scavenging activity equal to that of α-

Table 2. Antioxidant Activity, Superoxide Dismutase like Activity, and Tyrosinase Activity Inhibition of **3a-k**.

Compound					DPPH Radical Scavenging Assay[a]	Superoxide Dismutase like Activity Assay[c]	Tyrosinase Activity Inhibition Assay[e]
3	R^5	R^6	R^7	R^8	Scavenging Rate (%)[b]	Inhibition Rate (%)[d]	Inhibition Rate (%)[f]
3a	OH	H	H	H	0.5	–	4.2
3b	H	OH	H	H	0.0	–	0.0
3c	H	H	OH	H	5.8	2.9	30.6
3d	H	OCH_3	OH	H	61.5	4.4	4.2
3e	H	OH	OCH_3	H	57.8	0.0	8.8
3f	OH	OH	H	H	91.0	2.3	5.0
3g	OH	H	OH	H	28.9	0.0	93.5
3g	OH	H	OH	H	–	–	82.4 [g]
3h	OH	H	H	OH	92.6	0.5	4.6 [g]
3i	H	OH	OH	H	96.1	11.2	0.0 [h]
3j	H	OH	H	OH	10.5	1.9	8.9
3k	H	OCH_3	OH	OCH_3	87.0	0.0	3.1
3c-Ac	H	H	OH	H	–	–	21.5
α-Tocopherol					96.1	–	–
Ascorbic acid					–	5.7	–
Arbutin					–	–	3.5

a) Sample Concentration: 0.10 mM. b) Final Concentration: 0.040 mM. c) Sample Concentration: 0.10 mM.
d) Final Concentration: 0.0043 mM. e) Sample Concentration: 30.0 mM. f) Final Concentration: 1.0 mM.
g) Final Concentration: 0.10 mM. h) Final Concentration: 0.70 mM.

tocopherol, but not showed inhibition of tyrosinase activity. Rhododenol **3c** was found that inhibition rate of tyrosinase activity showed higher than that of arbutin, but rare antioxidant activity. 4-(4-Hydroxy-3-methoxyphenyl)-2-butanol **3d** derived from zingeron showed middle antioxidant activity and tyrosinase activity inhibition effect equal to arbutin. Displaced hydroxyl group and methoxy group was not influenced activities. In the antioxidant activity, *ortho* or *para* hydroquinone moiety of phenyl group plays important role. 4-(2,4-Dihydroxyphenyl)-2-butanol **3g** showed highest tyrosinase activity inhibition rate, but antioxidant activity was low. 4-(3,5-Dihydroxyphenyl)-2-butanol **3j** showed low antioxidant activity and lower tyrosinase activity inhibition rate than that of **3g**. 4-(4-Hydroxyphenyl)-2-butyl acetate **3c-Ac**, which was protected hydroxyl group of side chain with acetyl group, showed lower tyrosinase activity inhibition than that of **3c**. That is, 2-butanol group was also important in the activity.

CONCLUSION

Horner-Wadsworth-Emmons reaction of hydroxybenzaldehydes with dimethyl 2-oxopropylphosphonate was simply proceeded without anhydrous condition in the presence of DBU to give the corresponding α,β-unsaturated ketones in moderate yields. Although compounds **3g,j** bearing of dihydroxyphenyl group like as resorcinol showed lower antioxidant activity, **3g** was highest inhibition of tyrosinase activity.

EXPERIMENTAL

General Procedures. ^{1}H NMR spectra were obtained on a JEOL JNM-EX400 spectrometer in $CDCl_3$ and CD_3OD operating at 400 MHz with Me_4Si as internal standard.

Materials. Tetrahydrofuran (THF) was purified by distillation from benzophenone ketyl under an argon atmosphere before use.

Synthesis of 4-(Benzyloxyphenyl)-3-buten-2-ones 2a-k.

A solution of aryl aldehydes **1a-k** (3 mmol), dimethyl 2-oxopropylphosphonate (0.60 g, 3.6 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.91 g, 6 mmol) in THF (15 mL) was stirred at room temperature for 24 hours. Reaction mixture was quenched by the addition of 2M HCl. The mixture was extracted with Et_2O, washed with water and saturated NaCl, dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The residue was chromatographed on preparative TLC ($CHCl_3$:Et_2O=95:5) to give 4-(benzyloxyphenyl)-3-buten-2-ones **2a-k**.

4-(2-Benzyloxyphenyl)-3-buten-2-one (2a): 0.60 g (79%); ^{1}H NMR ($CDCl_3$) 2.34 (s, 3H, CH_3), 5.16 (s, 2H, CH_2), 6.76 (d, J=16.4 Hz, 1H, olefinic H), 6.97 (d, J=8.8 Hz, 1H, ArH), 7.32-7.50 (m, 7H, ArH), 7.56 (d, J=7.3 Hz, 1H, ArH), 7.95 (d, J=16.4 Hz, 1H, olefinic H).

4-(3-Benzyloxyphenyl)-3-buten-2-one (2b): 0.58 g (77%); ^{1}H NMR ($CDCl_3$) 2.36 (s, 3H, CH_3), 5.07 (s, 2H, CH_2), 6.68 (d, *J*=16.1 Hz, 1H, olefinic H), 7.01 (d, *J*=8.3 Hz, 1H, ArH), 7.14 (s, 1H, ArH), 7.24-7.48 (m, 7H, ArH), 7.46 (d, *J*=16.1 Hz, 1H, olefinic H).

4-(4-Benzyloxy-3-methoxyphenyl)-3-buten-2-one (2d): 0.79 g (93%); ^{1}H NMR ($CDCl_3$) 2.36 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 5.19 (s, 2H, CH_2), 6.59 (d, 1H, olefinic H), 6.88 (d, *J*=8.3 Hz, 1H, ArH), 7.05 (d, *J*=8.3 Hz, 1H, ArH), 7.09 (s, 1H, ArH), 7.32-7.47 (m, 6H, olefinic H and ArH).

4-(3-Benzyloxy-4-methoxyphenyl)-3-buten-2-one (2e): 0.72 g (85%); ^{1}H NMR ($CDCl_3$) 2.34 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 5.17 (s, 2H, CH_2), 6.50 (d, *J*=16.4 Hz, 1H, olefinic H), 6.89 (d, *J*=8.1 Hz, 1H, ArH), 7.10 (s, 1H, ArH), 7.13 (d, *J*=8.3 Hz, 1H, ArH), 7.26-7.47 (m, 5H, ArH), 7.40 (d, *J*=16.4 Hz, 1H, olefinic H).

4-(2,3-Dibenzyloxyphenyl)-3-buten-2-one (2f): 0.99 g (93%); ^{1}H NMR ($CDCl_3$) 2.23 (s, 3H, CH_3), 5.08 (s, 2H, CH_2), 5.17 (s, 2H, CH_2), 6.56 (d, *J*=16.6 Hz, 1H, olefinic H), 7.05 (s, 1H, ArH), 7.06 (d, *J*=2.2 Hz, 1H, ArH), 7.17 (dd, *J*=3.4 and 5.9 Hz, 1H, ArH), 7.30-7.49 (m, 10H, ArH), 7.71 (d, *J*=16.6 Hz, 1H, olefinic H).

4-(2,4-Dibenzyloxyphenyl)-3-buten-2-one (2g): 0.97 g (91%); ^{1}H NMR ($CDCl_3$) 2.32 (s, 3H, CH_3), 5.05 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.59 (s, 1H, ArH), 6.60 (d, *J*=8.1 Hz, 1H, ArH), 6.69 (d, *J*=16.6 Hz, 1H, olefinic H), 7.26-7.41 (m, 10H, ArH), 7.51 (d, *J*=8.3 Hz, 1H, ArH), 7.87 (d, *J*=16.4 Hz, 1H, olefinic H).

4-(2,5-Dibenzyloxyphenyl)-3-buten-2-one (2h): 0.76

g (71%); ^{1}H NMR ($CDCl_3$) 2.34 (s, 3H, CH_3), 5.03 (s, 2H, CH_2), 5.11 (s, 2H, CH_2), 6.69 (d, J=16.6 Hz, 1H, olefinic H), 6.90 (d, *J*=9.0 Hz, 1H, ArH), 6.96 (dd, *J*=2.9 and 9.0 Hz, 1H, ArH), 7.17 (d, J=2.9 Hz, 1H, ArH), 7.30-7.44 (m, 10H, ArH), 7.90 (d, *J*=16.4 Hz, 1H, olefinic H).

4-(3,4-Dibenzyloxyphenyl)-3-buten-2-one (2i): 1.01 g (94%); ^{1}H NMR ($CDCl_3$) 2.34 (s, 3H, CH_3), 5.18 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 6.53 (d, *J*=16.4 Hz, 1H, olefinic H), 6.92 (d, *J*=8.3 Hz, 1H, ArH), 7.09 (d, *J*=8.3 Hz, 1H, ArH), 7.13 (s, 1H, ArH), 7.26-7.44 (m, 10H, ArH), 7.39 (d, *J*=16.4 Hz, 1H, olefinic H).

4-(3,5-Dibenzyloxyphenyl)-3-buten-2-one (2j): 1.06 g (99%); ^{1}H NMR ($CDCl_3$) 2.37 (s, 3H, CH_3), 5.05 (s, 4H, CH_2), 6.65 (d, *J*=16.4 Hz, 1H, olefinic H), 6.66 (t, *J*=2.0 Hz, 1H, ArH), 6.77 (d, *J*=2.2 Hz, 2H, ArH), 7.31-7.43 (m, 10H, ArH), 7.41 (d, *J*=16.1 Hz, 1H, olefinic H).

4-(4-Benzyloxy-3,5-dimethoxyphenyl)-3-buten-2-one (2k): 0.83 g (89%); ^{1}H NMR ($CDCl_3$) 2.38 (s, 3H, CH_3), 3.86 (s, 6H, OCH_3), 5.06 (s, 2H, CH_2), 6.63 (d, *J*=16.1 Hz, 1H, olefinic H), 6.76 (s, 2H, ArH), 7.29-7.37 (m, 3H, ArH), 7.43 (d, *J*=16.1 Hz, 1H, olefinic H), 7.47 (d, *J*=6.8 Hz, 2H, ArH).

Synthesis of 4-(Hydroxyphenyl)-2-butanols 3a-k.

A solution of **2a-k** (1 mmol) and 10% palladium carbon (0.05 g) in ethanol (20 mL) was stirred at room temperature for 2 days under hydrogen atmosphere. After being filtered off palladium carbon through the celite pad, sodium borohydride (0.04 g, 1 mmol) was added to the filtrate. The mixture was stirred at room temperature for 5 h. Reaction mixture was quenched by the addition of 2M HCl. The mixture was extracted with Et_2O, washed with water and saturated NaCl, dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The residue was chromatographed on preparative TLC ($CHCl_3$:Et_2O=1:1) to give 4-(hydroxyphenyl)-2-butanols **3a-k**.

4-(2-Hydroxyphenyl)-2-butanol (3a): 0.08 g (49%); ^{1}H NMR ($CDCl_3$) 1.20 (d, *J*=6.3 Hz, 3H, CH_3), 1.67-1.81 (m, 2H, CH_2), 2.60-2.67 (m, 1H, CH_2), 2.89 (ddd, *J*=6.3, 10.3, and 14.2 Hz, 1H, CH_2), 3.17 (brs, 1H, OH), 3.71-3.77 (m, 1H, CH), 6.85 (d, *J*=8.8 Hz, 1H, ArH), 6.87 (d, *J*=7.3 Hz, 1H, ArH), 7.09 (dd, *J*=2.4 and 8.8 Hz, 1H, ArH), 7.10 (d, *J*=7.1 Hz, 1H, ArH), 7.84 (s, 1H, OH).

4-(3-Hydroxyphenyl)-2-butanol (3b): 0.11 g (67%); ^{1}H NMR ($CDCl_3$) 1.20 (d, J=6.1 Hz, 3H, CH_3), 1.72-1.78 (m, 2H, CH_2), 2.40-2.66 (m, 3H, CH_2 and OH), 3.72-3.84 (m, 1H, CH), 6.68 (d, J=7.1 Hz, 1H, ArH), 6.69 (s, 1H, ArH), 6.72 (s, 1H, ArH), 7.11 (t, *J*=7.1 Hz, 1H, ArH), 7.35 (brs, 1H, OH).

4-(4-Hydroxyphenyl)-2-butanol (3c): 0.15 g (88%); ^{1}H NMR ($CDCl_3$) 1.22 (d, *J*=6.3 Hz, 3H, CH_3), 1.66-1.81 (m, 2H, CH_2), 1.99 (brs, 1H, OH), 2.55-2.69 (m, 2H, CH_2), 3.83 (sext, *J*=6.1 Hz, 1H, CH), 6.25 (brs, 1H, OH), 6.74 (d, *J*=8.5 Hz, 2H, ArH), 7.02 (d, *J*=8.5 Hz, 2H, ArH).

4-(4-Hydroxy-3-methoxyphenyl)-2-butanol (3d): 0.15 g (77%); ^{1}H NMR ($CDCl_3$) 1.22 (d, *J*=6.3 Hz, 3H, CH_3), 1.61 (brs, 1H, OH), 1.67-1.81 (m, 2H, CH_2), 2.56-2.72 (m, 2H, CH_2), 3.82 (sext, *J*=6.1 Hz, 1H, CH), 3.86 (s, 3H, OCH_3), 5.60 (brs, 1H, OH), 6.68 (dd, *J*=1.5 and 8.3 Hz, 1H, ArH), 6.70 (d, *J*=1.5 Hz, 1H, ArH), 6.82 (d, *J*=7.8 Hz, 1H, ArH).

4-(3-Hydroxy-4-methoxyphenyl)-2-butanol (3e): 0.11 g (57%); ^{1}H NMR ($CDCl_3$) 1.21 (d, *J*=6.1 Hz, 3H, CH_3), 1.50-1.77 (m, 3H, CH_2 and OH), 2.53-2.70 (m, 2H, CH_2), 3.78-3.84 (m, 1H, CH), 3.85 (s, 3H, OCH_3), 5.74 (s, 1H, OH), 6.67 (d, *J*=8.3 Hz, 1H, ArH), 6.77 (d, *J*=8.8 Hz, 1H, ArH), 6.78 (s, 1H, ArH).

4-(2,3-Dihydroxyphenyl)-2-butanol (3f): 0.09 g (47%); ^{1}H NMR ($CDCl_3$) 1.23 (d, *J*=6.1 Hz, 3H, CH_3), 1.72-1.78 (m, 2H, CH_2), 2.22 (brs, 1H, OH), 2.64 (dt, *J*=4.6 and 14.2 Hz, 1H, CH_2), 2.87-2.95 (m, 1H, CH_2), 3.70-3.77 (m, 1H, CH), 5.93 (brs, 1H, OH), 6.63 (dd, *J*=2.4 and 6.6 Hz, 1H, ArH), 6.73-6.79 (m, 2H, ArH), 7.92 (brs, 1H, OH).

4-(2,4-Dihydroxyphenyl)-2-butanol (3g): 0.07 g (40%); ^{1}H NMR ($CDCl_3$) 1.17 (d, *J*=6.1 Hz, 3H, CH_3), 1.62-1.70 (m, 2H, CH_2), 2.55 (ddd, *J*=5.1, 7.1, and 13.9 Hz, 1H, CH_2), 2.67-2.76 (m, 1H, CH_2), 3.68-3.74 (m, 1H, CH), 6.32 (s, 1H, ArH), 6.33 (d, *J*=8.3 Hz, 1H, ArH), 6.90 (d, *J*=7.8 Hz, 1H, ArH).

4-(2,5-Dihydroxyphenyl)-2-butanol (3h): 0.11 g (58%); ^{1}H NMR (CD_3OD) 1.18 (d, J=6.1 Hz, 3H, CH_3), 1.57-1.73 (m, 2H, CH_2), 2.51-2.63 (m, 2H, CH_2), 3.71 (sext, J=6.1 Hz, 1H, CH), 6.45 (dd, *J*=2.9 and 8.5 Hz, 1H, ArH), 6.55 (d, *J*=2.9 Hz, 1H, ArH), 6.58 (d, *J*=8.5 Hz, 1H, ArH).

4-(3,4-Dihdyroxyphenyl)-2-butanol (3i): 0.06 g

(79%); ^{1}H NMR ($CDCl_3$) 1.19 (d, J=5.9 Hz, 3H, CH_3), 1.65-1.74 (m, 2H, CH_2), 2.47-2.64 (m, 2H, CH_2), 3.76 (q, J=5.9 Hz, 1H, CH), 6.56 (d, J=7.8 Hz, 1H, ArH), 6.68 (s, 1H, ArH), 6.73 (d, J=8.1 Hz, 1H, ArH).

4-(3,5-Dihydroxyphenyl)-2-butanol (3j): 0.09 g (51%); ^{1}H NMR (CD_3OD) 1.17 (d, J=6.3 Hz, 3H, CH_3), 1.60-1.75 (m, 2H, CH_2), 2.43-2.61 (m, 2H, CH_2), 3.72 (sext, J=6.3 Hz, 1H, CH), 6.08 (t, J=2.0 Hz, 1H, ArH), 6.15 (d, J=2.0Hz, 2H, ArH).

4-(4-Hydroxy-3,5-dimethoxyphenyl)-2-butanol (3k): 0.09 g (38%); ^{1}H NMR ($CDCl_3$) 1.24 (d, J=6.1 Hz, 3H, CH_3), 1.43 (brs, 1H, OH), 1.72-1.79 (m, 2H, CH_2), 2.56-2.64 (m, 1H, CH_2), 2.66-2.74 (m, 1H, CH_2), 3.82-3.89 (m, 1H, CH), 3.88 (s, 6H, OCH_3), 5.41 (s, 1H, OH), 6.43 (s, 2H, ArH).

DPPH Radical Scavenging Assay.

The measurement of DPPH radical scavenging effect was performed to according the established procedure[2].

Sample compounds were dissolved in ethanol to obtain the concentration of 0.1 mM. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical was dissolved in ethanol to obtain the concentration of 0.2 mM. To a sample solution (100 μL) on 96 well transparent microplate was added ethanol (100 μL) and DPPH solution (50 μL). The mix solution was mixed on a plate-mixer for 1 minute. The mix solution was stand at 25 °C for 30 min under dark, followed by measuring absorbance with a microplate reader at 570 nm. The sample blank test (**B**) was carried out with ethanol instead of sample solution with similar procedure. The blank test of sample (**C**) was similarly done with ethanol instead of DPPH solution. The blank test of sample blank (**D**) was similarly done with ethanol instead of sample and DPPH solution, respectively. DPPH radical scavenging rate was calculated as follows.

DPPH Radical Scavenging Rate (%) =
{(**B**-**D**)-(**A**-**C**)}/(**B**-**D**)*100

A: Abs. of Sample, **B**: Abs. of Sample Blank, **C**: Abs. of Blank of Sample, **D**: Abs. of Blank of Sample Blank

SOD-like Activity Assay.

SOD-like activity was determined by the nitroblue tetrazolium (NBT) reduction method with a SOD Activity Detection Kit (Wako Pure Chemical Ind. Ltd.)[3]. Sample compounds were dissolved in DMSO to obtain the concentration of 0.10 mM. To a sample solution (10 μL) on 96 well transparent microplate was added Color-producing Solution (100 μL). The mix solution was mixed on a plate-mixer for 1 minute. To the solution was added Enzyme Solution (100 μL). The mix solution was mixed on a plate-mixer for 1 minute, followed by incubating accurately for 28 minutes at 37 °C in gaseous phase. To the solution was added Stop Solution (20 μL). The mix solution was mixed on a plate-mixer for 5 minutes, followed by measuring absorbance with a microplate reader at 560 nm. The sample blank test (**B**) was carried out with DMSO instead of sample solution with similar procedure. The blank test of sample (**C**) was similarly done with Blank Solution instead of Enzyme Solution. The blank test of sample blank (**D**) was similarly done with DMSO and Blank Solution instead of sample and Enzyme Solution, respectively. Inhibition rate was calculated as follows.

Inhibition Rate (%) = {(**B**-**D**)-(**A**-**C**)}/(**B**-**D**)*100

A: Abs. of Sample, **B**: Abs. of Sample Blank, **C**: Abs. of Blank of Sample, **D**: Abs. of Blank of Sample Blank

Tyrosinase Activity Inhibition Assay.

Tyrosinase activity was determined by the dopachrome method with L-3-(3,4-dihydroxyphenyl)alanine (L-DOPA) as substrate[3]. The reaction mixture of L-DOPA (1.66 mM in 0.2 M phosphate buffer solution (PBS, pH 6.8), 2.8 mL), enzyme tyrosinase from mushroom (20 units/mL in PBS, 0.1 mL) and the sample (30 mM DMSO solution, 0.1 mL) was incubated at 25 °C for 10 min. The mixture was measured absorbance at 475 nm. The sample blank test (**B**) was carried out with DMSO instead of sample solution with similar procedure. The blank test of sample (**C**) was

similarly done with PBS instead of enzyme solution. The blank test of sample blank (**D**) was similarly done with DMSO and PBS instead of sample and enzyme solution, respectively. The percentage inhibition of tyrosinase activity was calculated as follows.

Tyrosinase Activity Inhibition Rate (%) =
{(**B**-**D**)-(**A**-**C**)}/(**B**-**D**)*100

A: Abs. of Sample, **B**: Abs. of Sample Blank, **C**: Abs. of Blank of Sample, **D**: Abs. of Blank of Sample Blank.

REFERENCES

1) R. Kawaguchi, K.-G. Kim, and H.-K. Kim, *Yakugaku Zasshi*, **62**, 4-6 (1942). H. Ando, M. S. Matsui, and M. Ichihashi, *Int. J. Mol. Sci.*, **11**, 2566-2575 (2010). T. Inoue, Y. Ishidate, M. Fujita, M. Kubo, M. Fukushima, and M. Nagai, *Yakugaku Zasshi*, **98**, 41-46 (1978). T. Fujita, H. Hatamoto, M. Miyamoto, T. Iwasaki, and S. Takafuji, *Biotec. Lett.*, **20**, 1057-1061 (1998). T. Akihisa, A. Takeda, H. Akazawa, T. Kikuchi, S. Yokokawa, M. Ukiya, M. Fukatsu, and K. Watanabe, *Chem. Biodiv.*, **9**, 1475-1489 (2012). M. Sasaki, M. Kondo, K. Sato, M Umeda, K. Kawabata, Y. Takahashi, T. Suzuki, K. Matsunaga, and S. Inoue, *Pigment Cell Melanoma Res.*, **27**, 754-763 (2014).
2) H. Tominaga, Y. Kobayashi, T. Goto, K. Kasemura, and M. Nomura, *Yakugaku Zasshi*, **125**, 371-375 (2005).
3) T. Tada, M. Nomura, K. Shimomura, and Y. Fujihara, *Biosci. Biotech. Biochem.*, **60**, 1421-1424 (1996).