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Biomimic transformation of resveratrol

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Abstract—Resveratrol was treated with several kinds of peroxidases and inorganic reagents so as to prepare ε-viniferin. Among several inorganic reagents, which were investigated in this study, thallium(III) nitrate in methanol gave (±)-ε-viniferin in the yield of 68%. On the other hand, peroxidases did not lead to ε-viniferin, but some stilbenedimers such as pallidol, resveratrol *trans*-dehydrodimer, and leachianol F were obtained.

1. Introduction

There are many oligostilbenes isolated from Vitaceaeous plants, and they are generated from a stilbene, resveratrol (**1**). Some of them are the enantiomers of oligostilbenes from plants of other families, such as Dipterocarpaceae, Leguminosae, Cyperaceae, and Gnetaceae.[1] For instance, (+)-ε-viniferin (**2a**), a resveratrol dimer, and (+)-hopeaphenol (**3a**), a resveratrol tetramer, are obtained from Vitaceaeous plants, but their enantiomers (**2b** and **3b**, respectively), are from plants of other families. It is estimated that the stereochemistry of the oligostilbenes from Vitaceaeous plants are initially regulated when two molecules of resveratrol (**1**) oxidatively couple to form (+)-ε-viniferin (**2a**), and based on the stereochemistry of **2a**, further biotransformations are estimated to occur (Fig. 1).

In our previous papers, we reported the transformation from (+)-ε-viniferin (**2a**) to (−)-vitisin B (**4**), (+)-vitisin C (**5**), (+)-hopeaphenol (**3a**), and (−)-isohopeaphenol (**6**) by using horseradish peroxidase,[2,3] and from (+)-ε-viniferin (**2a**) to (+)-ampelopsin A (**7**), (+)-ampelopsin B (**8**), (−)-ampelopsin D (**9**), and (+)-ampelopsin F (**10**) under acidic conditions.[3,4] But we have never obtained ε-viniferin (**2**) by the transformation of resveratrol (**1**). Resveratrol (**1**) is supposed to dimerize oxidatively by peroxidase or phenoloxidase in plant cells to afford ε-viniferin (**2**). Langcake and Pryce reported the treatment of resveratrol (**1**) with horseradish peroxidase.[5] According to their result, ε-viniferin (**2**) was not obtained by the reaction but resveratrol-*trans*-dehydrodimer (**11**) was. Sako et al. also investigated the nonenzymatical oxidative coupling of resveratrol with several inorganic oxidative reagents, but

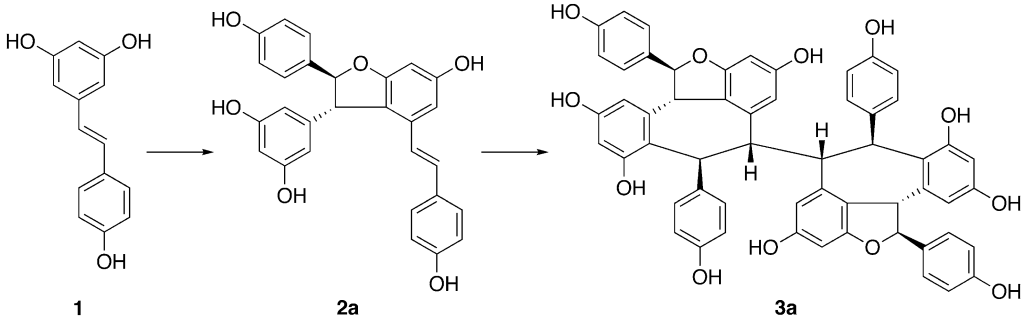

Figure 1. Plausible biogenesis of oligostilbenes.

Keywords: Resveratrol; Peroxidase; ε-Viniferin; Thallium(III) nitrate.
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they reported that the formation of ε-viniferin (**2**) was not observed.[6]

In this study, to substantiate the hypothesis to form (+)-ε-viniferin from resveratrol (**1**) in Vitaceaeous plants, we investigated the participation of peroxidase and inorganic oxidizing reagents in oligomerization of resveratrol (**1**). As the result, the treatment of thallium(III) nitrate led to ε-viniferin (**2**) in good yield, and potassium hexacyanoferrate, and iron(III) chloride also gave **2**, whereas no commercially available peroxidases, which were tested in this study, provided ε-viniferin (**2**).

2. Results and discussion

2.1. Synthesis of resveratrol (1)

In this study, synthetic resveratrol (**1**) under the procedure shown in Scheme 1 was used for the transformation with oxidizing reagents. The starting material, methyl 3,5-dihydroxybenzoate (**13**), was *tert*-butyldimethylsilylated followed by reduction of the methoxy carbonyl group with lithium aluminum hydride to afford 3,5-bis(*tert*-butyldimethylsilyloxy)benzyl alcohol (**14**). Compound **14** was converted to the benzyl chloride (**15**), and **15** was transformed into phosphonate (**16**). Resveratrol (**1**) was obtained by the reaction of **16** and 4-*tert*-butyldimetylsilyloxybenzaldehyde (**18**),[7] which was prepared from 4-hydroxybenzaldehyde (**17**), followed by deprotection. No *cis*-isomer of **1** was found in this preparation.

2.2. Treatment of resveratrol (1) with oxidizing reagents

Resveratrol (**1**) was treated with several inorganic oxidizing reagents in certain solvents as shown in Table 1. These reagents are usually used for phenol oxidation.[8] Among them, thallium(III) nitrate (TTN) in methanol at −50 °C transformed resveratrol (**1**) to (±)-ε-viniferin (**2**), consuming 30% of the starting material, within 5 min along with 56% of recovered resveratrol (**1**). But when this reaction was carried out at over 0 °C, the reaction gave just a complex mixture. The product obtained by the reaction at −50 °C was analyzed by HPLC equipped with CD detector using a chiral column. As the result, the product was revealed to be a mixture of (+)- and (−)-ε-viniferins (**2a** and **2b**) as shown in Figure 2. Potassium hexacyanoferrate(III) is also a good reagent to form ε-viniferin (**2**), and

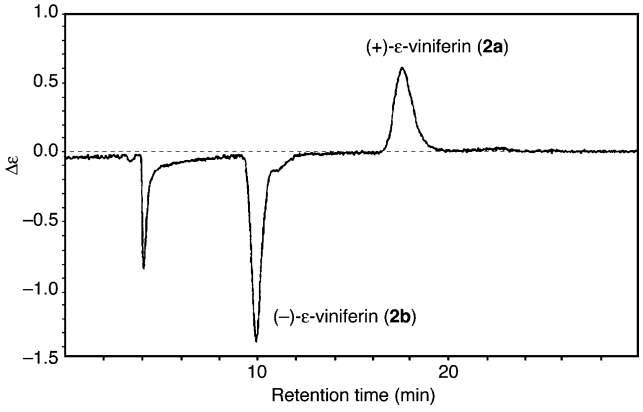
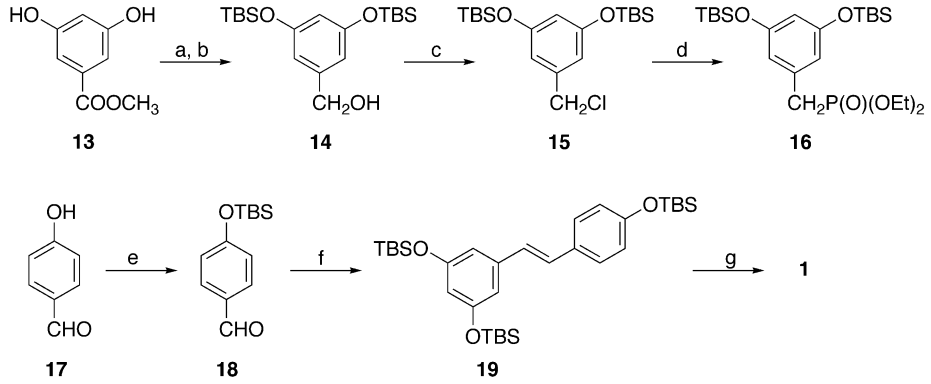


Figure 2. Analysis of the reaction mixture of TTN treatment.

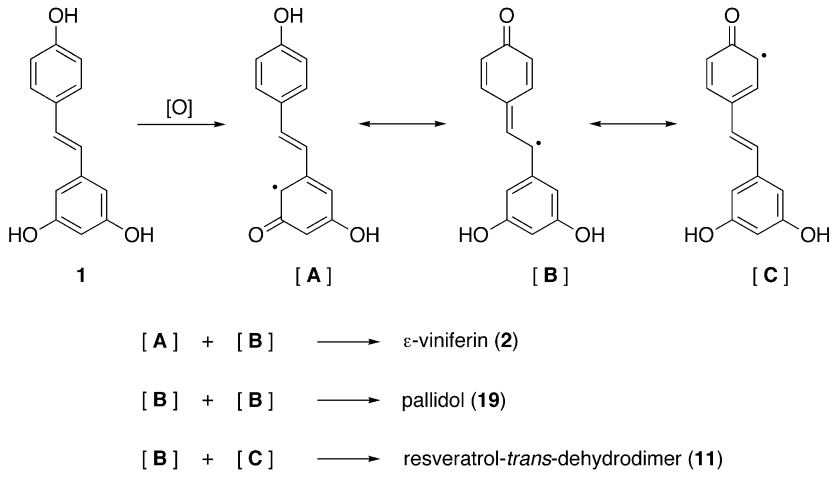


Scheme 1. Synthesis of resveratrol (**1**). Reaction conditions: (a) TBSCl, imidazole, DMF, rt; (b) $LiAlH_4$, THF, 0 °C; (c) *p*-TsCl, Et_3N, DMAP, CH_2Cl_2, rt; (d) $P(OEt)_3$, NaI; (e) TBSCl, imidazole, DMF, rt; (f) **16** NaH, THF, −55 °C; (g) concd HCl, EtOH, rt.

Table 1. Treatment of resveratrol (**1**) with oxidizing reagents

Reagents	Solvent	Temperature (°C)	Time	**2** (%)	**11** (%)	**12** (%)
$Tl(NO_3)_3$	MeOH	−50	5 min	30.1	—	—
$Tl(NO_3)_3$	MeOH	−30	5 min	3.9	—	—
$K_3[Fe(CN)_6]$	MeOH	25	10 min	21.9	22.5	15.7
$Ce(SO_4)_2$	MeOH	−50	26 h	3.7	8.4	—
$FeCl_3$	Acetone	25	20 h	0.9	97.0	1.5
MnO_2	CH_2Cl_2	25	24 h	—	91.0	9.0

The percentage in this table means the 'degree of transformation (%DT)'. See Section 3.



Scheme 2. Hypothesis for the dimerization of resveratrol (**1**).

cerium(IV) sulfate, and iron(III) chloride afforded ε-viniferin (**2**), though the yields were low. On the other hand, iron(III) chloride, and manganese(IV) oxide mainly gave (±)-resveratrol-*trans*-dehydrodimer (**11**). The mechanism for the dimerization of resveratrol (**1**) is suggested as shown in Scheme 2. When the reaction occurs in a short period, as in the case using TTN, the intermediate [**A**] would generate predominantly and then it reacts with intermediate [**B**] to afford ε-viniferin (**2**). Resveratrol-*trans*-dehydrodimer (**11**) is supposed to form by the reaction of intermediate [**B**] and [**C**]. On the basis of our result obtained when we used iron(III) chloride, and mangan(IV) oxide, and the result reported,[6] during the long reaction time and at comparatively higher temperature when **11** was mainly provided, sterically less hindered intermediate [**C**] would be easily involved in the reaction. But an appropriate account for the distinction of the reactivity cannot be made only with this evidence (Table 1).

2.3. Treatment of resveratrol (1) with peroxidases

ε-Viniferin (**2**) is supposed to generate from resveratrol (**1**) via oxidative coupling with peroxidase or phenoloxidase via the proposed intermediates shown in Scheme 2. In order to investigate the participation of peroxidase in the formation of ε-viniferin (**2**), resveratrol (**1**) was treated with several commercially available peroxidases in two kinds of solvents. In this study, we used peroxidases from soybean, fungus (*Arthromyces ramosus*), and horseradish. The reactions were carried out at 27 °C, because when we tried it at 37 °C, the reaction gave only a complex mixture. As the result, ε-viniferin (**2**) was not obtained by using any of the peroxidases tested, though several resveratrol dimers were obtained (Table 2). In all cases, (±)-resveratrol-*trans*-dehydrodimer (**11**)[5] and (±)-pallidol (**12**)[9,10] were major products and compounds **20** and **21** (Fig. 3), all of which were estimated to form via two molecules of intermediate

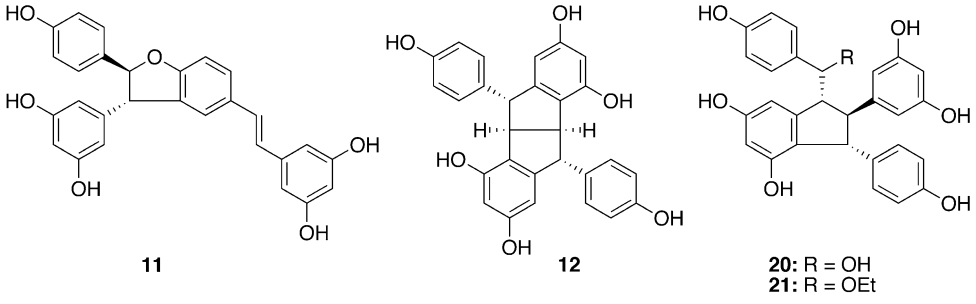


Figure 3. Products obtained by the treatment of resveratrol (**1**) with oxidizing reagents and peroxidases.

Table 2. Diversity of the products obtained by the treatment of resveratrol (**1**) with peroxidases

Origins of peroxidases	Solvent	**11** (%)	**12** (%)	**20** (%)	**21** (%)
Glycine max	aq Acetone	21.4	7.2	1.8	—
Arthromyces ramosus	aq Acetone	18.4	7.4	4.6	—
Horseradish	aq Acetone	12.6	10.2	—	—
Glycine max	aq EtOH	12.1	9.5	5.2	8.6
Arthromyces ramosus	aq EtOH	13.1	5.0	4.6	8.2

The percentage in this table means the 'degree of transformation (%DT)'. See Section 3.

[**B**] in Scheme 2, were obtained as minor products. Accordingly, some specific oxidizing enzymes would be involved to form ε-viniferin (**2**) in the plants. The reaction time in each case was short enough (0.5–1.5 h) to form ε-viniferin (**2**), if the reaction proceeded like that with inorganic oxidizing reagents. Furthermore, the pattern of the reaction was the same as those, which were observed when (+)-ε-viniferin (**2a**) was treated horseradish peroxidase to form (−)-vitisin B, (+)-vitisin C, (+)-hopeaphenol, and (−)-isohopeaphenol.[2,3] So, judging from the evidence, no intermediate [**A**] seemed to be generated in those enzymatic reactions, and the reaction proceeded via the less hindered intermediates [**B**] and [**C**]. Because resveratrol-*trans*-dehydrodimer (**11**) was resulted from intermediate [**B**] and [**C**], and other products, a mixture of (±)-leachianols F and G (**20**),[11] and a mixture of (±)-quadrangularin B and C (**21**),[9] were formed via two molecules of intermediate [**B**].

3. Experimental

3.1. General

UV and IR spectra were recorded on JASCO Ubest V-560 (cell length 10 mm) and FT-IR-410 spectrophotometers, respectively. Optical rotations were measured with a JASCO P-1020 polarimeter (cell length 100 mm). ^{1}H and ^{13}C NMR spectra were recorded on JEOL ALPHA-600 (^{1}H: 600 MHz and ^{13}C: 150 MHz), JEOL ECP-500 (^{1}H: 500 MHz and ^{13}C: 125 MHz), and JEOL ALPHA-400 (^{1}H: 400 MHz and ^{13}C: 100 MHz) spectrometers. Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to solvent signal (chloroform-*d*: δ_H 7.26 and δ_C 77.0, methanol-d_4: δ_H 3.30 and δ_C 49.0, acetone-d_6: δ_H 2.04 and δ_C 24.9) as internal standards, respectively. LR and HR FAB-MS were obtained with JEOL JMS HX-110 using *m*-nitrobenzyl alcohol as matrix. Analytical TLC was performed on silica gel 60 F_{254} (Merck). Column chromatography was carried out on silica gel BW-820MH (Fuji Silysia Chemicals, Co. Ltd). ODS (Develosil ODS UG-5, ϕ20×250 mm, Nomura Chemical, Seto, Japan), C-8 (Develosil C8-5, ϕ20×250 mm, Nomura Chemical, Seto, Japan), and C-8 (YMC-Pack C8, ϕ20×250 mm, YMC, Kyoto, Japan) columns were used for preparative HPLC, and C-8 (Develosil C8-5, ϕ4.6×250 mm, Nomura Chemical, Seto, Japan) column were used for analytical HPLC. Peroxidases from soybean (*Glycine max*), *A. ramosus* were purchased from Sigma and peroxidase from horseradish were from Wako Pure Chemicals, Osaka, Japan.

3.2. Synthesis of resveratrol (1)

3.2.1. 3,5-Bis(*tert*-butyldimetylsilyloxy)benzyl alcohol (14). To a solution of methyl 3,5-dihydroxybenzoate (**13**, 50.0 g) in DMF (300 mL), imidazole (21.6 g) and *tert*-butyldimethylsilyl chloride (TBSCl) (48.8 g) was added and stirred at rt for 21 h. The reaction solution was extracted with ethyl acetate, and the ethyl acetate layer was washed with water and brine, dried over anhydrous magnesium sulfate. The solvent was removed in vacuo, and the crude product was purified by silica-gel column chromatography using hexane/ethyl acetate 97:3 as an eluent to afford methyl 3,5-bis(*tert*-butyldimetylsilyloxy)benzoate (**23**) (115 g, 97.5%). Compound **23** (109 g) was dissolved to THF (350 mL), and lithium aluminum hydride (5.6 g) was added at 0 °C. After 20 h, the reaction mixture was extracted with ethyl acetate, and the ethyl acetate layer was washed with water and brine, dried over anhydrous magnesium sulfate, and evaporated. The residue was purified by silica-gel column chromatography using hexane/ethyl acetate 95:5 as an eluent to give alcohol **14** (89.9 g, 88.7%).

3,5-Bis(tert-butyldimetylsilyloxy)benzoate (**23**). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.10 (2H, d, J=2.2 Hz), 6.50 (1H, t, J=2.2 Hz), 3.86 (3H, s), 0.96 (18H, s), 0.18 (12H, s); EI-MS *m*/*z* 396 (M^+).

3,5-Bis(tert-butyldimetylsilyloxy)benzyl alcohol (**14**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.44 (2H, d, J=2.2 Hz), 6.23 (1H, t, J=2.2 Hz), 4.54 (2H, s), 0.95 (18H, s), 0.17 (12H, s); EI-MS *m*/*z* 368 (M^+).

3.2.2. 3,5-Bis(*tert*-butyldimetylsilyloxy)benzyl chloride (15). *p*-Toluenesulfonyl chloride (44.4 g) was added to the CH_2Cl_2 solution (400 mL) of alcohol (**14**, 71.4 g), triethylamine (32.5 mL), and 4-dimethylaminopyridine (DMAP) (11.9 g) at 0 °C. The reaction solution was stirred at rt for 12 h, and extracted with chloroform. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate and evaporated. The crude product was purified by silica-gel chromatography using hexane/ethyl acetate 95:5 as an eluent to give chloride **15** (45.7 g, 61.0%).

3,5-Bis(tert-butyldimetylsilyloxy)benzyl chloride (**15**). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.45 (2H, d, J=1.8 Hz), 6.26 (1H, t, J=1.8 Hz), 4.43 (2H, s), 0.96 (18H, s), 0.18 (12H, s); EI-MS *m*/*z* 386, 388 (M^+).

3.2.3. Diethyl [3,5-bis(*tert*-butyldimetylsilyloxy)phenyl] methylphosphonate (16). To a mixture of chloride (**15**, 31.5 g) and triethyl phosphite (23.7 mL), NaI (1.3 g) was added, and stirred at 135 °C for 3 h. Triethyl phosphite (23.7 mL) and NaI (1.3 g) was added again and stirred another 3 h. The reaction solution was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate, and evaporated. The crude product was purified by silica-gel chromatography using hexane/ethyl acetate 7:3 as an eluent to afford phosphonate (**16**) (34.2 g, 86.1%).

Diethyl [3,5-bis(tert-butyldimetylsilyloxy)phenyl]methylphosphonate (**16**). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.43 (2H, d, J=2.2 Hz), 6.26 (1H, t, J=2.2 Hz), 4.00 (4H, m), 3.03 (2H, d, J=21.6 Hz), 1.25 (6H, t, J=7.0 Hz), 0.94 (18H, s), 0.19 (12H, s); EI-MS *m*/*z* 488 (M^+).

3.2.4. 4-(*tert*-Butyldimetylsilyloxy)benzaldehyde (18). To a solution of methyl 3,5-dihydroxybenzoate (**17**, 16.8 g) in DMF (100 mL), imidazole (11.3 g) and TBSCl (25.0 g) was added and stirred at rt for 21 h. The reaction solution was extracted with ethyl acetate, and the ethyl acetate layer was washed with water and brine, dried over anhydrous magnesium sulfate. The solvent was removed in vacuo, and the crude product was purified by silica-gel column chromatography using hexane/ethyl acetate 95:5 as an eluent to afford compound **18** (18.9 g, 58.1%).

4-(tert-Butyldimetylsilyloxy)benzaldehyde (**18**). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.89 (1H, s), 7.79 (2H, d, J=8.8 Hz), 6.95 (2H, d, J=8.8 Hz), 0.99 (9H, s), 0.25 (6H, s); EI-MS m/z 236 (M^+).

3.2.5. 3,5,4′-Tris(*tert*-butyldimetylsilyloxy)stilbene (19). A solution of phosphonate (**16**, 23.4 g) in THF (40 mL) was added dropwise to a suspension of NaH (2.45 g) in THF (20 mL) at −55 °C for 1 h, and the reaction mixture was stirred at 0 °C for 1 h. The mixture was cooled to −55 °C again, and a solution of compound **18** (12.5 g) in THF (20 mL) was added dropwise for 1 h. The reaction temperature was raised to 0 °C for 3 h, and was kept at 0 °C for 20 h. The solution was stirred at rt for 6 h, neutralized with 2 M HCl, and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over anhydrous magnesium sulfate, and evaporated. The crude product was purified by silica-gel column chromatography using chloroform/methanol 97:3 as an eluent to give compound **19** (13.9 g, 50.8%).

3,5,4′-Tris(tert-butyldimetylsilyloxy)stilbene (**19**). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 (2H, d, J=8.7 Hz), 6.95 (1H, d, J=16.5 Hz), 6.84 (1H, d, J=16.5 Hz), 6.82 (2H, d, J=8.7 Hz), 6.59 (2H, d, J=2.2 Hz), 6.24 (2H, d, J=2.2 Hz), 0.96 (27H, s), 0.19 (18H, s); EI-MS m/z 570 (M^+).

3.2.6. Resveratrol (1). To a solution of compound **19** (5.7 g) in ethanol (12 mL), a mixture of concd HCl (20 mL) and ethanol (100 mL) was added, and stirred at rt for 26 h. The reaction solution was evaporated and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over anhydrous magnesium sulfate, and evaporated. The crude product was purified by silica-gel chromatography using chloroform/methanol 95:5 as an eluent to give resveratrol (**1**) (1.8 g, 79.0%).

3.3. Evaluation of the reactivity

In this study, 'Degree of transformation' was defined as follows. This term means the amount of resveratrol (**1**), which was consumed to form stilbenedimers, and the values (%DT) are used throughout the following investigations.

Degree of transformation (%DT)=(mole of product×2)/(mole of resveratrol (**1**))×100.

3.4. Treatment of resveratrol (1) with oxidizing reagents

3.4.1. Treatment with thallium(III) nitrate. To a solution of resveratrol (5 mg) in methanol (1.5 mL), $Tl(NO_3)_3$ (10.7 mg) was added at −50 °C. The solution was stirred at −50 °C for 30 min under N_2 atmosphere. Water (1 mL) was added to the solution and the reaction mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, died over anhydrous magnesium sulfate, evaporated. The crude product was purified by preparative HPLC equipped with ODS column using a mixed solvent of methanol/water 6:4 as an eluent to give ε-viniferin (**2**, 1.5 mg, 30.1%DT), along with the recovered resveratrol (**1**, 2.8 mg, 56%).

The resulted ε-viniferin (**2**) was submitted to HPLC analysis by using a chiral column (Chiralpak AD, ϕ4.6×150 mm, Daicel Chemical Industries, Osaka) by using hexane/isopropyl alcohol/trifluoroacetic acid 80:20:0.5 as a mobile phase at flow rate of 1 mL/min.

3.4.2. Treatment with potassium hexacyanoferrate(III). To a solution of resveratrol (**1**, 7.7 mg) in methanol (1 mL), a mixed solution of K_2CO_3 (4.1 mg) and $K_3[Fe(CN)_6]$ (9.8 mg) in 0.5 mL of water was added at rt. After 10 min, the reaction mixture was analyzed by HPLC with C-8 column using a mixed solvent of methanol/water 6:4 as an eluent at 0.5 mL/min, and the amount of a product was quantified on the basis of the area of the peak. ε-Viniferin (**2**, Rt=10.0 min, 21.9%DT), resveratrol-*trans*-dehydrodimer (**11**, Rt=14.9 min, 22.5%DT), and pallidol (**12**, Rt=6.7 min, 15.7%DT) was detected along with recovered resveratrol (**1**, Rt=9.1 min, 39.9%).

3.4.3. Treatment with cerium(IV) sulfate. To a solution of resveratrol (**1**, 1.0 mg) in methanol (1 mL), $CeSO_4$ (1.4 mg) was added at −50 °. After 26 h, the reaction mixture extracted with ethyl acetate and water. The organic layer was washed with water, brine, and dried over anhydrous magnesium sulfate, and evaporated. The residue was analyzed by HPLC with C-8 column using a mixed solvent of methanol/water 6:4 as an eluent at 0.5 mL/min, and the amount of a product was quantified on the basis of the area of the peak. ε-Viniferin (**2**, 3.7%DT), and resveratrol-*trans*-dehydrodimer (**11**, 8.4%DT) was detected along with recovered resveratrol (**1**, 53%).

3.4.4. Treatment with iron(III) chloride. To a solution of resveratrol (**1**, 6.1 mg) in acetone (1 mL), $FeCl_3$ (4.4 mg) was added at rt. After 20 h, the reaction mixture was analyzed by HPLC with C-8 column using a mixed solvent of methanol/water 6:4 as an eluent at 0.5 mL/min, and the amount of a product was quantified on the basis of the area of the peak. ε-Viniferin (**2**, 0.9%DT), resveratrol-*trans*-dehydrodimer (**11**, 97%DT), and pallidol (**12**, 1.5%DT) was detected.

3.5. Treatment of resveratrol (1) with peroxidases

3.5.1. General procedure of treatment with peroxidases in aqueous acetone. A mixture of resveratrol (ca. 10 mg), acetone (1 mL), water (1 mL), 0.5 M phosphate buffer (pH 6.0) (0.4 mL) and enzyme solution (0.1 mL in 0.5 M phosphate buffer (pH 6.0)) was stirred at 27 °C for 5 min. Then 30% H_2O_2 (3 μL) was added to this reaction solution. After 30 min, the reaction solution was extracted with ethyl acetate. The organic layer was washed with water, and brine, dried over anhydrous magnesium sulfate, and evaporated. The residue was fractionated by preparative HPLC with C-8 column (YMC) using a mixed solvent of methanol/water 1:1.

3.5.1.1. Peroxidase from soybean (*Glycine max*). For this reaction, resveratrol (10.8 mg) and peroxidase from soybean (*G. max*) as a solution (4 mg/mL) were used. Crude product of 6.3 mg was obtained, and by the preparative HPLC of the product, resveratrol-*trans*-dehydrodimer (**11**, 2.3 mg, 21.4%), pallidol (**12**, 0.8 mg, 7.2%), and a mixture of leachianol F and G (**20**, 0.2 mg, 1.8%) were obtained.

3.5.1.2. Peroxidase from *Arthromyces ramosus*. For this reaction, resveratrol (10.4 mg) and peroxidase from *A. ramosus* as a solution (4 mg/mL) were used. Crude product of 8.5 mg was obtained, and by the preparative HPLC of the product, resveratrol-*trans*-dehydrodimer (**11**, 1.9 mg, 18.4%), pallidol (**12**, 0.8 mg, 7.4%), and a mixture of leachianol F and G (**20**, 0.5 mg, 4.6%) were obtained.

3.5.1.3. Peroxidase from horseradish. For this reaction, resveratrol (10.4 mg) and peroxidase from horseradish as a solution (10 mg/mL) were used. Crude product of 7.9 mg was obtained, and by the preparative HPLC of the product, resveratrol-*trans*-dehydrodimer (**11**, 1.3 mg, 12.6%), and pallidol (**12**, 1.1 mg, 10.2%) were obtained.

3.5.2. General procedure of treatment with peroxidases in aqueous ethanol. A mixture of resveratrol (50 mg), ethanol (5 mL), water (5 mL), 0.5 M phosphate buffer (pH 6.0) (2 mL) and enzyme solution (0.5 mL in 0.5 M phosphate buffer (pH 6.0)) was stirred at 27 °C for 5 min. Then 30% H_2O_2 (15 μL) was added to this reaction solution. After 30 min, wet 1 g of Amberlite XAD-2 (Organo, Tokyo) was added to the solution and eluted with water, 50% aqueous methanol, methanol, and acetone. The acetone elute was evaporated and fractionated by preparative HPLC with C-8 column (Develosil) using a mixed solvent of methanol/water 1:1.

3.5.2.1. Peroxidase from soybean (*Glycine max*). Peroxidase from soybean (*G. max*) was used as a solution (40 μg/mL). By the preparative HPLC, resveratrol-*trans*-dehydrodimer (**11**, 6.0 mg, 12.1%), pallidol (**12**, 4.9 mg, 9.5%), a mixture of leachianol F and G (**20**, 2.7 mg, 5.2%), and a mixture of quadrangularin B and C (**21**, 4.7 mg, 8.6%) were obtained.

3.5.2.2. Peroxidase from *Arthromyces ramosus*. Peroxidase from *A. ramosus* was used as a solution (4 mg/mL). By the preparative HPLC, resveratrol-*trans*-dehydrodimer (**11**, 6.5 mg, 13.1%), pallidol (**12**, 2.6 mg, 5.0%), a mixture of leachianol F and G (**20**, 2.4 mg, 4.6%), and a mixture of quadrangularin B and C (**21**, 4.5 mg, 8.2%) were obtained.

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