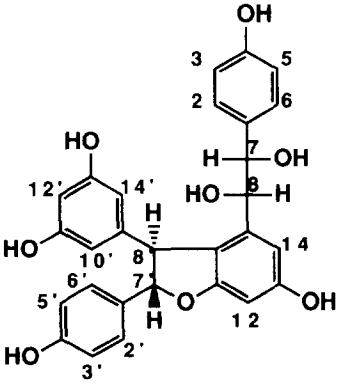
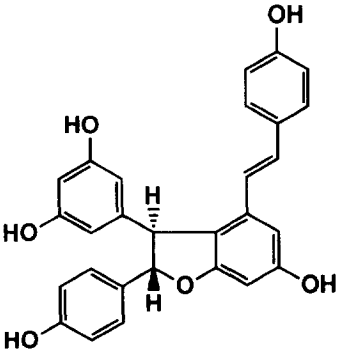
Table 1. ^{1}H and ^{13}C NMR Data of ε-viniferin diol (**1**) and ε-viniferin (**2**)[1]

Position No.	1		2	
1		135.0 (s)		130.0 (s)
2,6	6.77 (d, 8.6)	129.2 (d)	7.15 (d, 8.0)	128.6 (d)
3,5	6.62 (d, 8.6)	116.7 (d)	6.71 (d, 8.0)	116.1 (d)
4		157.5 (s)		158.0 (s)
7	4.35 (d, 4.0)	75.1 (d)[2]	6.91 (d, 16.0)	123.3 (d)
8	4.51 (d, 4.0)	75.9 (d)[2]	6.65 (d, 16.0)	130.1 (d)
9		142.3 (s)		136.3 (s)
10		119.8 (s)		119.7 (s)
11		162.4 (s)		162.3 (s)
12	6.34 (d, 2.0)	97.2 (d)	6.30 (d, 2.0)	96.7 (d)
13		159.7 (s)		159.4 (s)
14	6.68 (d, 2.0)	108.8 (d)	6.70 (d, 2.0)	104.1 (d)
1'		134.7 (s)		133.8 (s)
2',6'	7.17 (d, 8.5)	128.1 (d)	7.19 (d, 8.0)	127.8 (d)
3',5'	6.83 (d, 8.5)	115.8 (d)	6.80 (d, 8.0)	116.0 (d)
4'		158.6 (s)		158.0 (s)
7'	5.37 (d, 4.0)	94.3 (d)	5.40 (d, 5.0)	93.8 (d)
8'	4.40 (d, 4.0)	57.5 (d)	4.44 (d, 5.0)	57.0 (d)
9'		148.5 (s)		147.3 (s)
10',14'	6.18 (d, 2.5)	107.5 (d)	6.22 (s)	106.9 (d)
11',13'		160.5 (s)		159.7 (s)
12'	6.32 (t, 2.5)	102.6 (d)	6.22 (s)	101.9 (d)

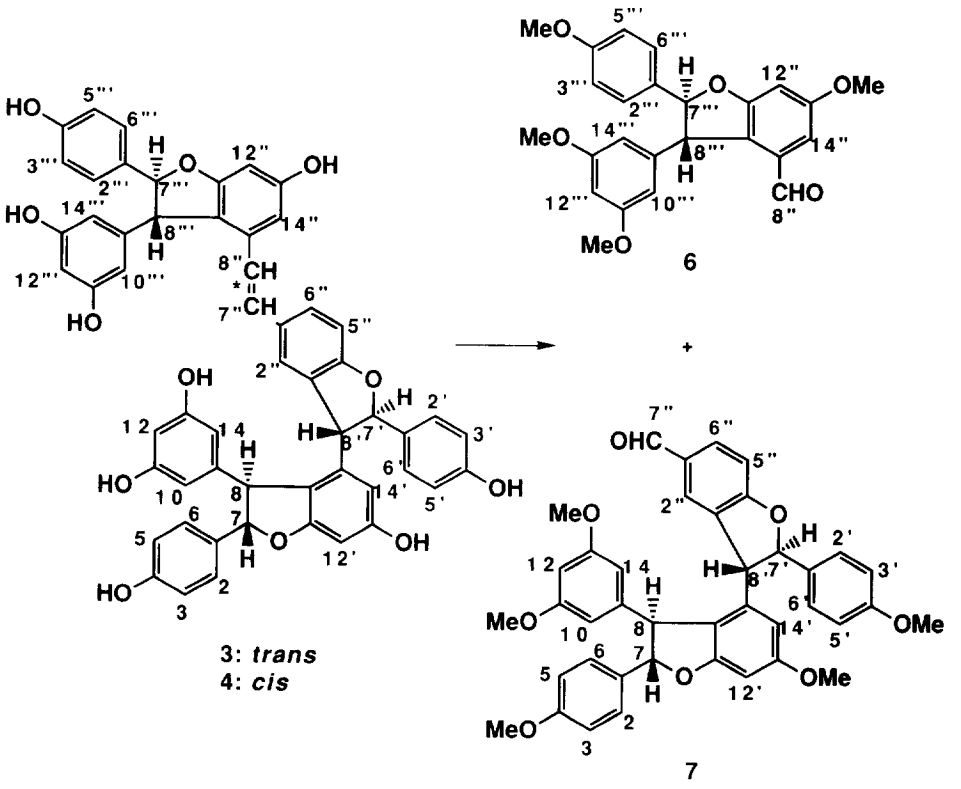
[1]Spectra were measured in acetone-d_6 at 500 and 125 MHz for ^{1}H and ^{13}C NMR. Assignments of the ^{1}H NMR signals were confirmed by decoupling experiments. [2]Assignment may be reversed.



1



2



benzene ring hydrogens (δ 6.90 (1H, d, J=8.4 Hz), 7.31 (1H, brd, J=1.8 Hz), 7.68 (1H, dd, J=8.4, 1.8 Hz)) in its ^{1}H NMR spectrum. Moreover, the ^{1}H NMR spectrum of the compound **7** exhibited the presence of two dihydrobenzofuran moieties in the molecule (δ 4.38, 5.41 (each 1H, d, J=4.8 Hz); 4.40, 5.59 (each 1H, d, J=6.6 Hz)). From detailed decoupling experiments of the ^{1}H NMR spectrum, three benzylic hydrogens at δ 4.38, 5.41 and 5.59 were found to long-range couple with the aromatic hydrogens at δ 6.01, 7.21 and 6.74, respectively, while the long-range coupling of the remaining benzylic hydrogen at δ 4.40 was not clearly observed. After the assignment of the ^{13}C NMR signals of the compound **7** by HMQC spectrum (Table 2), the HMBC spectrum was analyzed, which showed the following long range ^{13}C–^{1}H correlations: δ 56.9 (C-8)–6.01 (H-10, 14), 105.4 (C-10, 14)–4.38 (H-8), 126.6 (C-2, 6)–5.41 (H-7), 126.9 (C-2', 6')–5.59 (H-7'). These ^{13}C–^{1}H long-range couplings supported the benzylic couplings obtained by the decoupling experiments described above. Furthermore, the HMBC spectrum showed the cross peak between the C-14' signal at δ 106.6 and the H-8' benzyl hydrogen signal at δ 4.40, demonstrating the connectivity of C-8' and C-9' in the compound **7**.

The relative configurations of three dihydrobenzofuran groups of vitisin B (**3**) were concluded to be *trans* from NOEs between H-7–H-10(14), H-8–H-2(6); H-7'–H-14', H-8'–H-2'(6'); H-7'''–H-10'''(14'''), H-8'''–H-2'''(6''') in the ^{1}H NMR spectrum of a catalytic hydrogenation product (**8**) of the nonamethyl ether (**5**).

Table 2. ^{1}H and ^{13}C NMR Data of Vitisin B (**3**), *cis*-Vitisin B (**4**) and Their Derivative (**7**)[1]

Position No.	**3**[2]		**4**[2]		**7**[3]	
1		133.9 (s)		133.9 (s)		133.8 (s)
2,6	7.13 (d, 8.4)	128.2 (d)	7.01 (d, 8.8)	128.5 (d)	7.21 (d, 8.4)	126.6 (d)
3,5	6.76 (d, 8.4)	116.3 (d)	6.73 (d, 8.8)	116.3 (d)	6.89 (d, 8.4)	114.3 (d)
4		158.5 (s)		158.4 (s)		159.8 (s)
7	5.36 (d, 6.2)	94.8 (d)	5.21 (d, 5.9)	94.9 (d)	5.41 (d, 4.8)	93.3 (d)
8	4.33 (d, 6.2)	58.2 (d)	3.85 (d, 5.9)	57.8 (d)	4.38 (d, 4.8)	56.9 (d)
9		147.2 (s)		147.2 (s)		145.9 (s)
10,14	6.14 (d, 1.5)	107.5 (d)	5.93 (d, 2.2)	107.3 (d)	6.01 (d, 2.4)	105.4 (d)
11,13		160.0 (s)		159.6 (s)		161.2 (s)
12	6.13 (t, 1.5)	102.3 (d)	6.09 (t, 2.2)	101.9 (d)	6.09 (t, 2.4)	99.0 (d)
1'		132.7 (s)		132.5 (s)		131.6 (s)
2',6'	6.58 (d, 8.8)	127.8 (d)	6.60 (d, 8.8)	127.8 (d)	6.74 (d, 8.4)	126.9 (d)
3',5'	6.52 (d, 8.8)	116.0 (d)	6.54 (d, 8.8)	116.2 (d)	6.62 (d, 8.4)	114.0 (d)
4'		158.0 (s)		158.0 (s)		159.6 (s)
7'	5.42 (d, 5.1)	92.2 (d)	5.44 (d, 5.9)	92.3 (d)	5.59 (d, 6.6)	92.7 (d)
8'	4.25 (d, 5.1)	53.0 (d)	4.22 (d, 5.9)	52.9 (d)	4.40 (d, 6.6)	55.2 (d)
9'		142.5 (s)		142.3 (s)		139.9 (s)
10'		120.0 (s)		120.3 (s)		120.5 (s)
11'		162.7 (s)		162.8 (s)		161.8 (s)
12'	6.28 (d, 2.2)	96.7 (d)	6.28 (d, 2.2)	96.7 (d)	6.47 (d, 2.4)	94.7 (d)
13'		160.5 (s)		160.4 (s)		162.0 (s)
14'	6.09 (d, 2.2)	107.5 (d)	6.11 (d, 2.2)	107.3 (d)	6.21 (d, 2.4)	106.6 (d)
1"		132.7 (s)		131.7 (s)		132.4 (s)
2"	6.65 (d, 1.8)	125.5 (d)	6.54 (brs)	127.0 (d)	7.31 (brd, 1.8)	126.2 (d)
3"		132.3(s)		132.7 (s)		128.4 (s)
4"		160.2 (s)		160.0 (s)		164.2 (s)
5"	6.68 (d, 8.4)	110.7 (d)	6.56 (d, 8.4)	110.0 (d)	6.90 (d, 8.4)	110.3 (d)
6"	6.98 (dd, 8.4, 1.8)	126.8 (d)	6.91 (dd, 8.4, 1.8)	130.0 (d)	7.68 (dd, 8.4, 1.8)	133.2 (d)
7"	6.50 (d, 16.5)	124.2 (d)	5.96 (d, 13.2)	126.7 (d)	9.70 (s)	190.5 (d)
8"	6.68 (d, 16.5)	130.5 (d)	6.06 (d, 13.2)	131.5 (d)		
9"		136.8 (s)		137.5 (s)		
10"		120.1 (s)		120.3 (s)		
11"		162.8 (s)		162.7 (s)		
12"	6.24 (d, 1.8)	96.9 (d)	6.17 (d, 2.2)	96.8 (d)		
13"		159.6 (s)		159.4 (s)		
14"	6.58 (brs)	104.6 (d)	6.20 (d, 2.2)	108.8 (d)		
1"'		134.6 (s)		134.2 (s)		
2"',6"'	7.18 (d, 8.8)	127.8 (d)	7.12 (d, 8.8)	128.0 (d)		
3"',5"'	6.82 (d, 8.8)	116.5 (d)	6.76 (d, 8.8)	116.4 (d)		
4"'		158.3 (s)		158.5 (s)		
7"'	5.33 (d, 4.8)	94.7 (d)	5.30 (d, 4.8)	94.9 (d)		
8"'	4.36 (d, 4.8)	57.9 (d)	4.27 (d, 4.8)	57.9 (d)		
9"'		147.7 (s)		147.6 (s)		
10"',14"'	5.98 (d, 2.2)	107.0 (d)	5.97 (d, 2.2)	107.2 (d)		
11"',13"'		160.1 (s)		160.0 (s)		
12"'	6.06 (t, 2.2)	102.5 (d)	6.06 (t, 2.2)	102.5 (d)		

[1]Assinments were confirmed by 2D ^{1}H-^{1}H COSY, 2D ^{1}H-^{13}C COSY, HMQC, COLOC and HMBC spectra. [2]Spectra were measured in CD_3OD at 600 and 150 MHz for ^{1}H and ^{13}C NMR. [3]Spectra were measured in $CDCl_3$ at 600 and 150 MHz for ^{1}H and ^{13}C NMR. Methoxyl signals: 3.80 (4-OMe), 3.60 (11, 13-OMe), 3.77 (4'-OMe), 3.75 (13'-OMe); 55.2 (OMe x 2), 55.3, 55.5, 55.6.

In addition to the strong NOE between H-7' and H-14', NOEs were also detected between H-8–H-8', H-8–H-2" and H-8'–H-14'. Moreover, the following NOEs were unambiguously observed: H-8–H-7', H-8–H-2", H-7'–H-14', H-8'–H-14' and 11(13)-OMe–H-3'(5') in the NOE experiments of the degradative product (**7**). These results, together with the study using Dreiding stereomodel, implied the spatial relationship of C-7, 8 and C-7', 8'. Biogenetic consideration that vitisin B (**3**) is biosynthesized by oxidative coupling of two molecules of ε-viniferin (**2**) at C-7', 8' and C-3", 4", suggested the relative stereochemistry of C-7"' and C-8"' as described in the structure **3**.

The ^{1}H and ^{13}C NMR spectra of *cis*-Vitisin B (**4**), $[\alpha]_D$ -41.9°, FAB MS: *m/z* 907 [MH$^+$], resembled those of vitisin B (**3**) (Table 2). In the ^{1}H NMR spectrum, the former (**4**) clearly differs from the latter (**3**) in that the former (**4**) has *cis*-olefinic hydrogen signals at δ 5.96 and 6.06 (each 1H, d, J=13.2 Hz). The fact indicated that they are geometrical isomers of the double bond, and this was unambiguously substantiated by photochemical transformation from vitisin B (**3**) to *cis*-vitisin B (**4**). The H-8 signal in the *cis*-isomer (**4**) appeared at higher field relative to that of the *trans*-isomer (**3**), and the shift in the signal may be due to the shielding effect of the upper left stilbene part in the formula **4**.

Like vitisin A and *cis*-vitisin A,[4] these compounds (**3** and **4**) have characteristic feature that the C-3 atom of resveratrol molecule involves in the bond formation in resveratrol oligomers.

EXPERIMENTAL

General Procedure. IR spectra were recorded as KBr on a SHIMADZU IR-408 spectrometer. UV spectra were recorded on a SHIMADZU UV-260 spectrometer in MeOH. FAB and FD MS were measured on JEOL JMS-DX 303 mass spectrometer. Optical rotations were determined on a JASCO DIP-360 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM FX-500 and A-600 spectrometers using tetramethylsilane as an internal standard. Coupling constants are in hertz. Multiplicity: s, singlet; d, doublet; brd, broad doublet; t, triplet; dd, double doublet; m, multiplet.

Isolation of ε-Viniferin Diol, Vitisin B and cis-Vitisin B. Dried stems of *Vitis coignetiae* (25.5 kg) (collected in Miyagi Prefecture, Japan) were extracted with MeOH (75 l x 3) at room temperature to yield the extract (850 g). The MeOH extract (850 g) was partitioned with AcOEt (3 l) and water (3 l) to give AcOEt (730 g) and water solubles. The AcOEt solubles (210 g) were chromatographed over silica gel (1 kg), and the column was eluted with *n*-hexane–AcOEt mixtures. The *n*-hexane–AcOEt (2:8)-eluting fraction (25 g) was chromatographed over silica gel (150 g), and the column was eluted with the increasing polarity of $CHCl_3$–MeOH mixtures. A silica gel chromatography of the $CHCl_3$–MeOH (9:1)-eluting fraction followed by repeated HPLC work ((1) column: Tosoh TSK gel ODS-120A: 30 x 2.15 cm i.d.; solvent: CH_3CN–water (27.5:72.5); flow rate: 3 ml/min, (2) column: YMC-Pack C_8: 25 x 2 cm i.d.; solvent: MeOH–water (6:4); flow rate: 2 ml/min) afforded vitisin B (**3**) and *cis*-vitisin B (**4**) (80 and 25 mg, respectively). The *n*-hexane-AcOEt (2:8) and AcOEt-eluting fraction of the AcOEt solubles (9 g) was chromatographed over silica gel (eluting solvent: $CHCl_3$-MeOH). The $CHCl_3$-MeOH (87:13)-eluting fraction was separated by HPLC (column: Tosoh TSK gel ODS-120A: 30 x 2.15 cm i.d.; solvent: CH_3CN–water (20:80 and 25:75); flow rate: 3 ml/min) yielded ε-viniferin diol (**1**) (16 mg).

ε-Viniferin diol (**1**): an amorphous powder, $[\alpha]_D$ +136.0° (*c* 0.19, MeOH), FAB MS *m/z*: 489 [MH$^+$]; UV λ_{max} nm (log ε): 284 (3.34); IR ν_{max} cm^{-1}: 3270, 1600, 1515, 1450; ^{1}H and ^{13}C NMR (see Table 1).

Vitisin B (**3**): an amorphous powder, $[\alpha]_D$ -90.0° (*c* 2.28, MeOH); high-resolution FAB-MS *m/z* 907.2723 [MH$^+$]; UV λ_{max} nm (log ε): 285 (4.10), 320 (4.04); IR ν_{max} cm^{-1}: 3180, 1600, 1510, 1450; ^{1}H and ^{13}C NMR (see Table 2).

cis-Vitisin B (**4**): $[\alpha]_D$ -41.9° (*c* 0.72, MeOH), FAB-MS *m/z* 907 [MH$^+$]; ^{1}H and ^{13}C NMR (see Table 2).

Osmium Tetroxide Oxidation of ε-Viniferin. To a AcOEt solution (10 ml) of ε-viniferin (**2**) (50 mg) was added *t*-BuOH solution (0.2 ml) of osmium tetroxide (0.5% w/v) and 4-methylmorphiline water solution (60% w/v) (0.2 ml), and the mixture was stirred for 1 hr at room temperature. The reaction mixture was chromatographed over aluminum to give ε-viniferin diol (**1**) (34 mg) which was identified with the natural ε-viniferin diol (**1**) by direct comparison of their $[\alpha]_D$, FAB MS, ^{1}H and ^{13}C NMR.

Methylation followed by Ozonolysis of Vitisin B. To a solution of vitisin B (**3**) (80 mg) in acetone (10 ml) were added dimethyl sulfate (1 ml) and anhydrous potassium carbonate (200 mg). The reaction mixture was refluxed at 80°C for 12 hr, and after evaporation of solvent, it was chromatographed over silica gel to yield a nonamethyl ether (**5**) (76 mg) (FAB MS *m/z*: 1033 [MH$^+$]). The solution of the ether (**5**) (40 mg) in methylene chloride (10 ml) at 0°C was treated with an ozone-saturated methylene chloride solution (10 ml), and excess dimethyl sulfide was added to the resulting mixture. After evaporation of the solvent, the residue was chromatographed over silica gel to afford two compounds (**6** and **7**) (12 and 18 mg, respectively). The physicochemical data of the compound **6** were identical to those of the aldehyde obtained from the vitisin A–*cis*-vitisin A mixture.[4] **7**: EI MS *m/z*: 644 [M$^+$]; ^{1}H and ^{13}C NMR (Table 2).

Catalytic Hydrogenation of Vitisin B Nonamethyl Ether. A mixture of vitisin B nonamethyl ether (**5**) (30 mg) and PtO_2 (10 mg) in MeOH (10 ml) was shaken under H_2 for 24 hr at room temperature, and the reaction mixture, after filtration, was chromatographed over silica gel to yield a dihydro derivative (**8**) (28 mg); an amorphous powder, FAB MS: *m/z* 1035 [MH$^+$]; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 2.26 (1H, m, H-7"), 2.36 (2H, t, J=7.3 Hz, H-8"), 2.44 (1H, m, H-7"), 3.60 (6H, s, 11,13-OMe), 3.61 (6H, 11"',13"'-OMe), 3.71 (3H, s, 13'-OMe), 3.745 (3H, 4'-OMe), 3.747 (3H, s, 13"-OMe), 3.751 (3H, s, 4-OMe), 3.76 (3H, s, 4"'-OMe), 4.18 (1H, d, J=6.6 Hz, H-8"'), 4.27 (1H, d, J=6.6 Hz, H-8'), 4.40 (1H, d, J=4.4 Hz, H-8), 5.42 (1H, d, J=4.4 Hz, H-7), 5.42 (1H, d, J=6.6 Hz, H-7'), 5.43 (1H, d, J=6.6 Hz, H-7"'), 6.07 (2H, brs, H-10,14), 6.13 (1H, brs, H-12), 6.21 (1H, brs, H-14"), 6.23 (1H, d, J=1.8 Hz, H-14'), 6.25 (2H, d, J=1.8 Hz, H-10"',14"'), 6.27 (1H, t, J=1.8 Hz, H-12"'), 6.36 (1H, brs, H-12"), 6.37 (1H, brs, H-2"), 6.44 (1H, d, J=1.8 Hz, H-12'), 6.53 (1H, brd, J=8.1 Hz, H-6"), 6.55 (2H, d, J=8.1 Hz, H-3',5'), 6.62 (1H, d, J=8.1 Hz, H-5"), 6.67 (2H, d, J=8.1 Hz, H-2',6'), 6.85 (2H, d, J=8.1 Hz, H-3"',5"'), 6.86 (2H, d, J=8.1 Hz, H-3,5), 7.17 (2H, d, J=8.1 Hz, H-2"',6"'), 7.22 (2H, d, J=8.1 Hz, H-2,6).

Photochemical Transformation of Vitisin B to cis-Vitisin B. A solution of vitisin B (**3**) (10 mg) in MeOH (10 ml) was irradiated with a commertial fluorescent lamp (15 W) at room temperatrure for 4 hr. After removal of solvent, the residue wa separated by HPLC (column: YMC-Pack C_8: 25 x 2 cm i.d.; solvent: MeOH–water

(6:4); flow rate: 2 ml/min) to afford *cis*-vitisin B (**4**) (6 mg). The spectral data of the synthetic *cis*-vitisin B were identified with those of natural *cis*-vitisin B (**4**).

REFERENCES

1. Oshima, Y.; Kamijou, A.; Moritani, H.; Namao, K.; Ohizumi, Y.; Nakano, M; Terao, K. Symposium Papers, 33rd Symposium on the Chemistry of Natural Products, Osaka, 1991, pp. 393-400.
2. Yang, L.-L.; Yen, K.-Y.; Kiso, Y.; Hikino, H. *J. Ethnopharmacol.* **1987**, 19, 103-109.
3. Oshima, Y.; Ueno, Y.; Hikino, H.; Yang, L.-L.; Yen, K.-Y. *Tetrahedron* **1990**, 46, 5121-5126.
4. Oshima, Y.; Kamijou, A.; Moritani, H.; Namao, K.; Ohizumi, Y. *J. Org. Chem.* **1993**, 58, 850-853.
5. Oshima, Y.; Namao, K.; Kamijou, A.; Matsuoka, S.; Nakano, M.; Terao, K.; Ohizumi, Y. *Experientia* **1995**, 51, 63-66.
6. Langcake, P. L.; Pryce, R. J. *Experientia* **1977**, 33, 151-152.

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Chemo-enzymatic Synthesis of (*R*,*R*)-(–)-Pyrenophorin

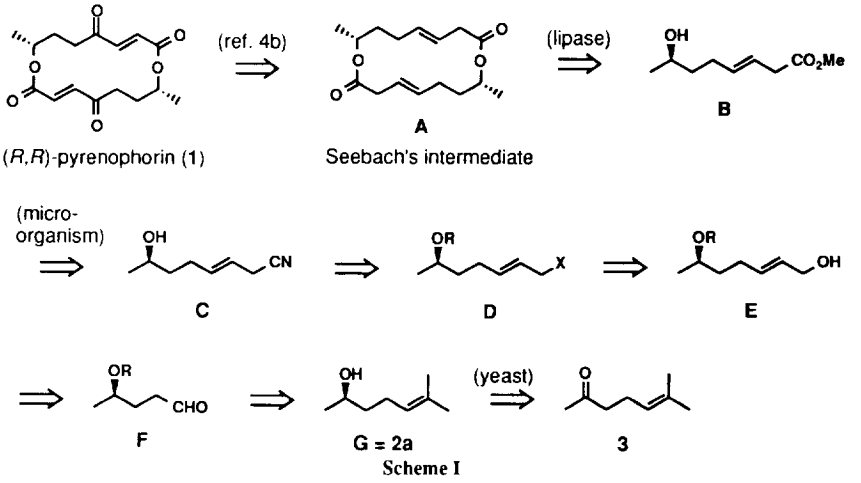
Takeshi Sugai, Osamu Katoh, and Hiromichi Ohta*

Department of Chemistry, Keio University, 3-14-1, Hiyoshi, Yokohama 223, Japan

Abstract: A chemo-enzymatic approach to (*R*,*R*)-(–)-pyrenophorin starting from commercially available 6-methyl-5-hepten-2-one is described. Firstly, (*R*)-6-methyl-5-hepten-2-ol (sulcatol) was prepared by interface-bioreactor mediated asymmetric reduction of the corresponding ketone by a yeast, *Pichia farinosa* IAM 4682 (51% yield, 90%*e.e.*). The sequential carbon-chain elongation *via* Horner-Emmons olefination of protected aldehyde and cyanation afforded all of carbon skeleton in the seco acid with a desired β,γ-(*E*)-double bond. By the aid of a microorganism, *Rhodococcus rhodochrous* IFO15564, the nitrile was efficiently hydrolyzed to give the corresponding carboxylic acid, (*R*,*E*)-7-hydroxy-3-octenoate, the key synthetic intermediate without affecting the position and configuration of the double bond (90% yield). Dimeric lactone structure was obtained by utilizing a lipase-catalyzed lactonization. While *Pseudomonas cepacia* lipase-catalyzed reaction worked in a moderate efficiency, higher yield of desired dimeric lactone (44%) was obtained by the use of an immobilized form of *Candida antarctica* lipase. The lactonization was accelerated in the presence of molecular sieves 4A. (*R*,*R*)-(–)-Pyrenophorin was obtained from this dimeric lactone (Seebach's intermediate) by the subsequent chemical transformation.

Introduction

(*R*,*R*)-(–)-Pyrenophorin (**1**) has been isolated[1] from *Pyrenophora avenae* as antifungal metabolite and is one of the representative of naturally occurring diolides.[2] Its functionalized dimeric lactone structure invoked the interests of synthetic chemists. So far the synthesis of racemic forms[3] as well as the optically active forms[4] and the seco acid syntheses[5] have been reported. Here we report an approach toward this molecule by the combination of chemical and enzymatic method. In the present synthesis, enzymatic methods were effectively applied in the following three key steps: 1) introduction of chirality; 2) installation of β,γ-unsaturated carboxylic acid in the synthetic intermediate; 3) dimeric lactonization.



Scheme I

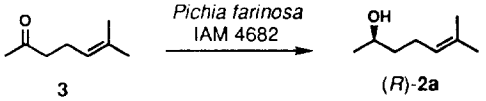
Synthetic Plan

The synthetic plan is shown in Scheme I. Pyrenophorin has been synthesized from the less functionalized β,γ-unsaturated dimeric lactone[4b] [Seebach's intermediate, (**A**)]. This lactone might be obtained by lipase-catalyzed lactonization of the corresponding optically active seco acid ester (**B**). The β,γ-unsaturated carboxylate functionality is planned to be obtained via microbial hydrolysis of the corresponding nitrile (**C**). The nitrile will be derived by one carbon homologation using cyanide *via* the nucleophilic substitution of allylic halide (**D**), which in turn will be readily available from allylic alcohol (**E**). The *trans* double bond of the precursor (**E**) is readily constructed from an aldehyde (**F**) by Horner-Emmons type two carbon homologation. We secured the source of chirality of the starting material in 6-methyl-5-hepten-2-ol [sulcatol, (**G** = **2a**)], of which preparation in optically active form with (*R*)-configuration had been reported by ourselves.[6a]

Interface-bioreactor Mediated Preparation of (*R*)-6-methyl-5-hepten-2-ol (sulcatol)

Optically active form of 6-methyl-5-hepten-2-ol [sulcatol, (**2a**)] is the pheromone components[7] of *Gnathotrichus sulcatus* and *Gnathotrichus retusus*, and also important starting materials for natural product synthesis.[8] So far a couple of biochemical procedures by the enzymatic / microbial reduction of the commercially available 6-methyl-5-hepten-2-one (**3**) have been reported, some of them have afforded the (*S*)-alcohol (Prelog rule product)[9] and others have afforded the (*R*)-enantiomer (*anti*-Prelog rule product).[6,9b]

All of these procedures, however, have suffered from the following drawbacks. Because of the toxicity of both substrate and product, which is characteristic for volatile ketones, the reduction proceeds only at a very low concentration of the substrate and product and with an excessively high weight of biocatalyst to the substrate. Those situation so far have made the biochemical reduction to be hardly utilized as the step for the preparative scale synthesis.



3 (*R*)-**2a**

Scheme II

The most conventional procedure for (*R*)-enantiomer which had been reported by ourselves by the use of stationary phase cells of yeast, *Pichia farinosa* IAM 4682 (Scheme II)[6a] was no exception. The reduction works only at a low concentration of substrate (< 0.1%) and the product (< 0.4%), and furthermore, only at the surprisingly high ratio of the wet cell mass / substrate (250 / 1) in a buffer solution.

As to apply these procedures to the preparations of the starting material of natural product synthesis, the scale-up of the reaction and the improvement of efficiency are the tasks to be firstly settled down. The key-point is how to avoid the toxicity of the substrate and product.

Oda and Ohta have proposed an interface-bioreactor[10] as the clue to solve this problem. Interface-bioreactor has been developed as an alternative to immobilized microorganisms in organic solvents. The microorganism is grown on a hydrophilic carrier (agar plate), and subsequently a solution of substrate in hydrophobic solvent is overlaid. We have already confirmed that *Pichia farinosa* IAM 4682 works in this system, for an enantioface selective protonation during the hydrolysis of enol ester.[12]

Thus, a solution of ketone (**3**, 0.5%) in isooctane was overlaid on the cells in stationary phase of *Pichia farinosa*, which was grown on the glucose-agar plate (pH 7.2, see experimental). Although no reduction could