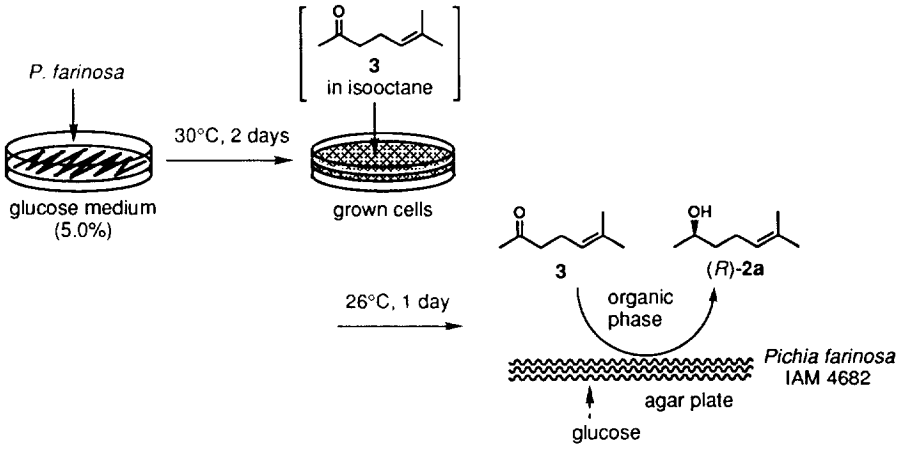
be observed when the concentration of the glucose in agar plate was 1%, a smooth reduction proceeded as shown in Scheme III when it was raised to 5%.



Scheme III

Through the pH profile experiments, the optimal pH for the reduction was found to be 6.5. At the higher concentration of glucose (10%), the reduction became slow. The enantiomeric excess (*e.e.*) of the product was found to be 84% and was in good coincidence with the one which had been obtained by the reduction in buffer solution with the same microorganism.[6a] It has been known that the *e.e.* of this product is higher when the reduction is carried out under an anaerobic condition. As expected, the *e.e.* of the product raised to 90%, when a solution which was degassed prior to the experiment was overlaid and the reaction vessel was tightly sealed. The results are shown in Table I.

Table I. Microbial Reduction of **3** on Interface-Bioreactor

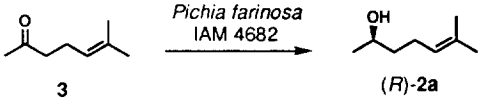
glucose in agar plate (%)	pH	solvent (mL)	**3** (mg)	conversion (%)	(*R*)-**2a** yield (%)	(*R*)-**2a** *e.e.* (%)
1.0	7.2	10	50	2	–	–
5.0	7.2	10	50	37	27	79
5.0	7.5	6	60	71	46	79
5.0	6.5	6	60	93	42	84
5.0	6.0	6	60	78	43	90
5.0	5.5	6	60	74	35	91
5.0	5.0	6	60	70	30	90
10.0	6.5	6	60	85	38	87
5.0[a]	6.5	6	60	90	51	90

a) anaerobic condition

In this way, the toxicity of substrate and product was alleviated by the use of interface-bioreactor. In the present case, reduction effectively proceeded at the lower ratio of the wet cell mass / substrate (50 / 1). Table

II represents the comparison of results obtained by conventional procedure with suspended cells in buffer solution and that with interface-bioreactor.

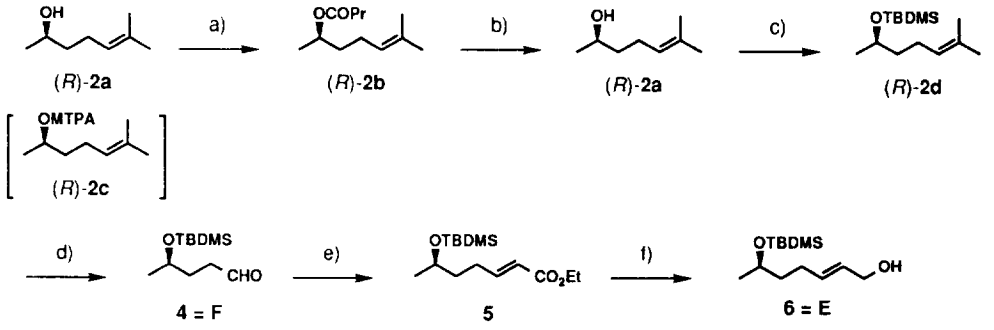
Table II. Comparison between Conventional Procedure and Interface-Bioreactor



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

	Conventional Procedure in Buffer Solution	Interface-Bioreactor mediated Reaction
substrate (%)	0.1 – 0.2	1.0
wet cell / substrate **3**	> 250 : 1	50 : 1
yield of (*R*)-**2a** (%)	52	51
e.e. of (*R*)-**2a** (%)	88	90

Another advantage with the use of interface-bioreactor is the improvement of the workup procedure. In the case of conventional procedure, the crude product should be subjected to azeotropic distillation prior to further purification, to avoid the contamination with the impurities from lipids and other hydrophobic metabolites from the excessive amount of yeast cells. On the other hand, in the present case, most of the product had already been transferred into organic phase and the isooctane solution could be directly charged on the column for silica gel chromatography. The contamination of cell material was negligible amount in the case of interface-bioreactor mediated reaction.



a) PPL, vinyl butanoate; b) K_2CO_3, H_2O / MeOH; c) TBDMSCl, imidazole / DMF; d) O_3 / CH_2Cl_2 then Me_2S; e) triethyl phosphonoacetate, LiCl, Hünig base / CH_3CN; f) DIBAL-H / CH_2Cl_2

Scheme IV

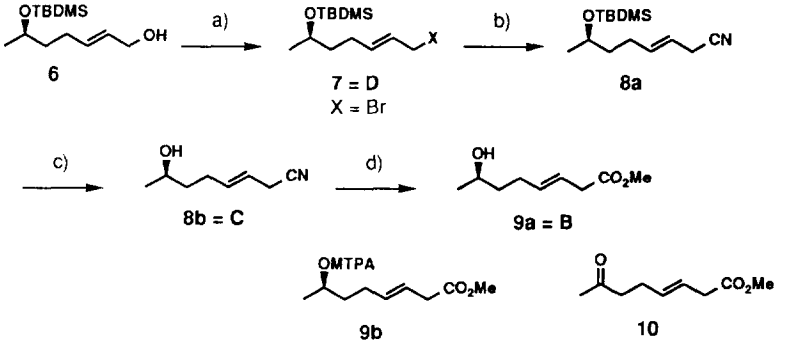
The Conversion to Allylic Alcohol (E)

The construction of allylic alcohol part (**E**) involving the desired (*E*) double bond was straightforward. Beforehand, the *e.e.* of the starting material **2a** was further enhanced to be over 98% by means of lipase-catalyzed transesterification.[6a] The hydroxyl group was protected as *t*-butyldimethylsilyl (TBDMS) ether to give **2d** in a quantitative yield. The double bond of **2d** was cleaved by ozonolysis with the subsequent reductive workup, and the resulting aldehyde **4** (= **F**) was homologated with a modified Horner-Emmons

procedure[12] to afford an α,β-unsaturated ester **5** in 74% yield from **2d**. The ester group was reduced with DIBAL-H to give the intermediate **6** (= **E**) with the desired (*E*) double bond in 97% yield.

The Conversion to Seco Acid Ester (B)

The next task was the further homologation of the alcohol **6** to the desired seco acid ester (**B**) with the retention of configuration of the chiral center and β,γ-position of (*E*) double bond. Among the synthetic methods so far reported,[13] we chose the combination of introduction of cyanide and subsequent microbial hydrolysis. Through an extensive study, the following procedure was found to be the best, *i.e.*, the activation of allylic alcohol as bromide[14] by the combined use of *N*-bromosuccinimide and triphenylphosphine in dichloromethane at –40 °C and the subsequent treatment of allylic bromide **7** (X = Br, = intermediate **D**) with copper(I) cyanide[15] at 80 °C without any solvent. The yield of desired nitrile **8a** (= **C**) was 58%. Many attempted alternatives[16] only resulted in the undesired product, which was derived by the simple elimination of leaving group (X = Cl, OMs, OTs). (*E*)-Configuration as well as the location in β,γ-position of double bond was confirmed by ^{1}H-NMR spectrum (see experimental). Activation of allylic alcohol as acetate and the conversion to cyanide through π-allylpalladium complex[17] proceeded with higher efficiency (76%), however, resulted in the E / Z mixture (7 / 1) of the product.



a) NBS, Ph_3P / CH_2Cl_2; b) CuCN; c) HF / acetonitrile; d) *R. rhodochrous* IFO 15564, then CH_2N_2

Scheme V

The hydrolysis of nitrile usually proceeds at high temperature with strong acid or base catalysis. In contrast, enzymatic hydrolysis,[18] which works at room temperature and at nearly neutral pH has recently been developed. In this case, to avoid the possible *E* / *Z* isomerization and / or migration of the double bond to α,β-position under those harsh reaction conditions of chemical hydrolysis, we attempted the microbial hydrolysis of nitrile **8a** with *Rhodococcus rhodochrous* IFO 15564, whose wide applicability toward aliphatic and aromatic nitriles had been exploited by us.[19]

First, nitrile **8a** was incubated with the grown cells of *R. rhodochrous* IFO15564. To our disappointment, almost no hydrolysis proceeded. The situation was overcome, by using hydroxy nitrile **8b** as the substrate, which was quantitatively obtained by the removal of silyl protective group of **8a**. The desired hydroxy ester was obtained in 95% yield after treatment with diazomethane of the corresponding hydroxy acid. No isomerization and migration of the double bond under the incubation and workup procedure was confirmed. The hydrolysis proceeded very smoothly within 0.5 h, and prolonged incubation caused the oxidation of

secondary alcohol part of the product to give a ketone **10**, due to a presence of oxidoreductase in the microorganism.

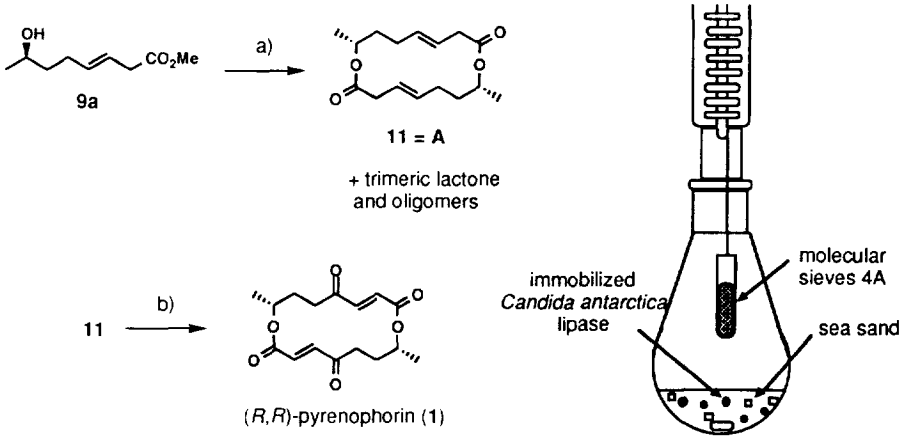
In this way, β,γ-unsaturated carboxylic ester with (*E*) configuration was effectively obtained by the combination of the introduction of cyano group to allylic system and the subsequent microbial hydrolysis. The scope and limitation of this protocol is now under investigation.

Synthesis of Pyrenophorin via Lipase-catalyzed Dimeric Lactonization

For the construction of the dimeric lactone, we decided to utilize the lipase-catalyzed lactonization in organic solvent among the expeditious methods for macrolactonization, because of the simplicity of procedure. Lipase-catalyzed lactonization / oligomerization has a distinctive advantage that the reaction is easily carried out by only mixing the substrates which possess hydroxyl groups, those with carboxyl groups, and lipase in an appropriate organic solvent. Accordingly, the lipase-catalyzed lactonization, oligomerization and related reaction have recently been studied.[5i,20]

Toward the synthesis of pyrenophorin and related dimeric lactones, Yamada[20a] and Sih[5i,20b] extensively studied the lipase-catalyzed lactonization of potential precursors. Those results seemed to be not promising, since the dimeric lactone was obtained from only the fully saturated hydroxy acid precursor. Two independent groups including ourselves, however, successfully applied lipase-catalyzed lactonization to the synthesis of naturally occurring macrocyclic lactone such as (*R*)-ferrulactone II,[20g] (*Z*)-3-dodecen-12-olide[20g] and (*R*)-recifeiolide.[20l] These results prompted us an attempt for lipase-catalyzed lactonization.

The hydroxy ester **9a** was treated with *Pseudomonas cepacia* lipase (Amano PS) in isooctane (5.8 mmol / L) in the presence of molecular sieves 4A at 65°C,[20d] for 5 days. The crude mixture of products was purified by silica gel column chromatography. The desired dimeric lactone **11** (= **A**, Seebach's intermediate, M^+ 280, 13%), trimeric lactone (M^+ 420, 12%), other oligomers were obtained together with the recovery of unreacted starting material (7%).



a) *Pseudomonas cepacia* lipase or *Candida antarctica* lipase; b) ref. 4b

Scheme VI

The same dimeric lactonization was performed efficiently by the use of immobilized form[20e] of *Candida antarctica* lipase[21] (Novo Nordisk, SP535). The lactonization was greatly accelerated by means of the vapor phase absorption of methanol and water on molecular sieves 4A, to give **11** (44%) and trimeric lactone (32%) without any recovery of starting material. It is noteworthy that the undesired products (trimers and other

oligomers) could be easily converted to the starting material without any effect on the chiral center and the double bond, by the enzyme-catalyzed hydrolysis with the same lipase in buffer.

The resulting dimeric lactone **11** was converted to (*R*,*R*)-(−)-pyrenophorin **1** [m.p. 170-171°C, $[\alpha]_D^{20}$ −47.6 (*c* 0.17, $CHCl_3$); lit.[1] m.p. 175°C, $[\alpha]_D$ −50.27 (*c* 1.5, $CHCl_3$)] according to Seebach's procedure in 22% yield.

Conclusion

(*R*,*R*)-(−)-Pyrenophorin was synthesized in 16 steps and 1.8% overall yield from commercially available 6-methyl-5-hepten-2-one. In this synthesis, three biocatalytic procedure: 1) interface-bioreactor mediated yeast reduction of aliphatic ketones as the introduction of chirality; 2) microbial hydrolysis of nitrile as the installation of β,γ-unsaturated carboxylic acid with (*E*) configuration; 3) lipase-catalyzed transesterification for the dimeric lactonization, were effectively demonstrated.

EXPERIMENTAL

All b.ps were uncorrected. IR spectra were measured as films on a Jasco IRA-202 spectrometer. ^1H NMR spectra were measured in $CDCl_3$ with TMS as the internal standard at 270 MHz on a JEOL JNM EX-270 spectrometer unless otherwise stated. Mass spectra were recorded on Hitachi M-80B spectrometer at 70 eV. Hitachi G-3000 Gas chromatograph was used for GLC analyses. Optical rotations were recorded on a Jasco DIP 360 polarimeter. Freshly distilled THF and Et_2O from sodium-benzophenone ketyl for anhydrous reaction. Wako Gel B-5F and silica gel 60 K070-WH (70-230 mesh) of Katayama Chemical Co. were used for prep TLC and column chromatography, respectively.

(R)-6-Methyl-5-hepten-2-ol (sulcatol) **2a**. The interface-bioreactor was set up as follows. A glucose-agar medium [containing glucose (5.0%), peptone (0.7%), yeast extract (0.5%), K_2HPO_4 (0.5%), agar (2.0%), pH 6.5, total volume 50 mL] was poured in a glass plate (56 cm^2) and solidified. On that plate, loopfuls of *P. farinosa* were streaked aseptically and grown at 30 °C for 2 days. Then a solution of **3** (60 mg, 0.48 mmol) in isooctane (6 mL) was overlaid, and the plate was sealed with a rid and left to stand at 25 °C. After 24 h, capillary GLC analysis showed that the reaction proceeded to 90%; GLC (column, TC-1, 6 m, 120 °C; N_2, 0.6 kg/cm^2) t_R 7.5 min (10%), 7.7 min (90%). Then the organic layer was recovered and the cell mass was washed with Et_2O. The organic solution was concentrated *in vacuo*. The residue was dissolved in hexane / Et_2O (5 / 1) and the solution was passed through a pad of SiO_2 (10 g) and afforded **2a** (30 mg, 50%), b.p. 105-120 °C/35 Torr (bulb-to-bulb distillation); $[\alpha]_D^{20}$ −14.5 (*c*=1.20, EtOH) [lit.[6a] $[\alpha]_D^{26}$ −16.7 (*c*=1.18, EtOH)]. Its IR and NMR spectra were identical with those reported previously.[6a] (*R*)-MTPA ester **2c**: ^1H NMR δ 1.26 (d, *J* = 6.3 Hz, 95%), 1.34 (d, *J* = 6.3 Hz, 5%). Therefore, the *e.e.* was determined to be 90%. The experiment using 50 plates in one time could be done without any difficulty, because the reaction smoothly proceeds in the glass plates which are stacked each other and left to stand in an incubator. A larger bioreactor (in an aluminum rectangle vessel, 683 cm^2)[11] was also available. Enhancement of the *e.e.* was carried out in the same manner as reported.[6a]

(R)-6-t-Butyldimethylsiloxy-2-methyl-2-heptene **2d**. To a stirred solution of **2a** [$[\alpha]_D^{18}$ -16.7 (*c*=1.24, EtOH), 3.86 g, 30.1 mmol] in DMF (10 mL) was added imidazole (5.8 g, 38 mmol) and TBDMS-Cl (5.3 g, 78 mmol) with ice-cooling, and the mixture was stirred overnight at room temp. The mixture was poured into ice-cooled water and extracted with Et_2O. The organic layer was washed with H_2O and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (80 g). Elution with hexane / Et_2O (40 / 1) afforded a colorless oil of **2d** (7.3 g, quant.). Analytical sample: b.p. 130-140

°C/2.5 Torr (bulb-to-bulb distillation); $[\alpha]_D^{19}$ –15.5 (*c*=1.29, $CHCl_3$); IR νmax 2970, 2940, 2860, 1460, 1375, 1255, 1140, 1080, 1035, 1005, 940, 890, 835, 780, 715, 665 cm^{-1}; ^1H NMR δ 0.05 (6H, s), 0.89 (9H, s), 1.12 (3H, d, *J* = 6.2 Hz), 1.30-1.50 (2H, m), 1.60 (3H, s), 1.68 (3H, s), 1.85-2.15 (2H, m), 3.78 (1H, tq, *J* = 6.2, 6.2 Hz), 5.11 (1H, tt, *J* = 1.3, 7.0 Hz); HRMS Found: 242.2076. Calc. for $C_{14}H_{30}OSi$: 242.2064. Due to its volatility, this compound could not give the correct elemental analysis.

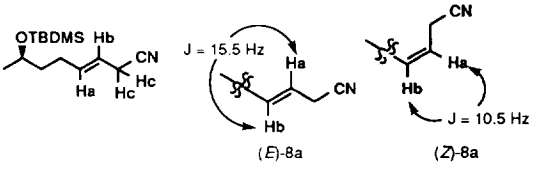
Ethyl (R,E)-6-t-Butyldimethylsiloxy-2-heptenoate **5**. To a mixture containing **2d** (3.6 g, 15 mmol), $NaHCO_3$ (40 mg) and CH_2Cl_2 (60 mL), O_3 gas (0.18 g / h) was bubbled for 4 h at -78 °C. After checking the disappearance of **2d** by TLC, Me_2S (7 mL) was added and the stirring was continued overnight at room temp. Solvent was removed *in vacuo*, the residue was diluted with H_2O, and extracted with *n*-hexane. The organic layer was washed with H_2O and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue, crude **4** (2.9 g), was used for next step without further purification. IR νmax 2970, 2940, 2860, 2720, 1730, 1460, 1375, 1255, 1140, 1100, 1040, 1005, 965, 940, 835, 810, 780, 665 cm^{-1}.

To a stirred solution of triethyl phosphonoacetate (5 mL, 28 mmol) in CH_3CN (230 mL), LiCl (1.4 g, 33 mmol), Hünig base (4 mL, 23 mmol), and crude **4** were added and the mixture was stirred overnight at room temp. After solvent was removed *in vacuo*, the residue was diluted with Et_2O. After the filtration through a pad of Celite, the organic layer was washed with H_2O and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (80 g). Elution with hexane / Et_2O (9 / 1) afforded **5** as a colorless oil (3.1 g, 74 % from **2d**), b.p. 150-160 °C/0.9 Torr (bulb-to-bulb distillation); $[\alpha]_D^{21}$ –15.6 (*c*=1.28, $CHCl_3$); IR νmax 2970, 2940, 2860, 1725, 1655, 1460, 1375, 1310, 1255, 1205, 1170, 1140, 1090, 1040, 1005, 985, 835, 810, 780, 710, 665 cm^{-1}; ^1H NMR δ 0.03 (3H, s), 0.04 (3H, s), 0.87 (9H, s), 1.12 (3H, d, *J* = 6.2 Hz), 1.27 (3H, t, *J* = 7.0 Hz), 1.40-1.65 (2H, m), 2.10-2.40 (2H, m), 3.80 (1H, tq, *J* = 6.2, 6.2 Hz), 4.16 (2H, q, *J* = 7.0 Hz), 5.80 (1H, ddd, *J* = 1.6, 1.6, 15.8 Hz), 6.97 (1H, dt, *J* = 7.0, 7.0, 15.8 Hz). (Found: C, 62.49; H, 11.38. Calc. for $C_{11}H_{24}O_2Si$: C, 62.89; H, 10.55%.)

(R,E)-6-t-Butyldimethylsiloxy-2-hepten-1-ol **6**. To a stirred solution of **5** (0.30 g, 1.0 mmol) in CH_2Cl_2 (20 mL), 1.5 M solution of DIBAL-H in toluene (1.6 mL, 2.4 mmol) was added over 5 min at –40 °C, and the mixture was stirred for 30 min at this temp. The mixture was quenched by slow addition of MeOH, and the solution was allowed to warm to room temp. The mixture was stirred with sat Rochelle salt aq solution (50 mL) for 2 h. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic solution was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (15 g). Elution with hexane / EtOAc (7 / 1) afforded **6** (0.25 g, 97%) as a colorless oil. Analytical sample: b.p. 150-160 °C/1.1 Torr (bulb-to-bulb distillation); $[\alpha]_D^{20}$ –14.4 (*c*=1.32, $CHCl_3$); IR νmax 3350, 2970, 2940, 2860, 1460, 1375, 1255, 1185, 1140, 1090, 1050, 970, 855, 810, 780, 710, 665 cm^{-1}; ^1H NMR δ 0.05 (6H, s), 0.89 (9H, s), 1.12 (3H, d, *J* = 6.2 Hz), 1.30-1.38 (1H, br.), 1.40-1.65 (2H, m), 1.85-2.15 (2H, m), 3.78 (1H, dq, *J* = 6.2, 6.2 Hz), 4.08 (2H, d, *J* = 4.6 Hz), 5.51-5.76 (2H, m). (Found: C, 63.75; H, 1.58. Calc. for $C_{13}H_{28}O_2Si$: C, 63.87; H, 11.54%.)

(R,E)-1-Cyano-7-t-butyldimethylsiloxy-3-heptene **8a**. To a stirred solution of **6a** (1.0 g, 4.1 mmol) in CH_2Cl_2 (8.0 mL), NBS (1.0 g, 5.6 mmol) and triphenylphosphine (1.26 g, 4.8 mmol) was added at –40 °C, and the stirring was continued for 20 min. The residue was diluted with Et_2O. After filtration, solvent was removed *in vacuo* and petroleum ether was added to the residue to filter off the precipitated triphenylphosphine oxide. Solvent was removed and **7** (1.30 g) was obtained as the residue. This was used for next step without further purification. IR νmax 2970, 2940, 1760, 1460, 1435, 1375, 1255, 1205, 1130, 1090, 1050, 1005, 970, 940, 835, 810, 780, 720, 700, 665 cm^{-1}.

CuCN(I) (0.80 g, 9.0 mmol) was added to a crude oil of **7**, and the mixture was heated with slow stirring at *ca.* 80 °C, and the stirring was continued for 1 h at that temp. After cooling down to room temp, sat $NaHCO_3$ aq solution was added to the mixture. After filtration through a pad of Celite, the mixture was extracted with EtOAc. The organic solution was washed with H_2O, sat $NaHCO_3$ aq solution, and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (100 g). Elution with hexane / EtOAc (40 / 1) afforded **8a** as an yellow oil (0.61 g, 58% from **6a**). Analytical sample: b.p. 155-165 °C/0.9 Torr (bulb-to-bulb distillation); $[\alpha]_D^{20}$ −15.9 (*c*=1.27, $CHCl_3$); IR νmax 2970, 2940, 2860, 2350, 1735, 1460, 1415, 1375, 1255, 1185, 1140, 1090, 1040, 1005, 970, 940, 835, 780, 710, 665 cm^{-1}; ^1H NMR δ 0.04 (3H, s), 0.05 (3H, s), 0.89 (9H, s), 1.12 (3H, d, *J* = 6.2 Hz), 1.35-1.60 (2H, m), 1.95-2.25 (2H, m), 3.05 (2H, dd, *J* = 1.5, 5.6 Hz), 3.78 (1H, tq, *J* = 6.2, 6.2 Hz), 5.35 (1H, dtt, *J* = 1.5, 5.6, 15.5 Hz), 5.83 (1H, dtt, *J* = 1.5, 6.6, 15.5 Hz); decoupling (270 Hz, $CDCl_3$) Hc irradiation: (*E*)-**8a**; δ 5.83 (1H, dt, $J_{Ha\text{-}Hb}$ = 15.5 Hz); *cf.* (*Z*)-**8a**; δ 5.70 (1H, dt, $J_{Ha\text{-}Hb}$ = 10.5 Hz) as shown below. (Found: C, 66.08; H, 11.49, N, 5.66%. Calc. for $C_{14}H_{27}O_2NSi$: C, 66.34; H, 5.53%.)



(R,E)-7-Cyano-5-hepten-2-ol **8b**. To a stirred solution of **8a** (1.00 g, 3.98 mmol) in CH_3CN (38 mL), HF aq solution (2.0 mL) was added at 0 °C, and the stirring continued for 20 min at that temp. The reaction was quenched by addition of sat $NaHCO_3$ aq solution, and extracted with Et_2O. The ether solution was washed with H_2O, sat $NaHCO_3$ aq solution, and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography (15 g). Elution with hexane / EtOAc (1 / 1) afforded **8b** as a colorless oil (0.56 g, quant). Analytical sample: b.p. 155-165 °C/0.9 Torr (bulb-to-bulb distillation); $[\alpha]_D^{20}$ −14.2 (*c*=0.98, $CHCl_3$); IR νmax 3500, 2970, 2940, 2860, 2350, 1735, 1460, 1415, 1375, 1185, 1130, 1090, 1015, 970, 940, 900, 850 cm^{-1}; ^1H NMR δ 1.20 (3H, d, *J* = 6.2 Hz), 1.30 (1H, br.), 1.50-2.60 (2H, m), 2.05-2.30 (2H, m), 3.05 (2H, dd, *J* = 1.5, 5.6 Hz), 3.81 (1H, tq, *J* = 6.2, 6.2 Hz), 5.40 (1H, dtt, *J* = 15.5, 1.5, 5.6 Hz), 5.83 (1H, dtt, *J* = 15.5, 1.5, 6.6 Hz). (Found: C, 69.04; H, 10.12, N, 9.85. Calc. for $C_8H_{30}NO$: C, 69.03; H, 9.41; N, 10.06%.)

Methyl (R,E)-7-hydroxy-3-octenoate **9a**. *R. rhodochrous* was incubated in a glucose medium [containing glucose (1.5 g), peptone (0.5 g), yeast extract (0.1 g), K_2HPO_4 (0.12 g), KH_2PO_4 (0.04 g) $MgSO_4$ (0.05 g), $FeSO_4$ (0.03 g), ε-caprolactam (0.1 g), pH 7.2, total volume 100 mL] for 2 days at 30 °C. The wet cells were harvested by centrifugation and washed with 0.1 M phosphate buffer (pH 6.0). The mixture of **8b** (570 mg, 4.09 mmol), wet cells (8.0 g) and 0.1 M phosphate buffer (pH 6.0, 57 mL) was stirred at 30 °C for 30 min. The mixture was acidified by the addition of hydrochloric acid, and EtOAc was added and the mixture was stirred for 30 min. After filtration with a pad of Celite, the organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic phase was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was treated with ethereal solution of diazomethane, and purified by SiO_2 flash column chromatography (25 g). Elution with hexane / EtOAc (2 / 1) afforded **9a** as a colorless oil (670 mg, 95% from **8b**), b.p. 155-165 °C/0.9 Torr (bulb-to-bulb distillation); $[\alpha]_D^{18}$ −12.5 (*c*=1.42, $CHCl_3$); IR νmax 3400, 2970, 2940, 2860, 2350, 1740, 1435, 1250, 1195, 1150, 1130, 1080, 1015, 970, 850 cm^{-1}; ^1H NMR δ 1.20 (3H, d, *J* = 6.2 Hz), 1.44-1.76 (2H, m), 2.05-2.25 (2H, m), 3.05 (2H, dd, *J* = 1.5, 5.6 Hz), 3.77

(3H, s), 3.90 (1H, m), 5.58-5.75 (2H, m). (Found: C, 62.72; H, 10.08. Calc. for $C_9H_{16}O_3$: C, 62.77; H, 9.36%.)

(4E,8R,12E,16R)-8,16-Dimethyl-1,9-dioxacyclohexadeca-4,12-diene-2,10-dione **11**. The mixture of **9a** (49 mg, 0.29 mmol), lipase PS (250 mg), molecular sieves 4A (500 mg) and anhydrous isooctane (50 mL) was stirred at 65 °C for 4 days. After filtration with a pad of Celite, the solution was concentrated *in vacuo*. The residue was purified SiO_2 flash column chromatography (6 g). Elution with hexane / Et_2O (3 / 1) afforded mixture of dimeric lactone and trimeric lactone. The mixture was purified by SiO_2 preparative TLC [developed with hexane / THF / EtOH (150 / 10 / 1) x 2, Rf 0.4] to afford **11** (5.3 mg, 13%); ^{1}H NMR (JEOL JNM α400, 400 MHz, $CDCl_3$) δ 1.20 (6H, d, *J* = 6.2 Hz), 1.58-1.74 (4H, m), 2.10-2.32 (4H, m), 2.90-2.95 (4H, m), 4.83 (2H, ddq, *J* = 3.1, 6.2, 6.2 Hz), 5.42-5.57 (4H, m); ^{13}C NMR (JEOL JNM α400, 100 MHz, $CDCl_3$) δ 20.54, 28.89, 34.15, 39.03, 70.17, 123.20, 132.58, 170.90; MS: m/e (%) = 280 (M^+, 11), 140 (100), 95 (55), 81 (52), 53 (37). Trimer (4.9 mg, 12%): Rf 0.3 with the same eluent for the development; MS: m/z (%) = 420 (M^+, 38), 262 (30), 149 (96), 140 (100), 95 (87), 81 (63), 53 (52).

Dimeric lactonization by using Candida antarctica lipase. The immobilized *Candida antarctica* lipase was prepared according to the reported procedure.[20c] The mixture of **9a** (106 mg, 0.62 mmol), immobilized *C. antarctica* lipase (500 mg), sea sand (1.1 g), and anhydrous isooctane (100 mL) was stirred at 65 °C for 17 h. The reaction apparatus was shown in scheme VI and molecular sieves 4A (2.5 g) was placed in a cylinder made by filter paper and hung in the reaction flask for the vapor phase absorption of methanol and water. The reaction mixture was worked-up in the same manner as for the *Pseudomonas* lipase-catalyzed lactonization to afford **11** (38 mg, 44%) and trimeric lactone (28 mg, 32%) without any recovery of starting material. The dimeric lactone **11** was recrystallized from *n*-pentane to give needles, m.p. 46.5-47°C, $[\alpha]_D^{21}$ –10.5 (*c*=0.27, $CHCl_3$) [lit.[4b] m.p. 68-69°C, $[\alpha]_D^{20}$ –55.0 (*c*=0.5, $CHCl_3$). The spectral data were in good accordance with those obtained as above. (Found: C, 68.16; H, 8.85. Calc. for $C_{16}H_{24}O_4$: C, 68.55; H, 8.63%.) Although the m.p. and specific rotation of our sample were different from those reported previously,[4b] our sample could successfully be converted to (*R*,*R*)-(–)-pyrenophorin in the same manner as described before.[4b] The discrepancy of physicochemical properties at this stage is not clear.

(3E,8R,11E,16R)-8,16-Dimethyl-1,9-dioxacyclohexadeca-3,11-diene-2,5,10,13-tetraone [(–)-Pyrenophorin] **1**. According to the reported procedure,[4b] **11** was converted to pyrenophorin **1** in 22% yield. M.p. 170-171°C (lit.[1] m .p. 175°C), $[\alpha]_D^{20}$ –47.6 (*c*=0.17, $CHCl_3$) [lit.[1] $[\alpha]_D$ –50.27 (*c*=1.5, $CHCl_3$)], $[\alpha]_D^{24}$ –62 (*c*=0.13, acetone) [lit.[4a] $[\alpha]_D$ –54.5 (acetone)], lit.[4c] $[\alpha]_D^{26}$ –72.9 (*c*=0.65, acetone)], lit.[4f] $[\alpha]_D^{20}$ –61.0 (*c*=0.66, acetone)]; ^{1}H NMR δ 1.29 (6H, d, *J* = 6.3 Hz), 2.0-2.2 (4H, m), 2.54 (2H, ddd, *J* = 4.3, 7.9, 14.2 Hz), 2.66 (2H, ddd, *J* = 3.9, 8.2, 14.2 Hz), 5.03 (2H, m), 6.49 (2H, d, *J* = 15.8 Hz), 6.94 (2H, d, *J* = 15.8 Hz). MS: m/z (%) = 308 (M^+, 4), 203 (3), 264 (5), 195 (4), 155 (43), 138 (26), 124 (13), 109 (19), 99 (57), 86 (100), 68 (18), 54 (66). The NMR and mass spectra were in good accordance with those reported previously.[4b] (Found: C, 62.06; H, 6.72. Calc. for $C_{16}H_{20}O_6$: C, 62.33; H, 6.54%.)

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Absolute Configuration of Leustroducsins

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Abstract: The absolute configuration of Leustroducsins was determined to be 4*S*, 5*S*, 8*R*, 9*R*, 11*R*, 16*R*, 18*S* by the modified Mosher's method.

A fervent interest is emerging in thrombopoietic agents both within the chemical and biological communities.[1] Recently thrombopoietin[2] was discovered as a physiological factor in normal thrombopoiesis by several groups, but reports[3] on thrombopoietic agents of low molecular weight are only a few. During the screening program in the quest for compounds which induce the production of colony-stimulating factors in KM-102 cells, Kohama *et al* isolated Leustroducsins (LSNs) A, B, and C[4] (**1a**, **1b**, and **1c**, respectively) from the culture broth of *Streptomyces platensis* SANK 60191. These compounds represent novel microbial metabolites belonging to the phoslactomycin (PLM)[5] family and were found to have inducing activities in the production of colony-stimulating factors by bone marrow stromal cells. It was observed that administration of LSN-B to mice induced thrombocytosis.[6] Their structures have been determined as **1a** - **1c**, but their absolute configurations are yet to be determined, since acquisition of crystals for X-ray analysis remains problematic. The absolute configurations of PLMs also have not been determined. Since LSNs are produced in certain microorganisms as a complex mixture containing PLM-F (**1e**), the task of obtaining each LSN separately in large scale was found to be arduous. Based on the finding that a single congener of the LSNs was obtained from treating a mixture of LSNs and PLM-F with porcine liver esterase in acetone-phosphate buffer (pH=6.7) at 37°C, LSNs and PLM-F can be expected to have the same absolute configurations except for the stereochemistry of the acyl group at the cyclohexane ring. After filtering off the enzyme, purification by a Cosmosil column afforded LSN-H (**1d**).[7] From the results of a biological assay, one noteworthy observation was that LSN-H was found to be 100 times less effective than LSN-B *in vitro*, but was found to show comparative thrombopoietic effects *in vivo*. LSN-H has potential to be a new lead compound for hematological disorders and could also find use as a tool in the analysis of the regulatory mechanism of hematopoiesis in bone marrow. Therefore the determination of absolute configuration of LSN-H could be very important. Kusumi and his co-workers[8] have reported on a modified Mosher's method in determining the absolute configuration of the secondary alcohol. In this paper we report on the absolute configuration of LSN-H (**1d**) as determined by the modified Mosher's method.

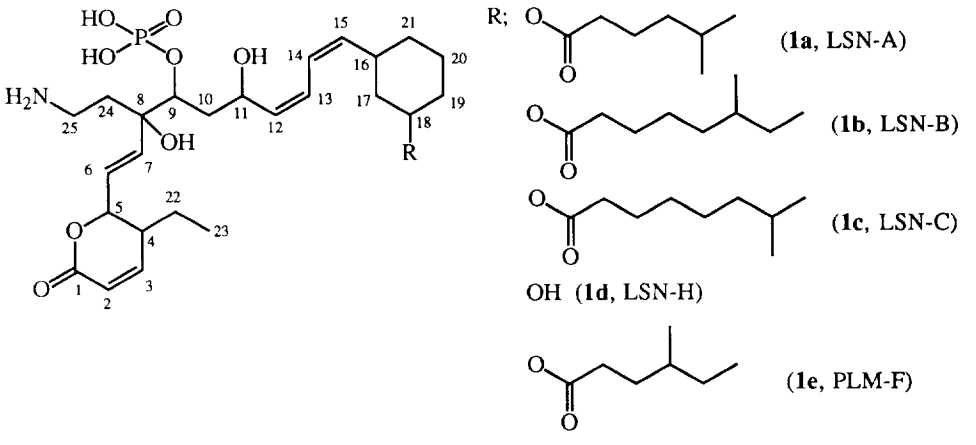

Fig.1. Structure of Leustroducsins and Phoslactomycin F

Dephosphorylation[9] of LSN-H in refluxing formamide-phosphate buffer gave formylated tetraol **2**, which was subsequently treated with *p*-toluenesulfonic acid in acetone to afford acetonide **3**. Acylation of **3** with *p*-bromobenzoyl chloride and triethylamine gave **4**. Both (+)-(*R*)- and (-)-(*S*)-MTPA esters of the 11-hydroxy group of **4** were prepared to afford **5a** and **5b**, respectively, and the proton signals of each derivative were assigned by the COSY technique. Analysis of the proton NMR spectra of **5a** and **5b** showed that the $\Delta\delta$ (δ_S - δ_R) (ppm) values of H-5 - H-10 were positive and the $\Delta\delta$ values of H-12 - H-16 were negative. The $\Delta\delta$ values obtained for the respective protons are shown in Fig. 2. The systematic arrangement of positive and negative $\Delta\delta$'s revealed the *R* configuration at C-11.

In order to obtain the objective MTPA esters for determination of the C-18 configuration, further selective derivatisation was desired. Thus the acetonide **3** was treated with benzoyl chloride and 4-dimethylaminopyridine (DMAP) to afford 11-O-benzoate **6** (y. 33%) along with 11,18-O-dibenzoate **7** (y. 30%). Interestingly, with DMAP as a base, the amide nitrogen was not acylated. Both (+)- and (-)-MTPA esters of the 18-hydroxy group of **6** were prepared to afford **8a** and **8b**, respectively. Inspection of the proton NMR spectra of **8a** and **8b** showed that the $\Delta\delta$ values of H-15 - H-17 were positive and the $\Delta\delta$ values of H-19 - H-20 were negative. The $\Delta\delta$ values obtained from these MTPA esters are shown in Fig. 3. Determination of the S configuration at C-18 was based on the systematic arrangement of positive and negative $\Delta\delta$'s. To determine the relative stereochemistries on the cyclohexane ring, the coupling constants of **8a** were investigated. The large coupling constants (J>10Hz) between H-16 and H-17a, H-16 and H-21a, H-18 and H17a, and H-18 and H19a indicated the cyclohexane ring to be a chair conformation with H-16, H-17a, H-18, and H-19a fixed in the axial orientation. Hence the absolute configuration at C-16 must be *R*. To determine the remaining relative stereochemistries, the NOESY spectrum of **8a** was studied. As depicted in Fig. 3 the following NOEs: H-7 and H-9; H-7 and an acetonide methyl; and H-9 and the latter methyl, were observed. Thus it was deduced that C-7 and H-9 on the five membered ring were of a *cis* relationship. The NOE observed between H-4 and H-5 indicated a *cis* relationship of both protons on the lactone ring.