# TWO NEW FUNGAL AZAPHILONES FROM TALAROMYCES LUTEUS, WITH MONOAMINE OXIDASE INHIBITORY EFFECT

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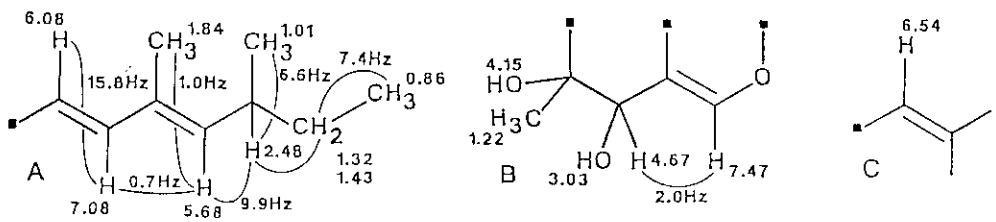
Abstract — Two new compounds with monoamine oxidase (MAO) inhibitory effect isolated from an ascomycete, Talaromyces luteus, have been deduced to be azaphilone compounds, (8R)-7-deacetyl-$O^8$,8-dihydro-7-epi-sclerotiorin and its (Z)-isomer at position 11 from their chemical and spectral data.

In a series of the survey of new biologically active fungal metabolites, the ethyl acetate extract of an ascomycete, Talaromyces luteus (Zukal) C. R. Benjamin strain IFM42239 gave the inhibitory activity of 33.6 % to the monoamine oxidase (MAO) from mouse liver at a concentration of $10^{-4}$ g/ml on an assay using Kraml method.[1]

To our knowledge, any metabolites with MAO inhibitory effect have not so far been isolated from this fungus. Repeated chromatographic separation of the extract on ordinary-phase and reversed-phase silica gel columns afforded two new compounds tentatively named TL-1 (1) and -2 (2) as the MAO inhibitory principles of this fungus. On this separation, 1 was obtained as pure crystallines, but 2 as only amorphous powder twenty percent mixed with 1 (the ratio of both constituents was estimated from the peak height of the $^1$H nuclear magnetic resonance ($^1$H-nmr) spectrum). The fifty percent inhibitory concentrations ($IC_{50}$) of 1 and 2 were 2.42 x $10^{-6}$ (6.6 x $10^{-6}$ M) and 3.87 x $10^{-6}$ g/ml, respectively, on the assay using modified Kraml method.

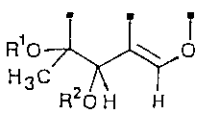
TL-1 (1), a yellow fine needle, mp 99-100°C, $[\alpha]_D^{20}$ -145° (c 1.0, chloroform), was positive to Beilstein test. The high resolution mass (hrms) spectrum indicated the molecular formular $C_{19}H_{23}O_4Cl$ for 1. The absorption maxima at 249 (log

ε, 4.24), 338 sh (4.27), 354 (4.31), 388 sh (4.38), 408 (4.44), 430 sh (4.33), and 460 nm sh (3.88) in the ultraviolet (uv) spectrum suggested the presence of a long conjugated unsaturation system in 1. The infrared (ir) spectrum suggested the presence of a hydroxyl group at 3400, a conjugated carbonyl group at 1630-1620, a conjugated carbon-carbon double bond at 1550, and a hydrogen-olefinic carbon bond at 960 and 840 $cm^{-1}$. The $^{13}C$ nuclear magnetic resonance ($^{13}C$-nmr) spectrum indicated that all carbons in 1 are classified into four methyls including one olefinic, one methylene, seven methines including five olefinic, six quaternary carbons including five olefinic, and one carbonyl carbons.
The $^{1}H$-nmr spectrum in deuteriochloroform ($CDCl_3$) gave the signals at 0.86 (3H, t, J=7.4 Hz), 1.01 (3H, d, J=6.6 Hz), 1.22 (3H, s), 1.32, 1.43 (each 1H, m), 1.84 (3H, d, J=1.0 Hz), 2.48 (1H, m), 3.03, 4.15 (each 1H, broad s, disappeared by an addition of deuterium oxide ($D_2O$)), 4.67 (1H, broad s, changed into a doublet signal (J=2.0 Hz) by an addition of $D_2O$), 5.68 (1H, dd, $J_1$=9.9, $J_2$=1.0 Hz), 6.08 (1H, d, J=15.8 Hz), 6.54 (1H, s), 7.08 (1H, d, J=15.8 Hz), and 7.47 ppm(1H, d, J=2.0 Hz). By the aid of spin decoupling and two-dimensional $^{1}H$-$^{1}H$ shift correlation ($^{1}H$-$^{1}H$ COSY) measurements, the presence of the following partial structures A, B, and C in 1 was suggested.

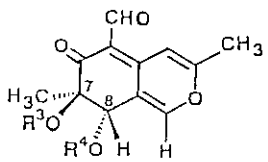



On catalytic hydrogenation with 5 % palladium-carbon, 1 gave a hydrogenated derivative (3), [$M^+$] 354, uv (MeOH) 237, 253, 365 nm. The $^{1}H$- and $^{13}C$-nmr spectra of 3 showed that tetrahydrogenation occurred in the moiety of partial structure A in 1. On acetylation with acetic anhydride and sodium acetate, 1 gave a monoacetate (4), [δ 2.27 ppm (3H, s)]. Comparison of the $^{1}H$-nmr spectrum of 4 with that of 1 indicated that the signals of a proton (4.67 ppm) attached to the carbon bearing a secondary hydroxyl group, an olefinic proton (7.47 ppm) at β-position to the secondary hydroxyl group, and a methyl group (1.22 ppm) attached to the carbon bearing a tertiary hydroxyl group in the partial structure

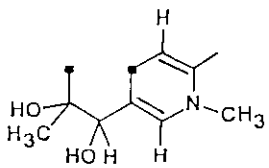
B are shifted to 5.94 (+1.27), 7.06 (-0.41), and 1.30 (+0.08) ppm, respectively. These $^1$H-nmr data suggested that the acetylation of a secondary hydroxyl group in the moiety of the partial structure B in 1 occurred to afford 4. On the other hand, on acetylation with acetic anhydride and perchloric acid, 1 gave diacetate (5), [δ 2.05, 2.22 (each 3H, s)]. Comparison of the $^1$H-nmr spectrum of 5 with that of 1 indicated that the signals of a hydrogen attached to a carbon bearing a secondary hydroxyl, an olefinic hydrogen, and a methyl attached to a carbon bearing a tertiary hydroxyl in the partial structure B are shifted to 6.78 (+2.11), 7.06 (-0.41), and 1.40 (+0.18) ppm, respectively. These $^1$H-nmr data suggested that the acetylation of both secondary and tertiary hydroxyl groups in the partial structure B in 1 occurred to afford 5. This finding in the $^1$H-nmr spectra of 1, 4, and 5 was quite similar to that in the $^1$H-nmr spectra of austdiol (6), an azaphilone from Aspergillus ustus, and its monoacetate (7) and diacetate (8).[2] Azaphilones which are fungal metabolites derived through polyketide are known to give vinylogous γ-pyridones with primary amines (or ammonia) because the divinyl ether system in the extended γ-pyrone structure in the azaphilone skeleton is easily replaced by primary amines (or ammonia) to afford enamine-type amino compounds.[3] On treatment with methylamine, 1 afforded an amino compound (9), $^1$H-nmr ($CDCl_3$) 3.74 (3H, s, $CH_3$-N), $^{13}$C-nmr ($CDCl_3$) 41.7 (q, $CH_3$-N). The $^1$H- and $^{13}$C-nmr spectra of 9 indicated that the divinyl ether in the moiety probably composed of the partial structures B and C in 1 was replaced by methylamine to afford 9. These facts showed that 1 may be a new compound belonging to the azaphilones.



1: $R^1$=$R^2$=H
4: $R^1$=H, $R^2$=Ac
5: $R^1$=$R^2$=Ac



6: $R^3$=$R^4$=H
7: $R^3$=H, $R^4$=Ac
8: $R^3$=$R^4$=Ac



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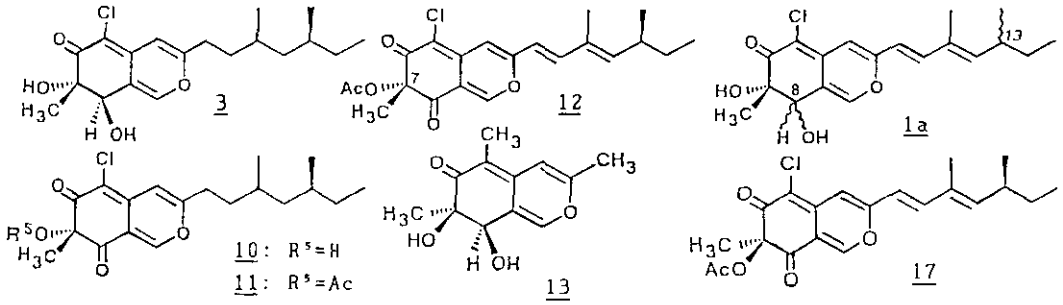
On oxidation with pyridinium chlorochromate,[4] 3 afforded a ketone (10). Comparison of the $^1$H-nmr spectrum of 10 with that of 3 indicated that oxidation of the secondary hydroxyl group in the partial structure B in 3 occurred to afford 10. On acetylation with acetic anhydride and pyridine, 10 gave an acetate (11),

which was identical with 7-epi-tetrahydrosclerotiorin derived from authentic 7-epi-sclerotiorin (12) (from Penicillium hirayamae[5]) in terms of $^1$H-nmr and electron-impact mass (ms) spectra and thin layer chromatographic (tlc) behavior. Therefore, the structure of 1 without its stereochemistry at positions 8 and 13 was disclosed to be 7-deacetyl-0[8],8-dihydro-7-epi-sclerotiorin (1a) which includes the partial structures A, B, and C.

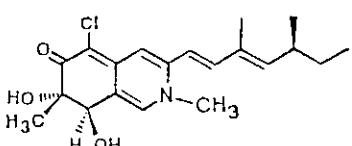



It is known that 6 having trans glycol at positions 7 and 8 affords no acetonide[2] on treatment with acetone in the presence of perchloric acid, but dihydrodeoxy-8-epi-austdiol (13) (from Aspergillus ustus) having cis glycol at positions 7 and 8 affords an acetonide.[6] On treatment in the same way, 1 was unchangingly recovered. Therefore, the glycol at positions 7 and 8 in 1 was suggested to have trans configuration. It is further known that the glycol in 6 has trans quasi-diequatorial conformation.[2,7] The differences in the $^1$H-nmr spectra between 1 and its acetyl derivatives (4 and 5) are quite similar to those between 6 and its acetyl derivatives (7 and 8)[2] as stated above. Consequently, the glycol in 1 was furthermore suggested to have trans quasi-diequatorial conformation. On treatment with racemic 2-phenylbutyric anhydride and pyridine, 1 afforded two diastereomeric products, the major (14) and the minor (15). The $^1$H-nmr spectra of 14 and 15 indicated that the secondary hydroxyl group at position 8 in 1 was esterified by 2-phenylbutyric acid to afford 14 and 15 during this reaction. On this reaction, the excess 2-phenylbutyric acid was recovered and gave a positive specific optical rotation ($[\alpha]_D^{23}$ +8.4° (c 1.21, benzene)), indicating that (-)-2-phenylbutyric acid was more selectively consumed to afford 14 than (+)-2-phenylbutyric acid to afford 15. This result on Horeau method[8] (the optical yield: 24.5 %) indicated that absolute configuration at position 8 in 1 is (R). Therefore, absolute configuration at position 7 in 1 was deduced to be (S). This conclusion on the configuration at position 7 in 1 was compatible with the fact

that 1 affords 7-epi-tetrahydrosclerotiorin (11). Though the relative stereostructure of the glycol in 1 is the same as that in 6, the absolute configuration of it in 1 (7S, 8R) is contrary to that in 6 (7R, 8S).[2]

On degradation with 5 % potassium hydroxide, 1 afforded a carboxylic acid (16), mp 92-93°C, $[\alpha]_D^{21}$ +66.3° (c 0.18, EtOH), whose physico-chemical and spectral data were identical with the corresponding data of (+)-(2E,4E)-4,6-dimethylocta-2,4-dienoic acid obtained from sclerotiorin (17) (from Penicillium sclerotiorum)[3a] and 12[5b] by the same manner. Therefore, the absolute configuration at position 13 in 1 was deduced to be (S) like that in 17 and 12. Accordingly, the final stereostructure of TL-1 including its absolute configuration has been deduced to be (8R)-7-deacetyl-$O^8$,8-dihydro-7-epi-sclerotiorin (1).

TL-2 (2) was obtained as an amorphous powder containing twenty percent of 1. Except for the presence of five signals at 5.51 (1H, d, J=9.5 Hz), 6.17 (1H, d, J=15.8 Hz), 6.57 (1H, s), 7.46 (1H, d, J=15.8 Hz), and 7.50 ppm (1H, d, J=2.1 Hz), all other signals in the $^1$H-nmr spectrum of 2 were identical with those of 1. On hydrogenation with 5 % palladium carbon, 2 afforded 3, indicating that 2 was a geometric isomer of 1 on the side chain. All signals in the $^{13}$C-nmr spectrum of 2 were assigned by the aid of two-dimensional $^{13}$C-$^1$H shift correlation ($^{13}$C-$^1$H COSY) nmr spectroscopy. Comparison of all signals in the $^{13}$C-nmr spectrum of 2 with those of 1 indicated that the signals of only two carbons at the positions 10 and 16 are significantly shifted to 133.8 (-8.5) and 20.1 (+7.7) ppm. This fact suggested that the γ-effect[9] which was present between the carbons at the positions 16 and 13 in 1 was absent in 2 and, instead, the γ-effect appeared between the carbons at positions 10 and 13 in 2. Therefore, 2 was estimated not to have (9E,11E)-diene system like 1 but to have (9E,11Z)-diene system in the side chain. Accordingly, the structure of TL-2 has been deduced to be (8R)-(11Z)-7-deacetyl-$O^8$,8-dihydro-7-epi-sclerotiorin (2).

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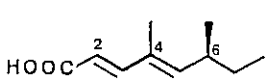


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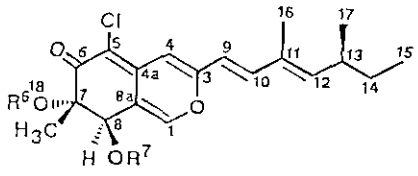
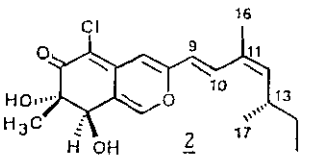


1: $R^6=R^7=H$
4: $R^6=H$, $R^7=Ac$
5: $R^6=R^7=Ac$
14: $R^6=H$, $R^7=(-)$-2-phenylbutyryl
15: $R^6=H$, $R^7=(+)$-2-phenylbutyryl



EXPERIMENTAL

Melting points were measured on a Yanagimoto micro-melting point apparatus (hot stage type) and were uncorrected, the optical rotations with a JASCO DIP-140 digital polarimeter. The uv spectra were recorded with a Hitachi U-3400 spectrophotometer, the ir spectra in KBr tablets with a Hitachi EPI-G3 grating infrared spectrophotometer, the ms and hrms spectra with a Hitachi M-60 or a Hitachi RMU-7M mass spectrometer, the $^1$H-nmr spectra in $CDCl_3$ solutions with a JEOL JNM-GX270 FT-NMR spectrometer at 270 MHz, and the $^{13}$C-nmr spectra in $CDCl_3$ solutions with a JEOL JNM-GX270 FT-NMR spectrometer at 67.8 MHz. Chemical shifts are expressed in δ(ppm) values from tetramethylsilane as an internal standard. The tlc analyses were carried out with silica gel plates (Merck Kieselgel 60 G).

Isolation of TL-1 (1) and -2 (2) Rice medium[10] (16 kg) was evenly divided into 80 cultivation bottles and autoclaved. T. luteus IFM42239 was stationary cultivated on rice medium in 80 bottles at 25°C for 4 weeks. Moldy rice was shaken in ethyl acetate (24 l) at room temperature for 5 h four times to afford the ethyl acetate extract (56.6 g). The ethyl acetate extract was separated by an ordinary-phase silica gel (Wako Gel C-200, 850 g) open column with $C_6H_6$, $CHCl_3$, $CHCl_3$-acetone (9:1 v/v), (3:1 v/v), and acetone. The fraction (10.97 g) eluted with $CHCl_3$-acetone (9:1) was evenly divided into five portions, and each portion was then chromatographed on an ordinary-phase silica gel (Fuji Gel CQ-3, 327g) column with n-hexane-acetone (88:12 v/v), (85:15 v/v), (75:25 v/v), and MeOH (flow rate 2.5 ml/min) two times. The fraction (803 mg) eluted with n-hexane-acetone (85:15) was further chromatographed on a reversed-phase silica gel (ODS) column (20 mm diameter x 250 mm length) with MeOH-$H_2O$ (3:1 v/v, flow rate 8.0 ml/min) two times to afford 2 (34 mg) twenty percent contaminated with 1, amorph. powder, and 1 (330 mg), which was recrystallized from n-hexane-acetone (9:1 v/v) to afford crystalline 1.

1, yellow fine needles, hrms: calcd for $C_{19}H_{23}O_4Cl$, $M^+$: 350.1284, found 350.1291, $M^+$+2: 352.1253, found 352.1252, $^{13}$C-nmr: 11.9(q, C-15), 12.3(q, C-16), 19.0(q, C-18), 20.2(q, C-17), 30.1(t, C-14), 35.0(d, C-13), 72.2(d, C-8), 77.4(s, C-7), 104.7(d, C-4), 107.2(s, C-5), 116.1(d, C-9), 118.9(s, C-8a), 131.9(s, C-11), 142.3(d, C-10), 142.7(s, C-4a), 144.3(d, C-1), 147.8(d, C-12), 159.2(s, C-3), 191.5(s, C-6).

2, yellow amorph., $^1$H-nmr: 0.86(3H, t, J=7.5Hz, $H_3$-15), 1.02(3H, d, J=6.7Hz, $H_3$-17), 1.22(3H, s, $H_3$-18), 1.32, 1.43(each 1H, m, $H_2$-14), 1.91(3H, d, J=1.2Hz, $H_3$-16), 2.64(1H, m, H-13), 3.03(1H, broad s, OH-8), 4.15(1H, broad s, OH-7), 4.67 (1H, broad s, H-8), 5.51 (1H, d, J=9.5Hz, H-12), 6.17(1H, d, J=15.8Hz, H-9), 6.57 (1H, s, H-4), 7.46(1H, d, J=15.8Hz, H-10), 7.50(1H, d, J=2.1Hz, H-1), $^{13}$C-nmr: 12.0(q, C-15), 19.0(q, C-18), 20.1(q, C-16), 21.0(q, C-17), 30.3(t, C-14), 34.1 (d, C-13), 72.1(d, C-8), 77.4(s, C-7), 105.4(d, C-4), 107.5(s, C-5), 118.9(s, C-8a), 119.0(d, C-9), 129.9(s, C-11), 133.8(d, C-10), 142.6(s, C-4a), 144.4(d, C-

1), 145.3(d, C-12), 159.0(s, C-3), 191.6(s, C-6).

Tetrahydro TL-1 (3) A solution of 1 (12.0 mg) in EtOH (2.0 ml) was added to a suspension of activated 5 % Pd-C (17 mg) in EtOH (1.0 ml). The reaction mixture was shaken under a $H_2$ gas for 30 min. The mixture was treated as usual to afford a crude product (11.0 mg), which was purified on an ODS column (20 mm diameter x 250 mm length) with MeOH-$H_2O$ (78:22 v/v, flow rate 10.0 ml/min) to afford 3 (5.2 mg), colorless amorph., ir: 3380, 1635, 1615, 1585, 1090, 840 $cm^{-1}$, ms m/z(%): 356($M^+$+2, 30.1), 354($M^+$, 83.2), $^1$H-nmr: 0.85(3H, d, J=6.0Hz, $H_3$-16 or 17), 0.86 (3H, t, J=7.6Hz, $H_3$-15), 0.93(3H, d, J=6.6Hz, $H_3$-16 or 17), 1.20(3H, s, $H_3$-18), 0.95-1.76(8H, m, $H_2$-10, 12, 14, H-11, 13), 2.52(2H, m, H-9), 2.90(1H, broad s, OH-8), 4.07(1H, broad s, OH-7), 4.65(1H, broad s, H-8), 6.47(1H, s, H-4), 7.47 (1H, d, J=2.0Hz, H-1), $^{13}$C-nmr: 11.2(q, C-15), 18.9(q, C-18), 19.7, 20.0(each q, C-16, 17), 29.2, 31.5, 33.8(each t, C-10 or 12 or 14), 29.8, 31.6(each d, C-11 or 13), 44.2(t, C-9), 72.4(d, C-7), 77.1(s, C-8), 103.7(d, C-4), 106.8(s, C-5), 119.0(s, C-8a), 142.4(s, C-4a), 144.8(d, C-1), 165.9(s, C-3), 191.7(s, C-6).

TL-1 monoacetate (4) Sodium acetate (5.6 mg) was added to a solution of 1 (7.0 mg) in acetic anhydride (0.1 ml). The reaction mixture was stirred at room temperature for 16 h. The mixture was poured into ice-water and extracted with $Et_2O$. The $Et_2O$ layer was treated as usual to give a crude product (6.8 mg), which was purified on an ODS column (10 mm diameter x 250 mm length) with MeOH-$H_2O$ (4:1 v/v, flow rate 2.5 ml/min) to afford 4 (4.5 mg), yellow amorph., ms m/z (%): 394($M^+$+2, 37.2), 392($M^+$, 100), $^1$H-nmr: 0.86(3H, t, J=7.4Hz, $H_3$-15), 1.01(3H, d, J=6.6Hz, $H_3$-17), 1.22-1.56(2H, m, $H_2$-14), 1.30(3H, s, $H_3$-18), 1.84(3H, d, J= 2.0Hz, $H_3$-16), 2.27(3H, s, Ac-8), 5.65(1H, broad d, J=9.9Hz, H-12), 5.94(1H, d, J=2.0Hz, H-8), 6.07(1H, d, J=15.8Hz, H-9), 6.55(1H, s, H-4), 7.03(1H, d, J=15.8 Hz, H-10), 7.06(1H, d, J=2.0Hz, H-1).

TL-1 diacetate (5) To a solution of 1 (4.3 mg) in acetic anhydride (0.5 ml), 1.0 μl of 60 % perchloric acid was added at -70°C. The reaction mixture was stirred at -70°C for 20 min. The mixture was poured into ice-water and extracted with $Et_2O$. The $Et_2O$ layer was treated as usual to give a crude product (4.3 mg), which was purified on an ODS column (10 mm diameter x 250 mm length) with MeOH-$H_2O$ (4:1 v/v, flow rate 2.5 ml/min) to afford 5 (2.6 mg), yellow amorph., ms m/z (%): 436($M^+$+2, 27.6), 434($M^+$, 72.9), $^1$H-nmr: 0.86(3H, t, J=7.3Hz, $H_3$-15), 1.01 (3H, d, J=6.6Hz, $H_3$-17), 1.22-1.54(2H, m, $H_2$-14), 1.40(3H, s, $H_3$-18), 1.83(3H, d, J=1.3Hz, $H_3$-16), 2.05(3H, s, Ac-7), 2.22(3H, s, Ac-8), 2.48(1H, m, H-14), 5.63 (1H, broad d, J=9.9Hz, H-12), 6.06(1H, d, J=15.8Hz, H-9), 6.50(1H, s, H-4), 6.78 (1H, d, J=2.0Hz, H-8), 6.99(1H, d, J=15.8Hz, H-10), 7.06(1H, d, J=2.0Hz, H-1).

Amino Compound (9) To a solution of 1 (27.2 mg) in MeOH (0.5 ml), 0.2 ml of 70 % $MeNH_2$ was added. The reaction mixture was stirred at room temperature for 10 min. The mixture was poured into cold water and lyophilized to give a crude mixture (27.1 mg), which was separated on an ODS column (20 mm diameter x 250 mm length) with MeOH-$H_2O$ (3:1 v/v, flow rate 10 ml/min) to afford 9 (12.3 mg) and the starting compound 1 (12.0 mg). To a solution of the recovered 1 (12.0 mg) in MeOH (0.5 ml), 0.2 ml of 70 % $MeNH_2$ was added. The reaction mixture was stirred at room temperature for 20 min. The mixture was treated in the same way as for the preceding treatment with $MeNH_2$ to afford 9 (7.0 mg) (19.3 mg of 9 was totally obtained from 27.2 mg of 1).

9, red amorph., uv(MeOH): 245, 317, 419 nm, ir: 3320, 1565, 1525, 1060 $cm^{-1}$, ms m/z(%): 365($M^+$+2, 24.2), 363($M^+$, 67.2), $^1$H-nmr: 0.88(3H, t, J=7.3Hz, $H_3$-15), 1.01 (3H, d, J=6.6Hz, $H_3$-17), 1.24-1.52(2H, m, $H_2$-14), 1.22(3H, s, $H_3$-18), 1.88(3H, s, $H_3$-16), 2.45(1H, m, H-13), 3.74(3H, s, $CH_3$-N), 4.71(1H, broad s, H-8), 5.72(1H,

broad d, J=9.6Hz, H-12), 6.23(1H, d, J=15.5Hz, H-9), 7.00(1H, d, J=15.5Hz, H-10), 7.01(1H, s, H-4), 7.24(1H, s, H-1), $^{13}$C-nmr: 12.0(q, C-15), 12.6(q, C-16), 18.6(q, C-18), 20.3(q, C-17), 30.1(t, C-14), 35.0(d, C-13), 41.7(q, $CH_3$-N), 73.7 (d, C-8), 76.3(s, C-7), 97.4(s, C-5), 107.5(d, C-4), 115.3(d, C-9), 123.5(s, C-8a), 131.6(s, C-11), 133.9(d, C-1), 144.4(d, C-10), 147.1(s, C-4a), 147.5(d, C-12), 148.1(s, C-3), 185.9(s, C-6).

Conversion of tetrahydro TL-1 (3) to 7-epi-tetrahydrosclerotiorin (11) Pyridinium chlorochromate[4] (14.8 mg) and powdered molecular sieves (Wako 3Å, 13.7 mg) were added to a solution of 3 (4.9 mg) in $CH_2Cl_2$ (0.1 ml). The reaction mixture was stirred at room temperature for 3 h. The mixture was poured into ice-water and extracted with $CH_2Cl_2$. The $CH_2Cl_2$ layer was treated as usual to give a crude product (4.7 mg), which was purified on an ODS column (10 mm diameter x 250 mm length) with MeOH-$H_2O$ (4:1 v/v, flow rate 2.5 ml/min) to afford compound 10 (0.3 mg), yellow amorph., $^1$H-nmr: 0.84(3H, d, J=6.9Hz, $H_3$-16 or 17), 0.87(3H, t, J=6.6 Hz, $H_3$-15), 0.94(3H, d, J=6.6Hz, $H_3$-16 or 17), 0.87-1.88(8H, m), 1.58(3H, s, $H_3$-18), 2.52(2H, m, $H_2$-9), 6.58(1H, s, H-4), 7.92(1H, s, H-1).

A solution of 10 (0.3 mg) in acetic anhydride (25 µl) and pyridine (25 µl) was left to stand at room temperature for 3 h. The reaction mixture was decomposed by an addition of cold water and extracted with $CH_2Cl_2$. The $CH_2Cl_2$ layer was washed with water and evaporated in vacuo to afford compound 11 (0.3 mg), which was identical with 7-epi-tetrahydrosclerotiorin in terms of $^1$H-nmr, ms, and tlc behavior (solvent: n-hexane-acetone (2:1 v/v)). Compound 11, yellow amorph., ms m/z(%): 396($M^+$+2, 6.3), 394($M^+$, 19.0), 354($M^+$+2-$CH_2CO$, 32.0), 352($M^+$-$CH_2CO$, 95.2), $^1$H-nmr: 0.85(3H, t, J=6.3Hz, $H_3$-15), 0.86(3H, d, J=6.6Hz, $H_3$-16 or 17), 0.93(3H, d, J=6.3Hz, $H_3$-16 or 17), 1.56(3H, s, $H_3$-18), 0.95-1.76(8H, m, $H_2$-10, 12, 14, H-11, 13), 2.18(3H, s, $H_3$-18), 2.50(2H, m, $H_2$-9), 6.60(1H, s, H-4), 7.92 (1H, s, H-1).

Conversion of 7-epi-tetrahydrosclerotiorin (11) from 7-epi-sclerotiorin (12) A solution of authentic 7-epi-sclerotiorin (12) (3.0 mg) in AcOEt (0.2 ml) was added to the suspension of activated 5 % Pd-C (3.0 mg) in AcOEt (0.1 ml). The reaction mixture was shaken under a $H_2$ gas flow for 10 min. The product mixture was treated as usual to afford 11 (3.0 mg), yellow amorph..

Treatment of TL-1 (1) with acetone in the presence of perchloric acid A solution of TL-1 (1) (2.0 mg) and 60 % perchloric acid (10 µl) in acetone (0.2 ml) was stirred at room temperature for 28 h. To the reaction mixture, $Na_2CO_3$ (20 mg) was added, and the mixture was stirred at room temperature for 3 min. The reaction mixture was poured into ice-water and extracted with $Et_2O$. The $Et_2O$ layer was treated as usual to afford an amorph. powder (2.5 mg), which was identical with the starting compound 1 in terms of tlc behavior (solvent: n-hexane-acetone (2:1 v/v), benzene-acetone (5:1 v/v), and $CHCl_3$).

Horeau method[2, 8] A solution of 1 (98.4 mg) and (±)-2-phenylbutyric anhydride (317.3 mg) in pyridine (1.65 ml) was stirred in the dark at room temperature for 17 h. Water (0.25 ml) was added to the reaction mixture to hydrolyze the excess of anhydride and the mixture was left to stand at room temperature for 1 h. Benzene (3.5 ml) was added to the mixture to dissolve esters. The mixture was titrated against 0.1 N NaOH solution (the factor: 1.108) with phenolphthalein as an indicator (16.1 ml of 0.1 N NaOH solution was required). After titration, the aqueous phase was separated from the organic phase. The aqueous phase was washed with $CHCl_3$ to remove traces of esters, acidified with 1 N HCl (3 ml), and extracted with benzene (5 ml) two times. The benzene layer was dried over $Na_2SO_4$ and filtered. After adjustment of the volume of the filtrate to 10.0 ml with

benzene, optical rotation of the filtrate was measured (specific rotation of the filtrate: $[\alpha]_D^{23}$ +8.4° (c 1.21, benzene)). Therefore, the esterification yield was 93.6%, and the optical yield was 24.5 %. The organic phase was washed with water, dried over $Na_2SO_4$, and evaporated in vacuo to afford a crude mixture of esters (160mg), which was separated on an ordinary-phase silica gel column (20 mm diameter x 250 mm length) with n-hexane-AcOEt (5:1 v/v, flow rate 4.0 ml/min) and on an ODS column(20mm diameter x 250 mm length) with MeOH-$H_2O$ (10:1 v/v, flow rate 8.0 ml/min) to afford esters 14 (57.7 mg) and 15 (8.0 mg).

14, yellow amorph., ms m/z(%): 498($M^+$+2, 38.1), 496($M^+$, 100), $^1$H-nmr: 0.85(3H, t, J=7.5Hz, $H_3$-15), 1.00(3H, d, J=6.4Hz, $H_3$-17), 1.02(3H, t, J=7.3Hz, $H_3$-4'), 1.16 (3H, s, $H_3$-18), 1.25-1.47(2H, m, $H_2$-14), 1.82(3H, d, J=1.2Hz, H-9), 1.95, 2.26 (each 1H, m, $H_2$-3'), 2.47(1H, m, H-13), 3.65(1H, t, J=7.8Hz, H-2'), 5.65(1H, broad d, J=9.5Hz, H-12), 5.92(1H, d, J=2.0Hz, OH-7), 6.03(1H, d, J=15.8Hz, H-9), 6.50(1H, s, H-4), 6.66(1H, d, J=2.0Hz, H-8), 6.98(1H, d, J=15.8Hz, H-10), 7.28-7.41(6H, m, H-1, Ph) (2', 3', 4' and Ph: (-)-2-phenylbutyryl part).

15, yellow amorph., ms m/z(%): 498($M^+$+2, 24.5), 496($M^+$, 61.1), $^1$H-nmr: 0.86(3H, t, J=7.3Hz, $H_3$-15), 0.94(3H, t, J=7.3Hz, $H_3$-4'), 1.00(3H, d, J=6.7Hz, $H_3$-17), 1.25(3H, s, $H_3$-18), 1.28-1.51(2H, m, $H_2$-14), 1.81(3H, d, J=1.2Hz, $H_3$-16), 1.89, 2.21(each 1H, m, $H_2$-3'), 2.48(1H, m, H-13), 3.65(1H, t, J=7.6Hz, H-2'), 5.63(1H, broad d, J=9.5Hz, H-12), 5.92(1H, d, J=2.0Hz, OH-7), 5.99(1H, d, J=15.8Hz, H-9), 6.22(1H, d, J=2.0Hz, H-8), 6.47(1H, s, H-4), 6.92(1H, d, J=15.8Hz, H-10), 7.29-7.41(6H, m, H-1, Ph) (2', 3', 4' and Ph: (+)-2-phenylbutyryl part).

(+)-(2E,4E)-4,6-Dimethylocta-2,4-dienoic acid (16) Compound 1 (180 mg) was slowly dissolved in warm 5 % KOH solution (10 ml), and the resulting solution was refluxed for 5 min. The reaction mixture was extracted with $CHCl_3$ two times to remove $CHCl_3$-soluble substances. The aqueous layer was acidified with 2 N $H_2SO_4$ to pH 3, and extracted with petroleum ether five times. The petroleum ether layer was evaporated in vacuo to give a crude acidic substance (48.5 mg), which was recrystallized from MeCN to afford 16 (13.8 mg), colorless crystals, mp 92-93°C (lit.[5b] 91-92°C), $[\alpha]_D^{21}$ +66.3° (c 0.18, EtOH) (lit.[5b] +66.3°), uv(EtOH) nm(log ε): 260(4.38) (lit.[5b] 261(4.44)).

Tetrahydro TL-1 (3) from TL-2 (2) A solution of 2 twenty percent contaminated with 1 (29 mg) in EtOH (3 ml) was added to a suspension of activated 5 % Pd-C (29 mg) in EtOH (3 ml), and the resulting suspension was treated in the same way as for hydrogenation of 1 to give a crude product (28.9 mg), which was purified on an ODS column (20 mm diameter x 250 mm length) with MeOH-$H_2O$ (10:1 v/v, flow rate 8.0 ml/min) to afford a pure product (14.3 mg), colorless amorph.. This product was identical with 3 in terms of $^1$H-nmr, $^{13}$C-nmr, and ms spectra.

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