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Fungal Metabolites. II. Structural Elucidation of Minor Metabolites, Valinotricin, Cyclonerodiol Oxide, and Epicyclonerodiol Oxide, from *Trichoderma polysporum*

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A new minor metabolite, valinotricin (**1**), along with cyclonerodiol oxide (**9**) and epicyclonerodiol oxide (**10**) was isolated from *Trichoderma polysporum*. The structure of valinotricin was elucidated as **1** by chemical and spectral studies, and then confirmed by synthesis. Chemical and spectroscopic studies of cyclonerodiol oxide and epicyclonerodiol oxide led to the structures **9** and **10**, respectively.

Keywords——*Trichoderma polysporum*; trichopolyn; valinotricin; cyclonerodiol; cyclonerodiol oxide; epicyclonerodiol oxide; valinol

In our previous paper[1] we reported the isolation of peptide antibiotics, hypelcins A and B, from *Hypocrea peltata* (JUNGH) SACC., which causes damage to cultivated *Lentinus edodes* (BERK.) SING. (Japanese name: Shiitake). *Trichoderma polysporum* (LINK ex PERS.) RIFAI (strain TMI 60146) also has a strong inhibitory activity against *L. edodes* and we showed that the active substances were trichopolyns I and II.[2] *Trichoderma* species produce many kind of metabolites such as sesquiterpenes[3] and peptide antibiotics.[4] In the course of a continuing search for new fungal metabolites, we found that a culture filtrate of *T. polysporum* also contains an ester, as well as sesquiterpenes and peptides. This paper deals with the isolation and structural elucidation of several *T. polysporum* metabolites, a new ester, valinotricin (**1**), and sesquiterpenes, cyclonerodiol (**8**), cyclonerodiol oxide (**9**) and epicyclonerodiol oxide (**10**).

The method of extraction is shown in Chart 1. Sephadex LH-20 column chromatography of the neutral fraction gave fraction (fr.) A, fr. B, and fr. C. Fraction B was chromatographed on a silica gel column to give trichopolyns.[2] Fraction C was fractionated by repeated silica gel chromatography and preparative thin-layer chromatography (TLC) to afford an ester, valinotricin (**1**) and sesquiterpenes, cyclonerodiol (**8**), which has also been isolated from *Trichothecium*,[5] *Gibberella*,[6] and *Fusarium*[7] species, cyclonerodiol oxide (**9**) and epicyclonerodiol oxide (**10**), and a peptide, TP-I.

Valinotricin (**1**) was isolated as needles, mp 128—129.5 °C, $[\alpha]_D^{19}$ −65.7° ($c = 1.0$, MeOH). The infrared (IR) spectrum showed strong absorption at 1745 (ester), 1690 and 1550 cm^{-1} (amide), while the proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed two isopropyl groups [δ 0.96—1.04 (3H × 4), 1.84, 2.30 (each 1H, m)], two formyl groups (δ 8.55 and 8.59) and two amide protons (δ 8.80 and 9.40). Amino acid analysis of the complete hydrolysate (6 N HCl, 110 °C, 24 h) of valinotricin showed the presence of valine. The mass spectrum (MS) (*m/z* 258, M^+) and elemental analysis showed the molecular formula to be $C_{12}H_{22}N_2O_4$. These results lead to the ester (**1**) or amide (**2**) structure for valinotricin.

The MS of valinotricin gave fragmentation peaks at *m/z* 128, 114, and 100, indicating the ester structure, **1**. Selective hydrolysis of valinotricin using 0.2 N NaOH–MeOH afforded *N*-

T. polysporum culture broth (6200 l)

mycelium

culture filtrate

Amberlite XAD-2 column
eluted with MeOH and acetone
concd. *in vacuo* and partitioned
between EtOAc and H_2O

EtOAc layer

aq. layer

washed with aq. $NaHCO_3$
and dil. HCl

neutral fraction

Sephadex LH-20 column
eluted with MeOH

A (14.7 g)

B (68.7 g)

C (74.1 g)

SiO_2 column

trichopolyn (36.6 g)

C_1 (9.94 g)

C_2 (2.26 g)

C_3 (12.43 g)

C_4 (4.04 g)

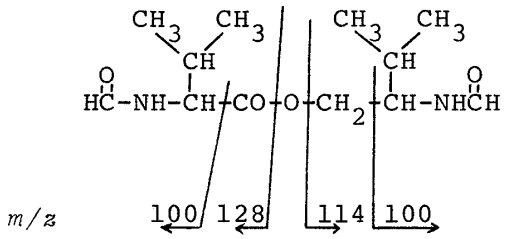
8 (3.996 g)
10 (33 mg)

9 (42 mg)

1 (708 mg)

TP-I (22 mg)

Chart 1



1

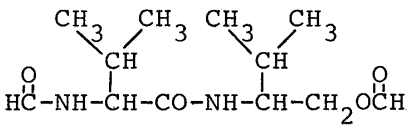


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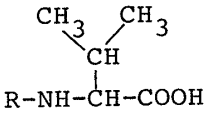
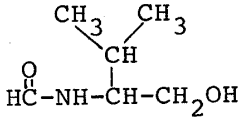
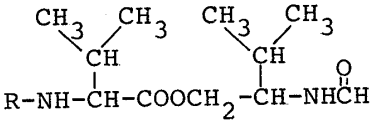
Chart 2







3 : R= 

4 : R= 

5

6 : R= 

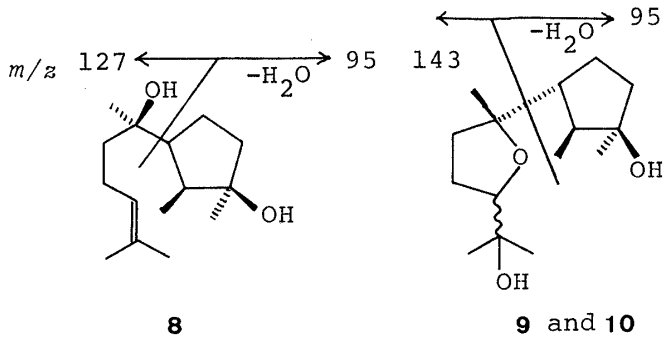
7 : R= H

Chart 3

formyl-L-valine (**3**), mp 153—155 °C, $[\alpha]_D^{20}$ +7.6° (c=0.66, MeOH) [lit.,[8] mp 148—149 °C, $[\alpha]_D^{25}$ +13° (EtOH)] and *N*-formyl-L-valinol (**5**), oil, $[\alpha]_D^{21}$ −27.9° (c=0.43, MeOH). The structures of these products were confirmed by synthesis. These results lead to the structure **1** for valinotricin.

The structure **1** was confirmed by synthesis as follows. *N*-Formyl-L-valinol (**5**) was prepared by formylation[9] of L-valinol.[10] *N*-Formyl-L-valine (**3**)[8] and *N*-formyl-L-valinol (**5**) were coupled using dicyclohexylcarbodiimide and 4-aminopyridine[11] to give valinotricin (**1**) in a low yield (9%). In order to increase the yield of the coupling reaction, benzyloxycarbonyl-L-valine (**4**) and **5** were treated with 1-ethoxycarbonyl-2-dihydroquinoline (EEDQ) and 4-dimethylaminopyridine to give **6** (58%). Hydrogenolysis of the ester (**6**) gave an unstable compound **7**, which was immediately formylated with HCOOH–Ac_2O to give valinotricin (**1**) (70% from **6**). This is the second reported example of a natural product containing valinol. The first peptide antibiotic containing valinol, trichotoxin A-40, was isolated from *Trichoderma viride*.[12]

Cyclonerodiol oxide (**9**) and epicyclonerodiol oxide (**10**) had the same molecular formula, $C_{15}H_{28}O_3$, on MS (each m/z 257, $M^+ + 1$). Similarities in the spectral data [IR, ^{1}H-NMR, and ^{13}C-NMR] indicated a close structural relationship between the two compounds, **9** and **10**. Spectra of cyclonerodiol oxide (**9**) indicated the presence of a hydroxy group (IR: 3600 and 3440 cm^{-1}), five methyl groups consisting of a secondary methyl (^{1}H-NMR: δ 1.04, d, J= 7 Hz) group and four tertiary methyl (^{1}H-NMR: δ 1.13, 1.15, 1.21, and 1.25, each s) groups, and a methine group bearing an oxygen atom (^{1}H-NMR: δ 3.76). The nature of the carbon skeleton of cyclonerodiol oxide (**9**) was revealed by its MS, which was similar to that of cyclonerodiol (**8**) and showed a characteristic peak due to the dimethylcyclopentanol C_7



8 **9** and **10**

Chart 4

fragment at m/z 95 ($C_7H_{13}O-H_2O$). The peak at m/z 143 showed that cyclonerodiol oxide has a C_8 fragment which has one more oxygen atom than that of cyclonerodiol (m/z 127). This was supported by the fact that treatment of cyclonerodiol with *m*-chloroperbenzoic acid gave cyclonerodiol oxide and epicyclonerodiol oxide. Oxidation of cyclonerodiol oxide and epicyclonerodiol oxide gave the γ-lactone (**11**), mp 85—87 °C, which was identical with the ozonolysis product of cyclonerodiol (**8**).[5,7] These results established that cyclonerodiol oxide and epicyclonerodiol oxide have the structures **9** and **10**, respectively, except for the stereochemistry at C-10. The physical data for the oxides (**9** and **10**) are similar to those for a minor metabolite, $C_{15}H_{28}O_3$, isolated from *Trichothecium* species by Nozoe,[5] who proposed the same planar structure as the oxide (**9** and **10**) for it.

In order to determine the configuration at C-10 of the oxides (**9** and **10**), their ^{13}C-NMR spectra were compared with those of ocotillon-type triterpenes (**12**—**17**).[13] The signal of C-24 in the *cis* form of the triterpenes was shifted to higher field than in the *trans* form. The same shift was observed at the C-10 signal in the sequiterpene oxides, corresponding to C-24 in the

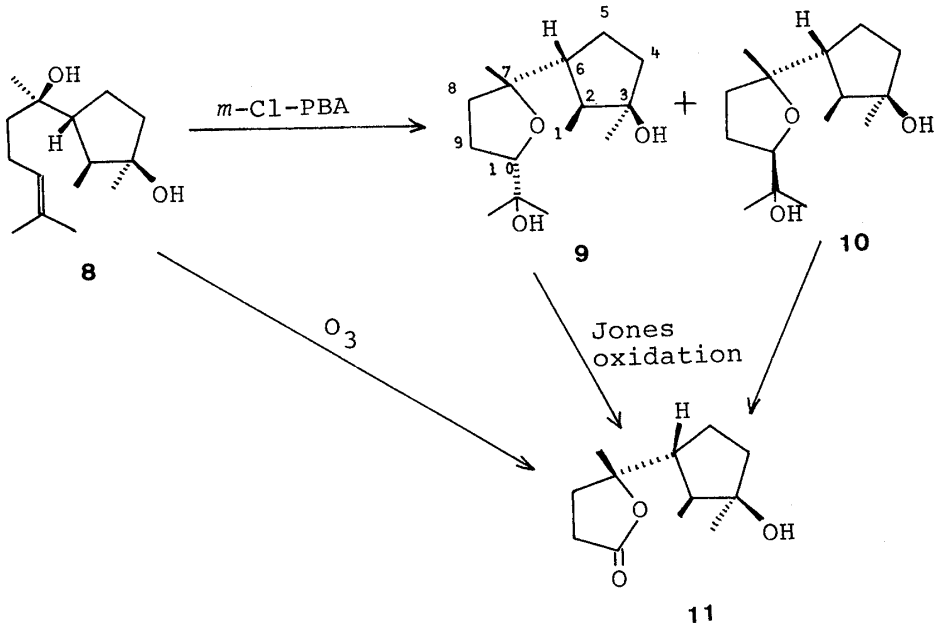


Chart 5

triterpenes (Fig. 1). Therefore, cyclonerodiol oxide (**9**) has a *cis* form and epicyclonerodiol oxide (**10**) has a *trans* form in the tetrahydrofuran ring. Cyclonerodiol oxide and epicyclonerodiol oxide thus have the structures **9** and **10**, respectively.

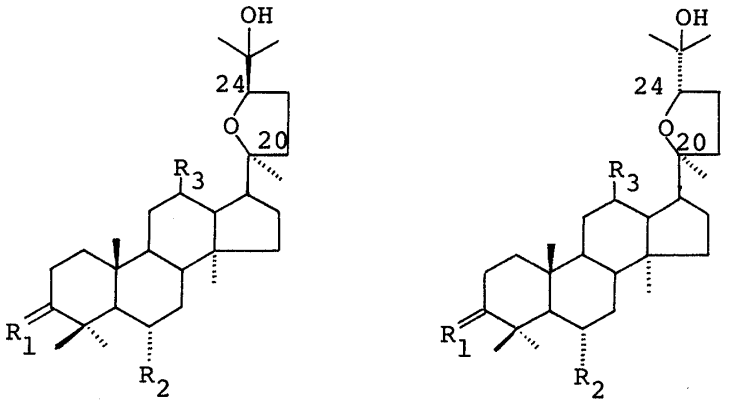
Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a Union Giken PM-201 automatic digital polarimeter. IR spectra were measured on a Hitachi IR-215 machine. ^{13}C-NMR and ^{1}H-NMR spectra were obtained on JEOL JNM FX-200, JEOL JNM PS-100 and Hitachi R-42FT instruments, respectively. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. MS were taken with a JEOL D-300 machine. Amino acid autoanalysis was done with a JEOL JIC-6AH machine. TLC and preparative TLC were performed on precoated silica gel plates 0.25 and 0.5 mm in thickness (Kieselgel 60, Merck), respectively. Column chromatography was performed on Kieselgel 60 (70—230 mesh, Merck), Sephadex LH-20 (Pharmacia), and Amberlite XAD-2 (Organo).

Microorganisms and Cultural Conditions——A preculture (3—5 l) of *Trichoderma polysporum* (LINK ex PERS.) Rifai TMI 60146 grown on the medium described in a previous communication[2] was used as the inoculum. Large-scale cultivation was carried out as follows. Medium: glucose (17.5 kg), ammonium tartrate (2.8 kg), KH_2PO_4 (1.4 kg), $MgSO_4 \cdot 7H_2O$ (700 g), $FeCl_3$ (70 g), H_2O (700 l), pH adjusted to 3.5 with HCl. Jar fermenter: 1000 l. Mechanical rotation: 67 rpm. Aeration: 200 l/min. Temperature: 28 °C. Incubation time: 4 d.

Extraction and Isolation of *Trichoderma* Metabolites——The culture filtrate (700 l) was passed through an Amberlite XAD-2 column (20 kg). The column was washed with H_2O (10 l) and then eluted with MeOH (20 l) and acetone (10 l). The MeOH and acetone eluates were combined and concentrated *in vacuo*. The residue was partitioned between H_2O (2 l) and EtOAc (10 l). The EtOAc layer was washed with aqueous $NaHCO_3$, dil HCl, and brine, then dried over $MgSO_4$ and evaporated *in vacuo* to give a neutral fraction (total yield: 170.2 g). A part (17.1 g) of the neutral fraction was chromatographed on a Sephadex LH-20 column (5 × 45 cm, MeOH) to afford three fractions: fr. A (0.94 g), fr. B (6.99 g), and fr. C (9.36 g). Fraction B (44.63 g) was chromatographed on a silica gel column (220 g, CH_2Cl_2 and acetone). The eluates with CH_2Cl_2–acetone (9 : 1–1 : 1, v/v) were combined and concentrated to give a residue (31.65 g). The residue was crystallized from CH_2Cl_2–hexane to give trichopolyn, 28.25 g, as needles. Fraction C (74.0 g) was chromatographed on a silica gel column (2 kg, CH_2Cl_2, acetone, and MeOH). The eluates which showed the same *Rf* values on TLC were combined and concentrated to afford fractions C_1 (9.94 g, CH_2Cl_2–acetone = 7 : 3), C_2 (2.26 g, CH_2Cl_2–acetone = 7 : 3), C_3 (12.43 g, acetone), and C_4 (4.04 g, acetone–MeOH = 1 : 1).

Valinotricin (1)——Fraction C_3 (12.4 g) was subjected to silica gel (380 g) column chromatography ($CHCl_3$–acetone = 2 : 1) to give valinotricin (708 mg) as needles (from EtOAc), mp 128—129.5 °C, $[\alpha]_D^{19}$ −65.7° (c = 1.1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3260 (–NH–), 1745 (ester), 1690 and 1550 (amide). ^{1}H-NMR (C_5D_5N) δ: 0.96—1.04 (12H, Me × 4), 1.84, 2.30 (each 1H, m), 4.38 (3H, m), 4.88 (1H), 8.55, 8.59 (each 1H, s), 8.80 (1H, m), 9.40 (1H, br d, J =



12: R_1=  , R_2=R_3= OH

13: R_1=  , R_2= H, R_3= OH

14: R_1= O, R_2=R_3= H

15: R_1=  , R_2=R_3= OH

16: R_1=  , R_2= H, R_3= OH

17: R_1= O, R_2=R_3= H

Chart 6

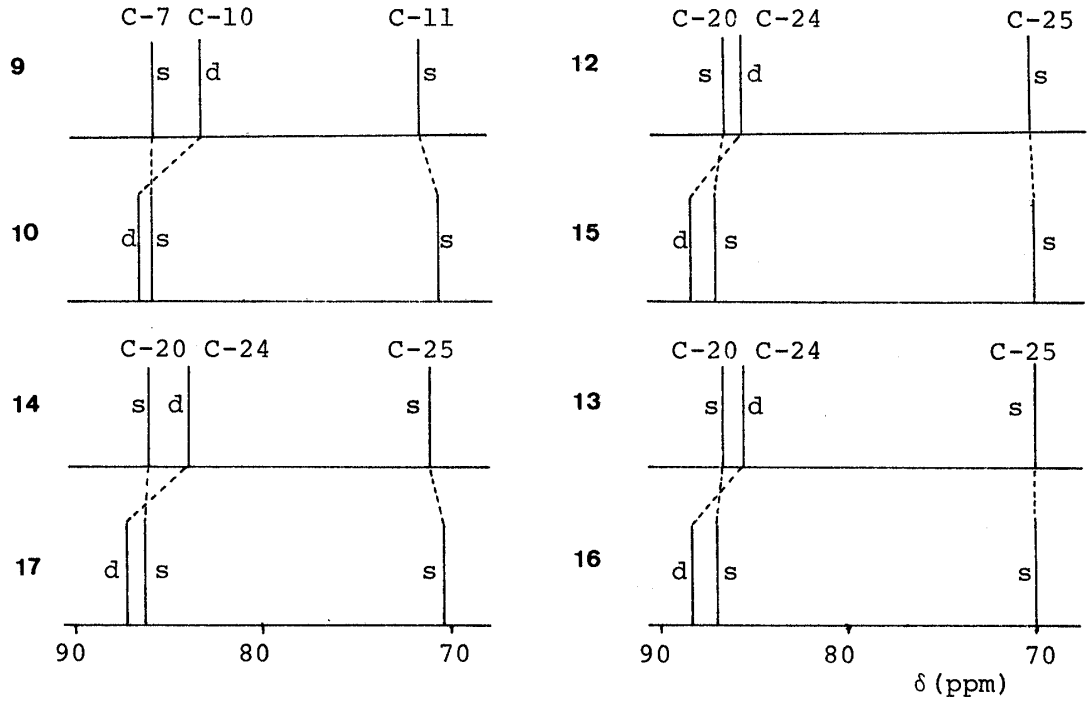


Fig. 1. Comparison of the ^{13}C-NMR Chemical Shifts of C-7, C-10, and C-11 in the Oxides **9** and **10** and the Corresponding Carbons, C-20, C-24, and C-25, in Ocotillon-Type Triterpenes (**12**—**17**)

8 Hz). ^{1}H-NMR (CD_3OD) δ: 0.93 (3H × 2, d, J=7 Hz), 0.97 (3H × 2, d, J=7 Hz), 1.82, 2.15 (each 1H, m), 4.02 (1H, m), 4.16 (2H, d, J=6 Hz), 4.40 (1H, d, J=6 Hz), 8.10 (2H, s). ^{13}C-NMR (C_5D_5N) δ: 18.0 (q), 18.6 (q), 19.4 (q), 19.6 (q), 29.7 (d), 31.0 (d), 52.3 (d), 56.8 (d), 65.5 (t), 161.8 (d), 161.9 (d), 172.1 (s). MS *m/z*: 258 (M^+), 215, 128, 114. *Anal.* Calcd for $C_{12}H_{22}N_2O_4$: C, 55.79; H, 8.58; N, 10.85. Found: C, 55.81; H, 8.75; N, 10.88.

Cyclonerodiol (8)——Fraction C_1 (9.9 g) was rechromatographed on a silica gel (300 g) column. The eluate

(CH_2Cl_2–acetone = 9 : 1) was evaporated to give a residue, which was repeatedly distilled using a glass tube oven, 162 °C, 2 mmHg, to give cyclonerodiol (3.99 g) as an oil, $[\alpha]_D^{27}$ −20.3° (c = 1.82, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3630—3490 (OH), 1710, 1670, 1445, 1381, 990, 920, 890. The properties and spectral data of this compound are in good agreement with those reported for cyclonerodiol.[7]

Cyclonerodiol Oxide (9)——Fraction C_2 (0.95 g) was purified by repeated column chromatography on silica gel ($CHCl_3$–acetone = 9 : 1 and ether) to give cyclonerodiol oxide (**9**), 42 mg, as needles (from petroleum ether), mp 47—50 °C, $[\alpha]_D^{22}$ −19.6° (c = 0.38, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3440, 1460, 1375, 1085, 920. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, d, J = 7 Hz), 1.13 (3H, s), 1.15 (3H, s), 1.21 (3H, s), 1.25 (3H, s), 3.76 (1H, t, J = 7 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.0 (q), 23.4 (q), 24.4 (q), 25.2 (t), 26.0 (q), 26.2 (t), 27.3 (q), 35.2 (t), 40.5 (t), 45.1 (d), 54.0 (d), 71.6 (s), 81.1 (s), 83.4 (d), 85.7 (s). Chemical ionization (CI) MS (isobutane) m/z: 257 (M^+ + 1), 239, 221, 143. *Anal.* High resolution CIMS Calcd for $C_{15}H_{29}O_3$ (M + H): 257.2117. Found: 257.2099.

Epicyclonerodiol Oxide (10)——Further fractionation of crude cyclonerodiol (205 mg) was carried out by preparative silica gel TLC ($CHCl_3$–MeOH = 95 : 5) to give epicyclonerodiol oxide as an oil (33 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580—3430 (OH), 1450, 1365, 1050, 905. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, d, J = 7 Hz), 1.11 (3H, s), 1.17 (3H, s), 1.21 (3H, s), 1.26 (3H, s), 3.7 (1H, m). ^{13}C-NMR ($CDCl_3$) δ: 13.8 (q), 24.1 (q), 25.4 (t), 26.1 (q), 26.3 (t), 26.9 (q), 27.8 (q), 34.8 (t), 40.4 (t), 45.1 (d), 54.3 (d), 70.3 (s), 81.2 (s), 85.9 (s), 86.7 (d), MS m/z: 256 (M^+), 197, 179, 143, 113, 95, 59. *Anal.* High resolution CIMS Calcd for $C_{15}H_{29}O_3$ (M + H): 257.2117. Found: 257.2094.

TP-I——Fraction C_4 (4.04 g) was chromatographed on a column (SiO_2 160 g, CH_2Cl_2–acetone = 7 : 3) to afford TP-I, 22 mg, as needles from CH_2Cl_2, mp 185—187 °C, IR ν_{max}^{KBr} cm^{-1}: 3260, 1640, 1550. ^{1}H-NMR (C_5D_5N) δ: 1.02 (3H, d, J = 7 Hz), 1.08 (6H, d, J = 7 Hz), 1.11 (3H, d, J = 7 Hz), 2.3—5.0 (6H), 6.34 (1H, t, J = 7 Hz), 8.70 (1H, d, J = 0.7 Hz), 8.76 (1H, d, J = 8 Hz), 9.26 (1H, d, J = 8 Hz).

Hydrolysis of the Ester 1 with Base——The ester **1** (169.2 mg) was stirred with 0.2 N NaOH–EtOH (10 ml) at room temperature for 12 h. The reaction mixture was neutralized with 1 N HCl and evaporated *in vacuo*. The residue was partitioned between H_2O and EtOAc. The EtOAc layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to give an oil, *N*-formyl-L-valinol (**5**), 48 mg, $[\alpha]_D^{21}$ −27.9° (c = 0.43, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600—3100 (–NH–), 1640 and 1505 (amide). ^{1}H-NMR ($CDCl_3$) δ: 0.93, 0.96 (each 3H, d, J = 7 Hz), 1.84 (1H, m). This compound was identical with synthetic *N*-formyl-L-valinol (IR and ^{1}H-NMR spectra). The aqueous layer was acidified with HCl, and extracted with EtOAc. The EtOAc layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo* to give crude crystals, which were recrystallized from water to give *N*-formyl-L-valine (**3**), 40.8 mg mp 153—155 °C, $[\alpha]_D^{20}$ +11.8° (c = 0.93, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3295—3080 (–NH–), 1695 (–COOH), 1600 and 1560 (amide). ^{1}H-NMR ($CDCl_3$–C_5D_5N = 1 : 1) δ: 1.07, 1.10 (each 3H, d, J = 7 Hz), 2.40 (1H, m), 4.89 (1H, dd, J = 10 and 5 Hz), 8.45 (1H, s), 8.76 (1H, d, J = 10 Hz), 11.16 (1H). MS m/z: 146 (M^+ + 1), 127 ($M^+ - H_2O$). *Anal.* Calcd for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.65. Found: C, 49.82; H, 7.95; N, 9.51. This compound was identical with synthetic *N*-formyl-L-valine (IR and ^{1}H-NMR spectra).

Hydrolysis of the Ester 1 with Acid——The ester **1** (7.1 mg) was heated in a sealed tube with 6 N HCl (2 ml) at 110 °C for 48 h. The hydrolysate was evaporated *in vacuo* to give a residue. The residue was analyzed with the amino acid autoanalyzer; the recovery of valine was 85%.

***N*-Formyl-L-valinol (5)**——Acetic anhydride (7 ml) was added to a solution of L-valinol (1.03 g) in formic acid (21 ml) at 0 °C for 30 min. After being stirred at 0 °C for 30 min and then at room temperature for 5 h, the reaction mixture was treated with water and evaporated *in vacuo* to give an oil, which was refluxed with 0.2 N NaOH–EtOH (50 ml) for 2 h. The reaction mixture was concentrated *in vacuo*, and extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and evaporated *in vacuo* to give *N*-formyl-L-valinol (1.13 g, yield 86%), oil, $[\alpha]_D^{26}$ −19.4° (c = 0.93, MeOH).

Synthesis of Valinotricin (1) from *N*-Formyl-L-valinol (5)——A solution of dicyclohexylcarbodiimide (157 mg) in CH_2Cl_2 (4 ml) was added to a solution of **3** (111 mg), **5** (101 mg) and 4-aminopyridine (72 mg) in CH_2Cl_2 (12 ml). After being stirred at room temperature for 22 h, the reaction mixture was filtered. The filtrate was evaporated *in vacuo* to give a residue. A solution of the residue in EtOAc (30 ml) was filtered. The filtrate was washed with 0.1 N HCl, H_2O and 5% $NaHCO_3$, then dried over $MgSO_4$, and evaporated *in vacuo* to give a residue (111 mg). The residue was purified by silica gel column chromatography to give valinotricin (**1**) (17.8 mg, yield 9%), which was identical with an authentic sample (IR, NMR, mixed mp).

Synthesis of Valinotricin from Benzyloxycarbonyl-L-valine (4)——Benzyloxycarbonyl-L-valine (193 mg), EEDQ (188 mg), and 4-dimethylaminopyridine (93 mg) were successively added to a solution of **5** (102 mg) in anhydrous THF–toluene (1 : 1, 4 ml). After being stirred at room temperature for 20.5 h, the reaction mixture was evaporated *in vacuo* and the residue was extracted with EtOAc. The EtOAc layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo* to give an oil (209 mg). Silica gel column chromatography of the oil gave **6** (162 mg, yield 58%) as needles (from Et_2O), mp 79.5—82 °C, $[\alpha]_D^{26}$ −40.3° (c = 0.94, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1740, 1720, 1690, 1505. ^{1}H-NMR ($CDCl_3$) δ: 0.88, 0.95 (each 3H, d, J = 7 Hz), 5.08 (2H, s). MS m/z: 364 (M^+). *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69. found: C, 62.42; H, 7.71; N, 7.49. A solution of **6** (145.6 mg) in MeOH–AcOH (20 : 1, 7 ml) was hydrogenated over Pd-black at room temperature. After removal of the catalyst by filtration, the filtrate was concentrated to give **7** as an oil. Acetic anhydride (0.5 ml) was added to a solution of **7** in formic acid (1.6 ml) at 0 °C.

After being stirred at 0 °C, for 30 min and then at room temperature for 6 h, the reaction mixture was evaporated *in vacuo* to give crystals (104.2 mg). Recrystallization from EtOAc–hexane gave valinotricin (**1**) (72.2 mg, yield 70%), as needles, mp 128—129 °C, $[\alpha]_D^{21}$ −65.0° ($c=0.83$, MeOH). *Anal.* Calcd for $C_{12}H_{22}N_2O_4$: C, 55.79; H, 8.58; N, 10.85. Found: C, 56.04; H, 8.79; N, 10.71. This product was identical with natural valinotricin (**1**) on direct comparison (TLC, IR, MS and ^{1}H-NMR).

Ozonolysis of Cyclonerodiol (8)——Ozone was passed through a solution of cyclonerodiol (**8**) (101 mg) in EtOAc (12 ml) at −40 °C for 15 min. After removal of the excess ozone by flushing with nitrogen, the reaction mixture was evaporated *in vacuo*, then water (12 ml) and zinc dust (80 mg) were added to the residue. The reaction mixture was stirred overnight at room temperature and extracted with $CHCl_3$ (10 ml × 3). The $CHCl_3$ layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a residue (38 mg), which was purified by preparative TLC to give the γ-lactone (**11**) (16 mg), mp 84.5—87 °C, $[\alpha]_D^{20}$ −48.3° ($c=0.39$, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3630, 1780, 1460, 1380, 1195. ^{1}H-NMR (C_5D_5N) δ: 1.04 (3H, d, $J=7$ Hz), 1.25 (3H, s), 1.39 (3H, s), 2.60 (2H, m). *Anal.* High resolution MS Calcd for $C_{12}H_{20}O_3$ (M^+): 212.1412. Found: 212.1436.

Oxidation of Cyclonerodiol (8)——*m*-Chloroperbenzoic acid (372 mg) was added to a solution of cyclonerodiol (300 mg) in Et_2O (30 ml) at 0 °C. After being stirred at 0 °C for 15 min and then at room temperature for 17 h, the reaction mixture was washed with 0.2 N NaOH (40 ml) and brine, then dried over $MgSO_4$, and concentrated *in vacuo* to give an oil. The oil was chromatographed on a silica gel column and preparative TLC plates to give cyclonerodiol oxide (65 mg, as needles), mp 50—54.5 °C, $[\alpha]_D^{22}$ −20.8° ($c=0.34$, MeOH), which was identical with natural cyclonerodiol oxide (**9**) (TLC, IR, MS, ^{1}H-NMR, and ^{13}C-NMR), and epicyclonerodiol oxide (51 mg), which was identical with natural epicyclonerodiol oxide (**10**) (TLC, IR, MS, ^{1}H-NMR, and ^{13}C-NMR).

Jones Oxidation of Cyclonerodiol Oxide (9)——The Jones reagent (0.1 ml) was added to a solution of cyclonerodiol oxide (26 mg) in acetone (2 ml) at room temperature. After being stirred for 1 h, the reaction mixture was diluted with H_2O (20 ml) and extracted with $CHCl_3$ (20 ml × 3). The $CHCl_3$ layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to give crude crystals (20.5 mg), which were recrystallized from Et_2O–hexane to give the γ-lactone (**11**) as needles (16.1 mg), mp 85—87 °C, $[\alpha]_D^{22}$ −47.7° ($c=0.37$, $CHCl_3$); this product was identical with an authentic sample (^{1}H-NMR, IR, and mixed mp).

Jones Oxidation of Epicyclonerodiol Oxide (10)——Epicyclonerodiol oxide (34.2 mg) was oxidized in the same manner as described above to give the γ-lactone (**11**) (23 mg), mp 84.5—87 °C, $[\alpha]_D^{22}$ −45.1° ($c=0.40$, $CHCl_3$).

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