Studies on Metabolites of Mycoparasitic Fungi. III.[1] New Sesquiterpene Alcohol from *Trichoderma koningii*

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A new sesquiterpene alcohol, named tricho-acorenol (1), was isolated from the culture broth of *Trichoderma koningii* OUDEMANS along with methyl benzoate (2), cyclonerodiol (3), cyclo-(L-Pro-L-Leu) (4), 4-hydroxyphenethyl alcohol (5), uracil (7), and a ceramide (6). The ceramide (6) was identified as (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytetracosanoylamino]-1,3,4-octadecanetriol by the use of ion-spray ionization MS (ISI-MS) and comparison of the optical rotation and the ^{1}H-NMR data with those of (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytetracosanoylamino]-1,3,4-hexadecanetriol. The structure of tricho-acorenol was elucidated to be (1*S*,4*S*,5*S*,7*R*)-1-isopropyl-4,8-dimethyl-spiro-[4.5]dec-8-en-7-ol on the basis of chemical and spectroscopic evidence.

Key words *Trichoderma koningii*; tricho-acorenol; acorane-type sesquiterpene; ceramide; (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytetracosanoylamino]-1,3,4-octadecanetriol; ion-spray ionization MS

In a previous paper,[1] we reported the isolation and structure elucidation of peptaibols, named trichokonins V—VIII, from the culture broth of *Trichoderma koningii* OUDEMANS, which is one of the fungi harmful to the cultivation of a medicinal mushroom, *Ganoderma lucidum* (FR.) KARST. (oriental crude drug "Lin-Chi"). In this paper, we wish to report the other metabolites obtained along with the peptaibols.

As reported in the previous paper,[1] the culture broth of *T. koningii* was separated into mycelia and medium by filtration and the medium was extracted with BuOH. The BuOH extract was separated by a combination of silica gel column chromatography and preparative TLC procedures to give seven compounds along with the peptaibols.

Among the seven compounds obtained, five (**2**—**5**, **7**) were identified as known ones, methyl benzoate (**2**), cyclonerodiol (**3**),[2] cyclo-(L-Pro-L-Leu) (**4**),[1,3] 4-hydroxyphenethyl alcohol (**5**), and uracil (**7**), by spectral analyses and comparison of the data with the literature values.

Tricho-acorenol (**1**), a colorless amorphous solid, $[\alpha]_D$ $-5.2°$ ($CHCl_3$), showed the molecular ion peak at *m/z* 222 in the EI-MS and its molecular formula was determined to be $C_{15}H_{26}O$ by high-resolution MS (HR-MS) measurement. The IR spectrum of **1** showed a hydroxyl absorption at 3600 cm^{-1} and the ^{1}H-NMR spectrum showed signals due to a hydroxy proton at δ_H 3.51 (1H, d, J=6.5 Hz, disappeared on addition of D_2O, 7-OH), a carbinol proton at δ_H 4.22 (1H, br s, $W_{1/2}$=19.5 Hz, 7-H), an olefinic proton at δ_H 5.39 (1H, m, 9-H), a vinylic methyl at δ_H 1.73 (3H, br s, 15-H_3), and three secondary methyls at δ_H 0.84 (J=7.0 Hz, 14-H_3), 0.86, and 0.93 (each J=6.5 Hz, 12-H_3, 13-H_3). The ^{13}C-NMR spectrum and the distortionless enhancement by polarization transfer (DEPT) experiment revealed the presence of four methyls, four methylenes, five (three sp^3, an oxygen-bearing, and an olefinic) methines, and two (an sp^2 and an sp^3) quaternary carbons. The hydroxyl group should be located

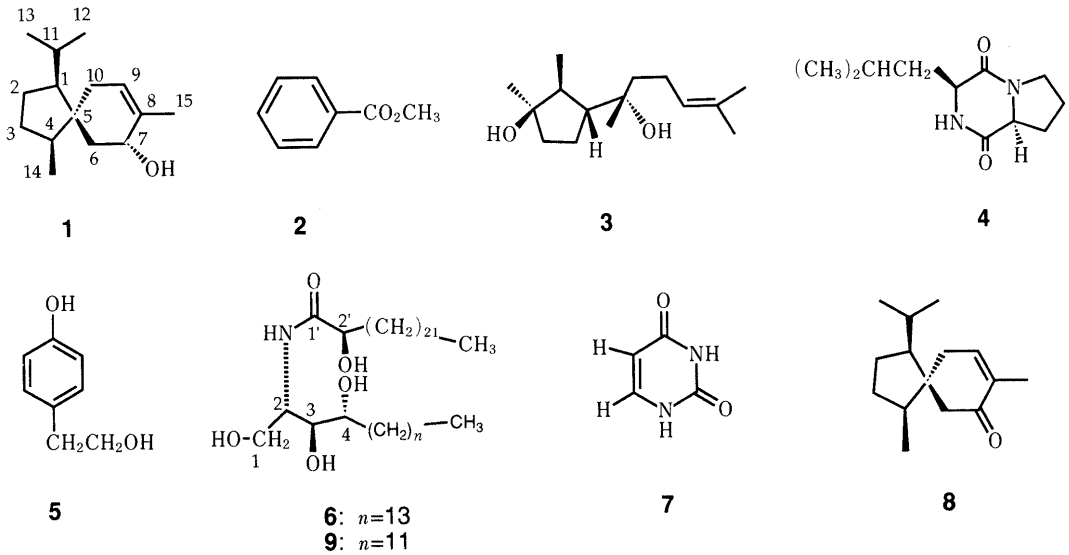

Chart 1

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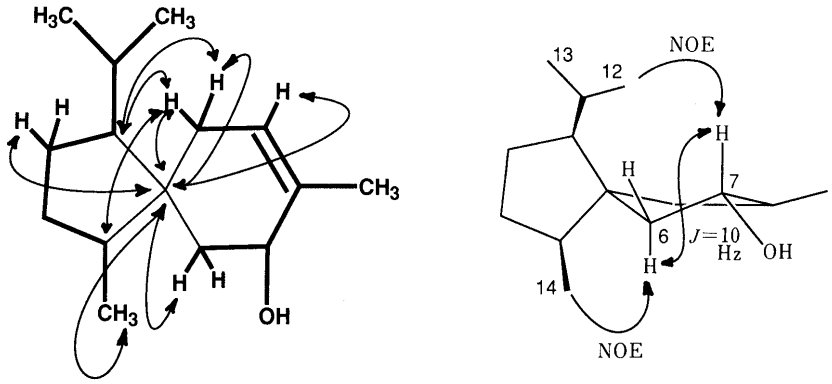

Fig. 1. Part of the Long-Range Correlations (Left) and NOE's (Right) Observed in the Long-Range ^1H–^{13}C COSY and Difference NOE Spectra of **1**
—: Connectivities deduced from the ^1H–^1H and ^1H–^{13}C COSY spectra and the decoupling experiments.

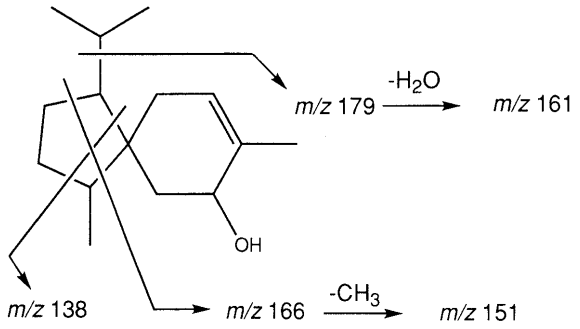

Fig. 2. EI-MS Fragmentation of **1**

at the allylic position because long-range couplings were observed between the carbinol proton and the vinylic methyl and olefinic protons. These data and the results of the ^1H–^1H and ^1H–^{13}C shift correlation spectroscopy (COSY) analyses suggested that **1** may be an acorane-type sesquiterpene,[4] 1-isopropyl-4,8-dimethyl-spiro[4.5]dec-8-en-7-ol. This was supported by the long-range correlations observed in the long-range ^1H–^{13}C COSY spectrum (Fig. 1, left). Also, in accordance with this structure, the EI-MS of **1** showed fragment ions at *m/z* 207 ($M^+ - CH_3$), 204 ($M^+ - H_2O$), 179, 166, 161, 151, and 138, which were reasonably interpreted as shown in Fig. 2.

Oxidation of **1** with manganese dioxide in methylene chloride afforded an α,β-unsaturated ketone (**8**), $C_{15}H_{24}O$ (M^+, *m/z* 220), $[\alpha]_D$ −22.0° ($CHCl_3$), which showed an IR absorption at 1655 cm^{-1} and a UV absorption at 238 nm (log ε, 3.51). Eventually **8** was identified as (−)-acorenone ($[\alpha]_D$ −22.3°), which had been isolated from *Acorus calamus*,[5,6] by comparison of the spectroscopic data. Thus, the absolute configurations at C-1, C-4, and C-5 in **1** were determined to be 1*S*, 4*S*, and 5*S*, respectively.

The configuration of the hydroxyl group in **1** was elucidated based on the proton–proton coupling constant values and the results of NOE experiments. The proton at δ_H 1.29 (dd, J=14.0, 10.0 Hz, 6-H) showed a *trans*-diaxial coupling with the carbinol proton at δ_H 4.22 (br s, $W_{1/2}$=19.5 Hz, 7-H). On the other hand, NOE enhancements were observed between the methyl protons at δ_H 0.86, 0.93 (12-H_3 and 13-H_3) and the carbinol proton at δ_H 4.22 (7-H), and between the methyl protons at δ_H 0.84 (14-H_3) and the proton at δ_H 1.29 (6-H) (Fig. 1, right). Therefore, the hydroxyl group in **1** must have α(pseudo equatorial)-orientation (7*R*). Thus, the absolute configuration of **1** was determined as 1*S*,4*S*,5*S*,7*R*.

The ceramide **6** was obtained as a colorless amorphous solid, $[\alpha]_D$ +11.1° (pyridine), and showed the quasi molecular ion peak at *m/z* 684 $(M+H)^+$ in the ion-spray ionization MS (ISI-MS). The IR spectrum of **6** showed absorptions at 3310 (br, NH and OH), 1620 (amide CO), and 1540 (amide NH) cm^{-1}, and the ^1H-NMR spectrum of **6** showed the presence of long aliphatic chain(s) (δ_H 1.27 and 1.32, both br s, total *ca.* 60H). On the other hand, the ^1H–^1H COSY spectrum revealed that **6** was a ceramide with an α-hydroxy acyl group and a 2-amino-1,3,5-trihydroxy alkane group.

Next, in order to determine the numbers of methylene groups at the acyl and amino-alcohol moieties, the collision-induced dissociation (CID) spectrum of the quasi molecular ion was measured. The characteristic fragment ions were observed at *m/z* 384 and 318 (ions derived from the α-fissions of the NH group) and at *m/z* 366, 300, 282, and 264 (ions due to dehydration from the *m/z* 384 and 318 ions) along with an ion at *m/z* 426 arising from the β-fission of the NH group (Fig. 3). Thus, the acyl moiety was identified as 2-hydroxytetracosanoyl, and the amino-alcohol as 4-hydroxysphinganine. From these data, the ceramide **6** was determined to be *N*-(2-hydroxytetracosanoyl)-4-hydroxysphinganine.

The stereochemistry of **6** was elucidated based on a comparison of its physical data with those of (2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytetracosanoylamino]-1,3,4-hexadecanetriol (**9**), which had been reported by Komori *et al.*[7] As shown in Table 1, the optical rotations and the ^1H-NMR data of both compounds were almost identical, despite the slight difference of their structures. Thus, the absolute configuration of **6** was concluded to be 2*S*,3*S*,4*R*,2′*R*. This ceramide has been reported to be a constituent of ceramide mixtures in bran and endosperm of rice grains and in leafy stems of rice, although the stereochemistry was not established.[8] Our present work provides the first example of the isolation and characterization of this compound from a natural source.

Experimental

Optical rotations were measured on a JASCO DIP-140 digital polarimeter at 26 °C. UV spectra were taken with a Shimadzu UV-160A

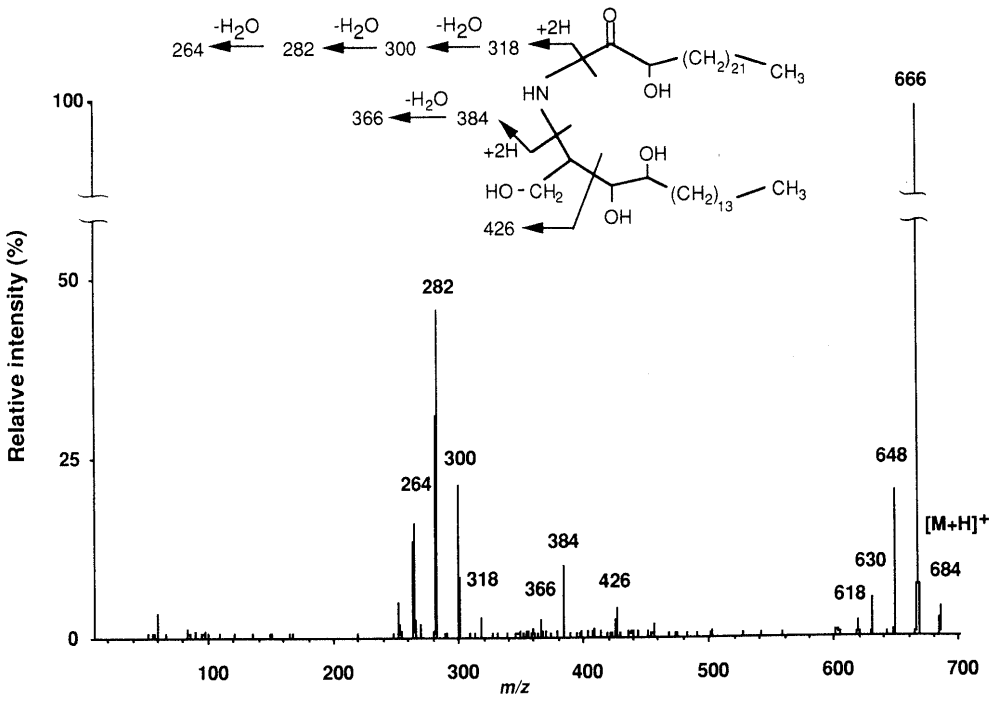

Fig. 3. CID Spectrum of the Quasi Molecular Ion of **6** ($m/z=684$)

Table 1. ^{1}H-NMR (in C_5D_5N) and $[\alpha]_D$ Data for **6** and **9**

	6	**9**[a]
$[\alpha]_D$ (in C_5H_5N)	11.1°	9.12°
δ_H (J in Hz)		
NH	8.54 d (9.0)	8.60 d (8.9)
1	4.50 dd (11.0, 4.5)	4.52 dd (10.7, 4.5)
	4.40 dd (11.0, 4.5)	4.43 dd (10.6, 5.0)
2	5.08 dddd (9.0, 4.5, 4.5, 4.5)	5.12 m
3	4.33 dd (6.0, 4.5)	4.36 dd (6.6, 4.6)
4	4.27 ddd (6.0, 6.0, 2.0)	4.29 m
2′	4.61 dd (7.5, 3.5)	4.63 dd (7.6, 4.0)
CH_3	0.88 t (6H, 6.0)	0.88 t (6H, 6.6)

a) Reference 7.

spectrophotometer in MeOH solution and IR spectra were taken with a Shimadzu IR-408 infrared spectrophotometer in $CHCl_3$ solutions unless otherwise noted. EI-MS and HR-MS measurements were done with a JEOL D-300 spectrometer using a direct inlet system at an ionization voltage of 70 eV. ISI-MS and CID spectra were obtained with a Perkin-Elmer Sciex API-III mass spectrometer. For CID experiments, Ar was used as a collision gas (collision energy, 25 eV). ^{1}H-, ^{13}C-, and two-dimensional (2D) NMR spectra were measured with a JEOL JNM-GX400 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are recorded in δ values and coupling constants in hertz (Hz). Multiplicities of ^{13}C-NMR signals were determined by the DEPT method and are indicated as s (singlet), d (doublet), t (triplet), and q (quartet).

Isolation of Compounds The extraction and separation procedure of the crude metabolites from the culture broth of *T. koningii* was described in the previous paper[1]; *i.e.*, the culture broth (36 l) was extracted with BuOH and the BuOH extract was subjected to column chromatography with $CHCl_3$–MeOH gradient mixtures (100:0—50:50) to give eight fractions (fr. 1 to fr. 8).

Fraction 2 ($CHCl_3$ eluate, 85 mg) was further purified by silica gel column chromatography with $CHCl_3$ to give tricho-acorenol (**1**, 30 mg).

Fraction 4 [$CHCl_3$–MeOH (95:5) eluate, 935 mg] was rechromatographed on a silica gel column with $CHCl_3$ (1.5 l) and $CHCl_3$–MeOH (99:1, 1.5 l; 95:5, 1.5 l) gradient mixtures to give five fractions [frs. 1—3, $CHCl_3$ eluate; fr. 4, $CHCl_3$–MeOH (99:1) eluate; fr. 5, $CHCl_3$–MeOH (99:1 and 95:5) eluate]. The fr. 2 (80 mg) and fr. 3 (88 mg) were further purified by preparative TLC with EtOAc–$CHCl_3$ (2:1), and fr. 2 gave methyl benzoate (**2**, 13 mg) and fr. 3 cyclonerodiol (**3**, 15 mg) and cyclo-(L-Pro–L-Leu) (**4**, 2 mg). Fraction 4 (122 mg) was purified by silica gel column chromatography with $CHCl_3$–EtOAc (6:4) to yield an additional crop of **3** (cyclonerodiol, 28 mg).

Fraction 6 [$CHCl_3$–MeOH (90:10) eluate, 3.5 g] was rechromatographed on a silica gel column with $CHCl_3$–acetone (95:5, 1 l; 90:10, 1 l; 85:15, 2 l; 75:25, 2 l) gradient mixtures, and the $CHCl_3$–acetone (85:15) eluate yielded 4-hydroxyphenethyl alcohol (**5**, 34 mg) and ceramide **6** (30 mg).

Fraction 7 [$CHCl_3$–MeOH (75:15) eluate, 787 mg] yielded uracil (**7**, 262 mg).

Tricho-acorenol [(1*S*,4*S*,5*S*,7*R*)-1-Isopropyl-4,8-dimethyl-spiro[4.5]-dec-8-en-7-ol, 1] Colorless amorphous solid, $[\alpha]_D$ −5.2° ($c=0.12$, $CHCl_3$). IR ν_{max} cm^{-1}: 3600 (OH). ^{1}H-NMR (acetone-d_6) δ: 0.84 (3H, d, $J=7.0$ Hz, 14-H_3), 0.86, 0.93 (each 3H, d, $J=6.5$ Hz, 12-H_3, 13-H_3), 1.09 (1H, m, 3-H), 1.27 (1H, m, 1-H), 1.29 (1H, dd, $J=14.0$, 10.0 Hz, 6-H), 1.46 (1H, tdd, $J=11.5$, 10.0, 4.0 Hz, 2-H), 1.58 (1H, ddq, $J=10.0$, 9.0, 6.5 Hz, 4-H), 1.70 (1H, m, 3-H), 1.70 (1H, m, 11-H), 1.72 (1H, m, 6-H), 1.73 (3H, br s, 15-H_3), 1.74 (1H, m, 2-H), 1.80 (1H, br d, $J=$ 9.5 Hz, 10-H), 2.10 (1H, ddq, $J=9.5$, 5.5, 2.5 Hz, 10-H), 3.51 (1H, d, $J=6.5$ Hz, 7-OH), 4.22 (1H, br s, $W_{1/2}=19.5$ Hz, 7-H), 5.39 (1H, m, 9-H). ^{13}C-NMR (acetone-d_6) δ: 15.0 (q, C-14), 20.0 (q, C-15), 23.8, 24.3 (each q, C-12, C-13), 27.0 (t, C-2), 30.1 (t, C-3), 31.5 (d, C-11), 33.4 (t, C-6), 36.7 (t, C-10), 46.2 (s, C-5), 48.0 (d, C-4), 61.2 (d, C-1), 68.6 (d, C-7), 124.6 (d, C-9), 138.6 (s, C-8). EI-MS m/z (%): 222 (M^+, 100), 207 (M^+-CH_3, 7), 204 (M^+-H_2O, 4), 179 (29), 166 (20), 161 (21), 151 (70), 138 (61). HR-MS: Found 222.1970, Calcd for $C_{15}H_{26}O$ 222.1984.

Oxidation of 1 with MnO_2 To a solution of **1** (3 mg) in CH_2Cl_2 (1.5 ml), MnO_2 (40 mg) was added and the mixture was stirred for 3 h at room temperature. After removal of insoluble material by filtration, the filtrate was concentrated and the residue was subjected to preparative TLC with $CHCl_3$–acetone (95:5) to give **8** (1 mg) as a colorless oil, $[\alpha]_D$ −22.0° ($c=0.047$, $CHCl_3$). UV λ_{max} nm (log ε): 238 (3.51). IR ν_{max} cm^{-1}: 1655 (CO). ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, d, $J=6.5$ Hz, 14-H_3), 0.86, 0.97 (each 3H, d, $J=6.5$ Hz, 12-H_3, 13-H_3), 1.76 (3H, m, 15-H_3), 2.13 (1H, ddq, $J=20.0$, 4.0, 0.7 Hz, 10-H), 2.30 (1H, d, $J=16.5$ Hz, 6-H), 2.35 (1H, d, $J=16.5$ Hz, 6-H), 2.67 (1H, ddq, $J=20.0$, 2.5, 2.0 Hz, 10-H), 6.66 (1H, m, 9-H). EI-MS m/z (%): 220 (M^+, 46), 177 (36), 164 (13), 150 (34), 136 (57), 135 (73), 109 (100), 82 (84).

Ceramide 6 Colorless amorphous solid, $[\alpha]_D$ +11.1° ($c=0.26$, C_5H_5N). IR ν_{max} (KBr) cm^{-1}: 3310 (br, OH and NH), 1620 (amide CO), 1540 (amide NH). ^{1}H-NMR (C_5D_5N) δ: 0.88 (6H, br t, $J=6.0$ Hz, 18-CH_3 and 24′-CH_3), 1.27, 1.32 (both br s, total *ca.* 60H, methylenes),

1.72 (1H, m, 6-H), 1.91 (1H, m, 6-H), 1.94 (1H, m, 5-H), 2.04 (1H, m, 3′-H), 2.22 (1H, m, 3′-H), 2.24 (1H, m, 5-H), 4.27 (1H, ddd, J=6.0, 6.0, 2.0 Hz, 4-H), 4.33 (1H, dd, J=6.0, 4.5 Hz, 3-H), 4.40 (1H, dd, J=11.0, 4.5 Hz, 1-H), 4.50 (1H, dd, J=11.0, 4.5 Hz, 1-H), 4.61 (1H, dd, J=7.5, 3.5 Hz, 2′-H), 5.08 (1H, dddd, J=9.0, 4.5, 4.5, 4.5 Hz, 2-H), 8.54 (1H, d, J=9.0 Hz, NH). ^{13}C-NMR (C_5D_5N) δ: 14.2 (q, 18-C and 24′-C), 26.6 (t, 6-C), 34.1 (t, 5-C), 35.7 (t, 3′-C), 53.0 (d, 2-C), 62.0 (t, 1-C), 72.4 (d, 2′-C), 73.0 (d, 3-C), 76.7 (d, 3-C), 175.3 (s, 1′-C). ISI-MS m/z: 684 $(M+H)^+$.

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References and Notes

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