

## Studies on Metabolites of Mycoparasitic Fungi. III.<sup>1)</sup> 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-hydroxy-tetracosanoylamino]-1,3,4-octadecanetriol by the use of ion-spray ionization MS (ISI-MS) and comparison of the optical rotation and the <sup>1</sup>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,<sup>1)</sup> 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,<sup>1)</sup> 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**),<sup>2)</sup> cyclo-(L-Pro-L-Leu) (**4**),<sup>1,3)</sup> 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, [ $\alpha$ ]<sub>D</sub> –5.2° (CHCl<sub>3</sub>), showed the molecular ion peak at *m/z* 222 in the EI-MS and its molecular formula was determined to be C<sub>15</sub>H<sub>26</sub>O by high-resolution MS (HR-MS) measurement. The IR spectrum of **1** showed a hydroxyl absorption at 3600 cm<sup>–1</sup> and the <sup>1</sup>H-NMR spectrum showed signals due to a hydroxy proton at  $\delta_H$  3.51 (1H, d, *J* = 6.5 Hz, disappeared on addition of D<sub>2</sub>O, 7-OH), a carbinol proton at  $\delta_H$  4.22 (1H, br s, *W*<sub>1/2</sub> = 19.5 Hz, 7-H), an olefinic proton at  $\delta_H$  5.39 (1H, m, 9-H), a vinylic methyl at  $\delta_H$  1.73 (3H, br s, 15-H<sub>3</sub>), and three secondary methyls at  $\delta_H$  0.84 (*J* = 7.0 Hz, 14-H<sub>3</sub>), 0.86, and 0.93 (each *J* = 6.5 Hz, 12-H<sub>3</sub>, 13-H<sub>3</sub>). The <sup>13</sup>C-NMR spectrum and the distortionless enhancement by polarization transfer (DEPT) experiment revealed the presence of four methyls, four methylenes, five (three *sp*<sup>3</sup>, an oxygen-bearing, and an olefinic) methines, and two (an *sp*<sup>2</sup> and an *sp*<sup>3</sup>) quaternary carbons. The hydroxyl group should be located

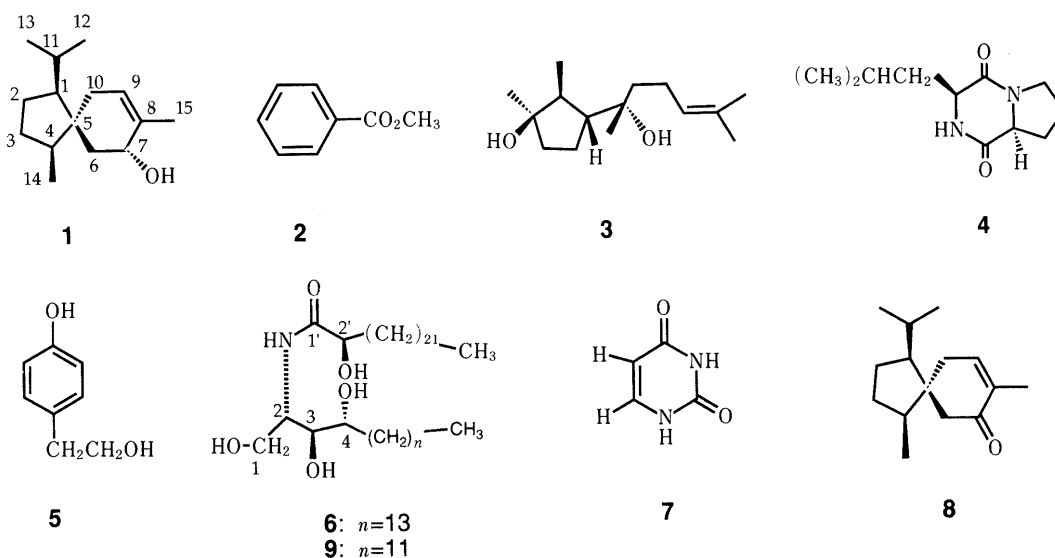


Chart 1

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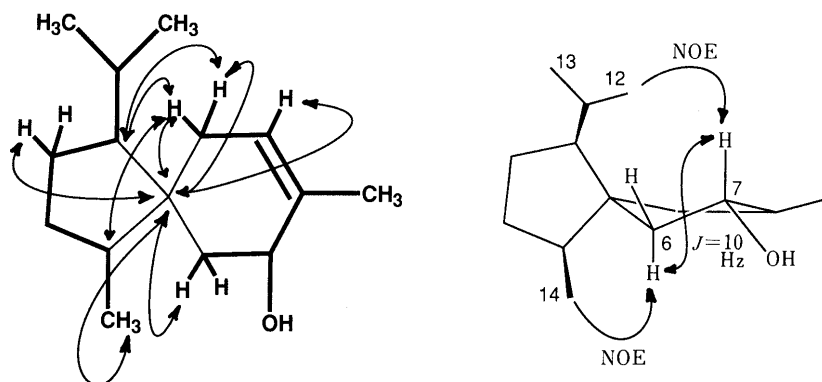


Fig. 1. Part of the Long-Range Correlations (Left) and NOE's (Right) Observed in the Long-Range  $^1\text{H}$ - $^{13}\text{C}$  COSY and Difference NOE Spectra of **1**  
 —: Connectivities deduced from the  $^1\text{H}$ - $^1\text{H}$  and  $^1\text{H}$ - $^{13}\text{C}$  COSY spectra and the decoupling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

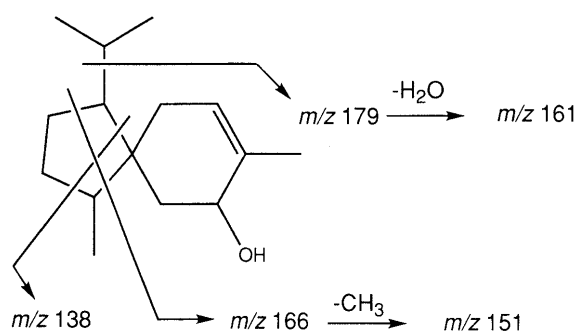


Fig. 2. EI-MS Fragmentation of **1**

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  $^1\text{H}$ - $^1\text{H}$  and  $^1\text{H}$ - $^{13}\text{C}$  shift correlation spectroscopy (COSY) analyses suggested that **1** may be an acorane-type sesquiterpene,<sup>4)</sup> 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  $^1\text{H}$ - $^{13}\text{C}$  COSY spectrum (Fig. 1, left). Also, in accordance with this structure, the EI-MS of **1** showed fragment ions at  $m/z$  207 ( $\text{M}^+ - \text{CH}_3$ ), 204 ( $\text{M}^+ - \text{H}_2\text{O}$ ), 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  $\alpha,\beta$ -unsaturated ketone (**8**),  $\text{C}_{15}\text{H}_{24}\text{O}$  ( $\text{M}^+$ ,  $m/z$  220),  $[\alpha]_D -22.0^\circ$  ( $\text{CHCl}_3$ ), which showed an IR absorption at  $1655 \text{ cm}^{-1}$  and a UV absorption at 238 nm ( $\log \epsilon$ , 3.51). Eventually **8** was identified as (–)-acorenone ( $[\alpha]_D -22.3^\circ$ ), which had been isolated from *Acorus calamus*,<sup>5,6)</sup> 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  $\delta_{\text{H}}$  1.29 (dd,  $J=14.0$ ,  $10.0 \text{ Hz}$ , 6-H) showed a *trans*-diaxial coupling with the carbinol proton at  $\delta_{\text{H}}$  4.22 (brs,  $W_{1/2}=19.5 \text{ Hz}$ , 7-H). On the other hand, NOE enhancements were observed between the methyl protons at  $\delta_{\text{H}}$  0.86, 0.93 (12- $\text{H}_3$  and 13- $\text{H}_3$ ) and the carbinol proton at  $\delta_{\text{H}}$  4.22 (7-H), and between the methyl protons at  $\delta_{\text{H}}$  0.84 (14- $\text{H}_3$ ) and the proton at  $\delta_{\text{H}}$  1.29 (6-H) (Fig. 1,

right). Therefore, the hydroxyl group in **1** must have  $\alpha$ (pseudo equatorial)-orientation (7*R*). Thus, the absolute configuration of **1** was determined as 1*S*,4*S*,5*S*,7*R*.

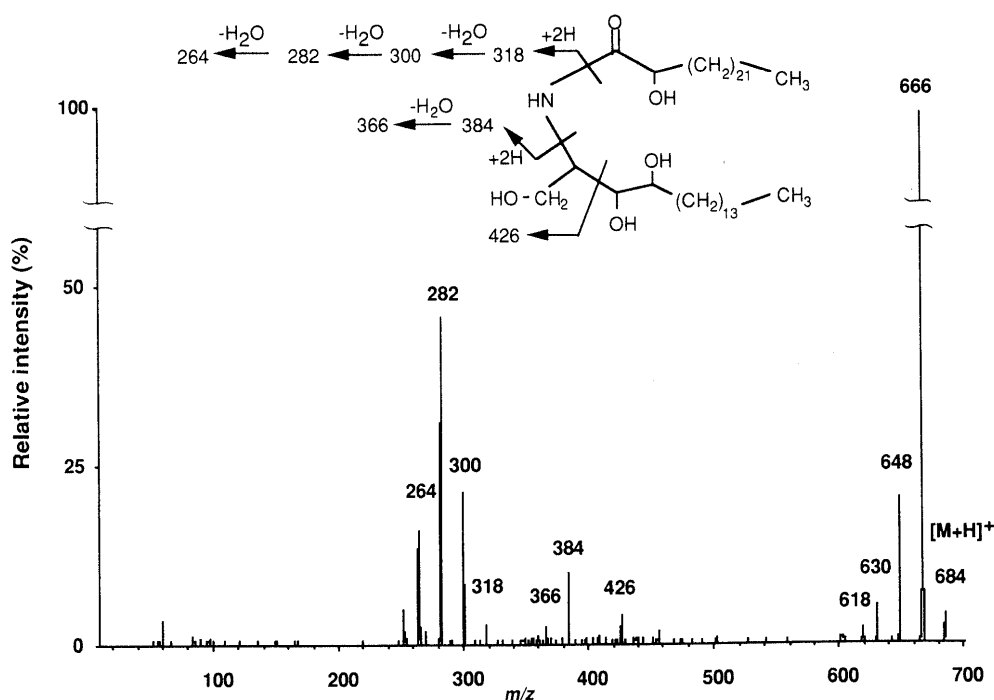
The ceramide **6** was obtained as a colorless amorphous solid,  $[\alpha]_D +11.1^\circ$  (pyridine), and showed the quasi molecular ion peak at  $m/z$  684 ( $\text{M} + \text{H}$ )<sup>+</sup> in the ion-spray ionization MS (ISI-MS). The IR spectrum of **6** showed absorptions at 3310 (br, NH and OH), 1620 (amide CO), and 1540 (amide NH)  $\text{cm}^{-1}$ , and the  $^1\text{H}$ -NMR spectrum of **6** showed the presence of long aliphatic chain(s) ( $\delta_{\text{H}}$  1.27 and 1.32, both brs, total *ca.* 60H). On the other hand, the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum revealed that **6** was a ceramide with an  $\alpha$ -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  $\alpha$ -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  $\beta$ -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.*<sup>7)</sup> As shown in Table 1, the optical rotations and the  $^1\text{H}$ -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.<sup>8)</sup> 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.  $^1\text{H-NMR}$  (in  $\text{C}_5\text{D}_5\text{N}$ ) and  $[\alpha]_D$  Data for **6** and **9**

	<b>6</b>	<b>9<sup>a)</sup></b>
$[\alpha]_D$ (in $\text{C}_5\text{H}_5\text{N}$ )	$11.1^\circ$	$9.12^\circ$
$\delta_H$ ( $J$ in Hz)		
NH	8.54 d (9.0)	8.60 d (8.9)
1	4.50 dd (11.0, 4.5) 4.40 dd (11.0, 4.5)	4.52 dd (10.7, 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)
$\text{CH}_3$	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  $\text{CHCl}_3$  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).  $^1\text{H}$ -,  $^{13}\text{C}$ -, 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  $\delta$  values and coupling constants in hertz (Hz). Multiplicities of  $^{13}\text{C}$ -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<sup>1)</sup>; i.e., the culture broth (36 l) was extracted with BuOH and the BuOH extract was subjected to column chromatography with  $\text{CHCl}_3$ -MeOH gradient mixtures (100:0–50:50) to give eight fractions (fr. 1 to fr. 8).

Fraction 2 ( $\text{CHCl}_3$  eluate, 85 mg) was further purified by silica gel column chromatography with  $\text{CHCl}_3$  to give tricho-acorenol (**1**, 30 mg).

Fraction 4 [ $\text{CHCl}_3$ -MeOH (95:5) eluate, 935 mg] was rechromatographed on a silica gel column with  $\text{CHCl}_3$  (1.5 l) and  $\text{CHCl}_3$ -MeOH (99:1, 1.5 l; 95:5, 1.5 l) gradient mixtures to give five fractions [frs. 1–3,  $\text{CHCl}_3$  eluate; fr. 4,  $\text{CHCl}_3$ -MeOH (99:1) eluate; fr. 5,  $\text{CHCl}_3$ -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- $\text{CHCl}_3$  (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  $\text{CHCl}_3$ -EtOAc (6:4) to yield an additional crop of **3** (cyclonerodiol, 28 mg).

Fraction 6 [ $\text{CHCl}_3$ -MeOH (90:10) eluate, 3.5 g] was rechromatographed on a silica gel column with  $\text{CHCl}_3$ -acetone (95:5, 1 l; 90:10, 1 l; 85:15, 2 l; 75:25, 2 l) gradient mixtures, and the  $\text{CHCl}_3$ -acetone (85:15) eluate yielded 4-hydroxyphenethyl alcohol (**5**, 34 mg) and ceramide **6** (30 mg).

Fraction 7 [ $\text{CHCl}_3$ -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,  $[\alpha]_D -5.2^\circ$  ( $c=0.12$ ,  $\text{CHCl}_3$ ). IR  $\nu_{\text{max}} \text{ cm}^{-1}$ : 3600 (OH).  $^1\text{H-NMR}$  (acetone- $d_6$ )  $\delta$ : 0.84 (3H, d,  $J=7.0$  Hz, 14- $\text{H}_3$ ), 0.86, 0.93 (each 3H, d,  $J=6.5$  Hz, 12- $\text{H}_3$ , 13- $\text{H}_3$ ), 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, brs, 15- $\text{H}_3$ ), 1.74 (1H, m, 2-H), 1.80 (1H, brd,  $J=9.5$  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, brs,  $W_{1/2}=19.5$  Hz, 7-H), 5.39 (1H, m, 9-H).  $^{13}\text{C-NMR}$  (acetone- $d_6$ )  $\delta$ : 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 ( $\text{M}^+$ , 100), 207 ( $\text{M}^+ - \text{CH}_3$ , 7), 204 ( $\text{M}^+ - \text{H}_2\text{O}$ , 4), 179 (29), 166 (20), 161 (21), 151 (70), 138 (61). HR-MS: Found 222.1970, Calcd for  $\text{C}_{15}\text{H}_{26}\text{O}$  222.1984.

**Oxidation of **1** with  $\text{MnO}_2$**  To a solution of **1** (3 mg) in  $\text{CH}_2\text{Cl}_2$  (1.5 ml),  $\text{MnO}_2$  (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  $\text{CHCl}_3$ -acetone (95:5) to give **8** (1 mg) as a colorless oil,  $[\alpha]_D -22.0^\circ$  ( $c=0.047$ ,  $\text{CHCl}_3$ ). UV  $\lambda_{\text{max}} \text{ nm}$  (log  $\epsilon$ ): 238 (3.51). IR  $\nu_{\text{max}} \text{ cm}^{-1}$ : 1655 (CO).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 0.83 (3H, d,  $J=6.5$  Hz, 14- $\text{H}_3$ ), 0.86, 0.97 (each 3H, d,  $J=6.5$  Hz, 12- $\text{H}_3$ , 13- $\text{H}_3$ ), 1.76 (3H, m, 15- $\text{H}_3$ ), 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 ( $\text{M}^+$ , 46), 177 (36), 164 (13), 150 (34), 136 (57), 135 (73), 109 (100), 82 (84).

**Ceramide **6**** Colorless amorphous solid,  $[\alpha]_D +11.1^\circ$  ( $c=0.26$ ,  $\text{C}_5\text{H}_5\text{N}$ ). IR  $\nu_{\text{max}} \text{ cm}^{-1}$ : 3310 (br, OH and NH), 1620 (amide CO), 1540 (amide NH).  $^1\text{H-NMR}$  ( $\text{C}_5\text{D}_5\text{N}$ )  $\delta$ : 0.88 (6H, brt,  $J=6.0$  Hz, 18- $\text{CH}_3$  and 24'- $\text{CH}_3$ ), 1.27, 1.32 (both brs, 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).  $^{13}\text{C-NMR}$  ( $\text{C}_5\text{D}_5\text{N}$ )  $\delta$ : 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 ( $\text{M} + \text{H}$ ) $^+$ .

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