A Glucosylceramide with a Novel Ceramide and Three Novel Ceramides from the Basidiomycete *Cortinarius umidicola*

Ji-Kai Liu*, Lin Hu, and Ze-Jun Dong

Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, People's Republic of China

ABSTRACT: A glucosylceramide with novel ceramide and three novel ceramide homologs were isolated from the basidiomycete *Cortinarius umidicola* and structurally characterized. The ceramide portion of the glucocerebroside consists of a rare (4*E*,8*E*)-9-methyl-4,8-sphingadienine sphingoid base. In contrast, the three ceramide homologs, while having the same sphingoid base, contain as FA residues 2-hydroxydocosanoic acid, 2-hydroxytricosanoic acid, and 2-hydroxytetracosanoic acid.

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In recent years, renewed attention has been paid to the constituents of basidiomycetes because of their possible medical usage (1). Antiviral, antibiotic, antiinflammatory, hypoglycemic, hypocholesterolemic, and hypotensive properties were ascribed to ingredients of such mushrooms (2). Sphingolipids, e.g., ceramides, cerebrosides, sphingomyelin, and gangliosides, are important building blocks of the plasma membrane of eukaryotic cells. Their function is to anchor lipid-bound carbohydrates to cell surfaces and to create an epidermal water permeability barrier, as well as to participate in antigen–antibody reactions and transmission of biological information (3). Some have shown antiulcerogenic, antihepatotoxic, antitumor, and immunostimulatory activities (4–6). In contrast to lower fungi, the glycolipids of higher fungi have been investigated less (7). The primary glycosphingolipids and ceramides isolated from higher fungi having an unsaturated sphingosine base (4,8-sphingadiene), saturated phytosphingosine, and 4-hydroxysphingosine have been investigated (8–11). *Cortinarius* (Cortinariaceae) is one of the largest genera, comprising hundreds of species and being widely distributed throughout the world (12). From the fruiting bodies of *Cortinarius* species, especially toadstools in Europe and Australia, a large number of toxins, pigments, cyclic polypeptides, bipyridyl compounds, anthraquinones, and triterpenoids have been isolated (13–16). In our continuing research on bioactive secondary metabolites of higher fungi in Yunnan, we investigated the glycosphingolipid and ceramide composition of fresh fruiting bodies of the mushroom *Cortinarius umidicola* Kauffm., which were grown under pine trees in the mountainous region near Kunming (Yunnan, P.R. China). Our investigation resulted in the isolation of a new glycosphingolipid containing a rare (4*E*,8*E*)-9-methyl-4,8-sphingadienine sphingoid base and three ceramides derived from an unusual 6-methyl-4-hydroxysphingosine, together with cerebrosides B and D (9,17,18). This report describes their isolation and structural elucidation.

*To whom correspondence should be addressed.
E-mail: jkliu@mail.kib.ac.cn

Abbreviations: CC, column chromatography; DEPT, distortionless enhancement by polarization transfer; HMBC, heteronuclear multiple bond correlation; LCB, long-chain base.

EXPERIMENTAL PROCEDURES

Chromatographic and instrumental methods. The m.p. were obtained on an XRC-1 apparatus (Sichuan University, Sichuan, P.R. China). Optical rotations were taken on a Horiba SEPA-300 automatic polarimeter (Horiba, Tokyo, Japan). The NMR spectra (^{1}H, ^{13}C, and 2-D NMR) were acquired on DRX-500 NMR instruments (Karlsruhe, Germany) at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR; tetramethylsilane was used as an internal standard and coupling constants were represented in hertz. Mass spectra were measured with a VG Autospec 3000 mass spectrometer (VG, Manchester, England). IR spectra were obtained in KBr pellets on a Bio-Rad FTS-135 IR spectrophotometer (Bio-Rad, Richmond, CA). GC–MS was performed with a Finnigan 4510 gas chromatograph–mass spectrometer (San Jose, CA) in the EI mode (ionizing potential, 70 eV) and a capillary column (30 m × 0.25 mm) packed with 5% phenyl/95% methylsilicone on 5% phenyl-dimethylsilicone (HP-5; Hewlett-Packard, Palo Alto, CA). Helium was used as carrier gas, column temperature was varied from 120 to 240°C with a slope of 5°C/min, and quantitative determination was based on the area of the GLC peaks.

Materials. Column chromatography (CC) was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Ltd., Qingdao, P.R. China). Reversed-phase CC was carried out on a LiChroprep® RP-18 column (40–63 μm; Merck, Darmstadt, Germany). All solvents were distilled before use.

Fresh fruiting bodies of *C. umidicola* were collected in Kunming (Yunnan, P.R. China) in August 2002 and identified by X.H. Wang at the Kunming Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (no. HKAS 41152) was deposited.

Extraction and isolation. The fresh fruiting bodies (25 kg) of *C. umidicola* were soaked in 95% ethanol at room tempera-

ture to destroy the enzymes. After filtration, the fruiting bodies were dried by air and finely crushed. The dried powders were extracted exhaustively with methanol (5 L × 3), then with chloroform/methanol (1:1, vol/vol; 5 L × 4) at room temperature. After concentrated to dryness *in vacuo*, the combined extracts were partitioned between water and ethyl acetate. The organic layer was concentrated under reduced pressure, affording a dark brown gum (120 g); the gum was dissolved in chloroform and then placed on a silica gel column eluted with petroleum ether containing increasing amounts of acetone. Twelve fractions were collected. Among them, the last fraction, eluted with acetone, was further chromatographed on a reversed-phase chromatography column (RP-18) eluting with MeOH/H_2O (9:1, vol/vol) to provide compound **1** (70 mg) and a mixture of **2** and **3** (118 mg). The fraction eluted with petroleum ether/acetone (1:10–1:9, vol/vol) was further purified on an RP-18 column eluting with MeOH/H_2O (95:5, vol/vol) to afford compounds **4**–**6** (mixture, 16.5 mg).

*(2*S*,3*R*,4*E*,8*E*)-1-(β-*D*-glucopyranosyl)-3-hydroxy-2-[(*R*)-2′-hydroxyhexadecanoyl]amino-9-methyl-4,8-heptadecadiene **1***. White amorphous powder (methanol), $[\alpha]_D^{20}$ +3.5° (*c* 0.15, $CHCl_3$). IR (KBr) ν_{max}: 3400 (OH), 2924, 2853, 2365, 2339, 1652 (HNC=O), 1544 (NH), 1077, 1035, 963, 721 (methylenes) cm^{-1}; negative FABMS *m/z* (relative intensity, %): 713 $[M]^-$ (100), 550 $[M - H - 162]^-$ (42), 488 (10), 456 $[M - 2H - COCH(OH)C_{14}H_{29}]^-$ (10), 443 $[M - NHCOCH(OH)C_{14}H_{29}]^-$ (7), 424 (13); ^1H and ^{13}C NMR spectra data are given in Table 1.

*Methanolysis of **1***. Compound **1** (12.8 mg) was refluxed with 3 mL 5% hydrochloride–methanol at 60°C for 6 h. Ten milliliters of cold water was poured into the reaction mixture, which was extracted three times with *n*-hexane (5 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue of the hexane phase was analyzed directly by GC–MS.

*Methyl (2*R*)-2-hydroxyhexanoate (**1a**)*. White solid, $[\alpha]_D^{20}$ –5.7° (*c* 0.18, $CHCl_3$). The retention time (t_R) of **1a** was 18.9 min. EI-MS (70 eV) *m/z* (relative intensity, %): 286 $[M]^+$ (2), 227 $[M - COOCH_3]^+$ (50), 125 (20), 111 (60), 103 (10), 97 (90), 90 (50), 83 (100), 69 (70), 57 (80), 55 (70), 43 (60).

*2-Acetoamino-1,3-diacetoxy-9-methyl-4,8-heptadecanediene (**1b**)*. The aqueous methanolic layer was neutralized with saturated Na_2CO_3, concentrated to dryness, and extracted with ether. The ether phase was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to afford a long-chain base (LCB), which was reacted with acetic anhydride/pyridine (1:1, vol/vol) at room temperature in 0.5 mL acetone overnight. The reaction mixture was diluted with water and then ex-

TABLE 1
^1H and ^{13}C NMR Spectral Data for Compound 1 in Pyridine-d_5[a]

Atom no.	^{13}C (multiplicity)	^1H (multiplicity, *J* in Hz)	^1H-^1H COSY	HMBC (selected)
Long-chain base				
1a	70.2 (CH_2)	4.68 (*m*)	H-1b, -2	H-2, -3, NH, 1″
1b		4.77 (*m*)	H-1a, -2	H-1″
2	54.6 (CH)	4.78 (*m*)	NH, H-1a, -1b, H-3	H-1, -3, -4
3	72.4 (CH)	4.75 (*m*)	H-2, -4	H-1, -2, -4
4	132.4 (CH)	5.94 (*dt*, 15.3)	H-3, -5	H-3, 6
5	132.0 (CH)	5.97 (*dd*, 15.3, 6.8)	H-4, -6	H-3, 7
6	33.1 (CH_2)	2.14 (*m*)	H-5, -7	H-4, 5
7	32.2 (CH_2)	2.14 (*m*)	H-6, -8	H-5, 8
8	124.2 (CH)	5.25 (*m*)	H-7	H-6, -7, -10, CH_3-18
9	135.6 (C)			H-7, CH_3-18
10	40.1 (CH_2)	2.00 (*m*)		H-8, CH_3-18
11–16	30.0–30.1 (CH_2)	1.25–1.41 (*br s*)		
17	14.1 (CH_3)	0.86 (*t*, 6.7)		
18	16.2 (CH_2)	1.61 (*s*)		H-8, -9, -10
NH		8.33 (*d*, 8.7)	H-2	H-1′
N-Acyl moiety				
1′	175.5 (C)			H-2, -2′, -3′, NH
2′	72.5 (CH)	4.57 (*dd*, 7.7)	H-3′	HN, H-3′, -4′
3′	35.7 (CH_2)	2.00 (*m*), 2.14 (*m*)	H-2′, -4′	H-2′, -4′
4′–15′	30.0–30.1 (CH_2)	1.25–1.31 (*br s*)		
16′-CH_3	14.4 (CH_3)	0.86 (*t*, 6.7)		
Sugar moiety				
1″	105.7 (CH)	4.88 (*d*, 7.9)	H-2″	H-1a, 1b, -2″
2″	75.1 (CH)	4.01 (*dd*, 9.0, 7.9)	H-1″, -3″	H-1″, -3″, -4″
3″	78.5 (CH)	4.20 (*t*, 9.0)	H-2″, -4″	H-1″, -2″
4″	71.5 (CH)	4.18 (*t*, 9.0)	H-5″, -3″	H-3″, -6″
5″	78.6 (CH)	3.88 (*ddd*, 9.0, 5.7, 2.5)	H-6″, -4″	H-3″, -4″, -6″
6″	62.7 (CH_2)	4.33 (*dd*, 11.5, 2.5)	H-5″	H-4″
		4.48 (*dd*, 11.5, 5.7)		

[a]HMBC, heteronuclear multiple bond correlation.

tracted with ethyl acetate. The residue of the organic phase was subjected to silica gel CC using petroleum ether/ethyl acetate (9:1, vol/vol) as eluent to produce a triacetate of the LCB (**1b**, 2.8 mg). EI-MS (70 eV) *m/z* (relative intensity, %): 423 $[M]^+$ (1), 381 $[M - Ac]^+$ (3), 363 $[M - CH_3COOH]^+$ (3), 303 $[M - 2CH_3COOH]^+$ (13), 144 $[AcOCH_2CHNHAc]^+$ (32), 102 (51), 84 (90), 55 (100).

1-O-*Methyl-D-glucopyranoside (**1c**)*. The remaining water layer was evaporated *in vacuo*. The residue was then chromatographed on the RP-18 column using methanol/water (1:9, vol/vol) to afford methyl glucopyranoside. $[\alpha]_D^{20}$ +76.0°(*c* 0.17, MeOH), negative FABMS *m/z* (relative intensity, %): 193 $[M - H]^+$ (100).

*(2*S*,3*R*,4*E*,8*E*)-1*-O-*(β-D-glucopyranosyl)-3-hydroxy-2-[(*R*)-2′-hydroxypalmitoyl]amino-9-methyl-4,8-hexadecadiene (= cerebroside B, **2**) and (2*S*,3*R*,4*E*,8*E*)-1*-O-*(β-D-glucopyranosyl)-3-hydroxy-2-[(*R*)-2′-hydroxyoctadecanoyl]amino-9-methyl-4,8-hexadecadiene (= cerebroside D, **3**)*. White amorphous powder (methanol), $[\alpha]_D^{20}$ +4.8° (*c* 0.18, $CHCl_3$). IR (KBr) ν_{max}: 3400 (OH), 2960, 1650 (HNC=O), 1540 (NH), 1100–1000, 720 cm^{-1}; negative FABMS *m/z* (relative intensity, %):755 $[M_1]^-$ (100), 726 $[M_2 - 1]^-$ (22), 592 $[M - H - 162]^-$ (38), 564 $[M - H - 162]^-$ (9); the NMR spectra of the mixture were consistent with those reported in the literature (3,11,12).

*(2*S*,3*S*,4*R*,2′*R*)-2-(2′-hydroxytetracosanoylamino)heptadecane-6-methyl-1,3,4-triol (**4**), (2*S*,3*S*,4*R*,2′*R*)-2-(2′-hydroxytricosanoylamino)heptadecane-6-methyl-1,3,4-triol (**5**) and (2*S*,3*S*,4*R*,2′*R*)-2-(2′-hydroxydocosanoylamino)heptadecane-6-methyl-1,3,4-triol (**6**)*. White amorphous powder (methanol), $[\alpha]_D^{20}$ −10.0° (*c* 0.18, $CHCl_3$). IR (KBr) ν_{max}: 3402 (OH), 1644, 1635, 1628, 1127, 1071, 1042, 721 cm^{-1}; negative FABMS *m/z* (relative intensity): 683 (83), 669 (75), and 655 (100), $[M]^-$ series. ^1H and ^{13}C NMR spectra data are given in Table 2.

*Methanolysis of **4–6***. The mixture of **4**, **5**, and **6** (11.3 mg) was refluxed with 3 mL 5% HCl–methanol at 60°C for 6 h. Ten milliliters of cold water was poured into the reaction mixture, which was extracted with *n*-hexane (5 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4. Concentration of the hexane phase yielded the mixture of FAME (3.4 mg), white solid, $[\alpha]_D^{20}$ −3.2° (*c* 0.09, C_5H_5N), which was analyzed by GC–MS. Result of the GC–MS analysis revealed the presence of three components. The most abundant one (49.2%) was characterized as methyl (2*R*)-2-hydroxytetracosanoate (**6a**), which displayed major ion peaks at *m/z* 398 $[M]^+$ and 339 $[M - COOCH_3]^+$; its t_R was 42.1 min. The second-most abundant one (47.8%) was identified as methyl (2*R*)-2-hydroxydocosanoate (**4a**), which displayed major ion peaks at *m/z* 370 $[M]^+$ and 311 $[M - COOCH_3]^+$; its t_R was 35.3 min. The minor one (3%) was identified as methyl (2*R*)-2-hydroxytricosanoate (**5a**). It displayed major ion peaks at *m/z* 398 $[M]^+$ and 339 $[M - COOCH_3]^+$, and its t_R was 38.7 min.

*2-Acetoamino-6-methyl-1,3,4-triacetoxyheptadecane (**4b**)*. The aqueous layer was neutralized with saturated $NaCO_3$, concentrated under reduced pressure to dryness, and extracted with ether. The ether phase was dried over anhydrous Na_2SO_4 and evaporated to afford LCB, which was reacted with acetic anhydride/pyridine (1:1, vol/vol) at room temperature overnight. The reaction mixture was diluted with water and then extracted with ethyl acetate. The residue of the organic phase was subjected to silica gel CC using petroleum ether/ethyl acetate (9:1, vol/vol) as eluent to produce a peracetate of the LCB (**4b**, 1.8 mg). EI-MS (70 eV) *m/z* (relative intensity, %): 485 $[M]^+$ (9), 425 $[M - CH_3COOH]^+$ (1), 365 $[M - 2CH_3COOH]^+$ (3), 305 $[M - 3CH_3COOH]^+$ (13), 245 $[M - 4CH_3COOH]^+$ (7).

TABLE 2
^1H and ^{13}C NMR Spectral Data for Compounds 4–6 in Pyridine-d_5[a]

Atom no.	^{13}C (multiplicity)	^1H (multiplicity, *J* in Hz)	^1H-^1H COSY (selected)	HMBC (selected)
Long-chain base				
1	62.1 (CH_2)	4.52 (*dd*, 10.6, 4.5) 4.43 (*dd*, 10.6, 5.2)	H-2	H-2, -3
2	53.0 (CH)	5.12 (*m*)	NH, H-2, -3	NH, H-1′, -1, -3
3	76.8 (CH)	4.40 (*dd*, 6.5, 4.0)	H-2, -4	H-1, -2, -4, -5
4	73.1 (CH)	4.35 (*m*)	H-3, -5	H-2, -3, -5, -6
5	35.8 (CH_2)	1.93 (*m*)	H-4, -6	H-3, -4, -6, CH_3-18
6	39.1 (CH)	1.69 (*m*)	H-7, H-3–19	H-4, -5, CH_3-18
7–16	29.6–32.2 (CH_2)	1.25–1.41 (*br, s*)		
17	14.3 (CH_3)	0.86 (*t*, 6.7)		
CH_3-18	11.2 (CH_3)	0.9 (*d*, 7.3)		
N-Acyl moiety				
1′	175.3 (C)			
2′	72.5 (CH)	4.63 (*dd*, 7.6, 4.0)	H-3′	HN, H-1′, -3′, -4′
3′	35.8 (CH_2)	2.24, 2.04 (*m*)	H-2′, -4′	H-2′, -4′
4′	25.9 (CH_2)	1.76 (*m*)	H-3′, -5′	H-2′, -3′
5′–21′*	29.6–32.2 (CH_2)	1.25–1.41 (*br, s*)		
22′**	14.3 (CH_3)	0.87 (*t*, 6.7)		
NH		8.58 (*d*, 8.8)	H-2	H-1′

[a]*21′ for **4,** 22′ for **5**, 23′ for **6**; ** 22′ for **4,** 23′ for **5**, 24′ for **6.** For abbreviation see Table 1.

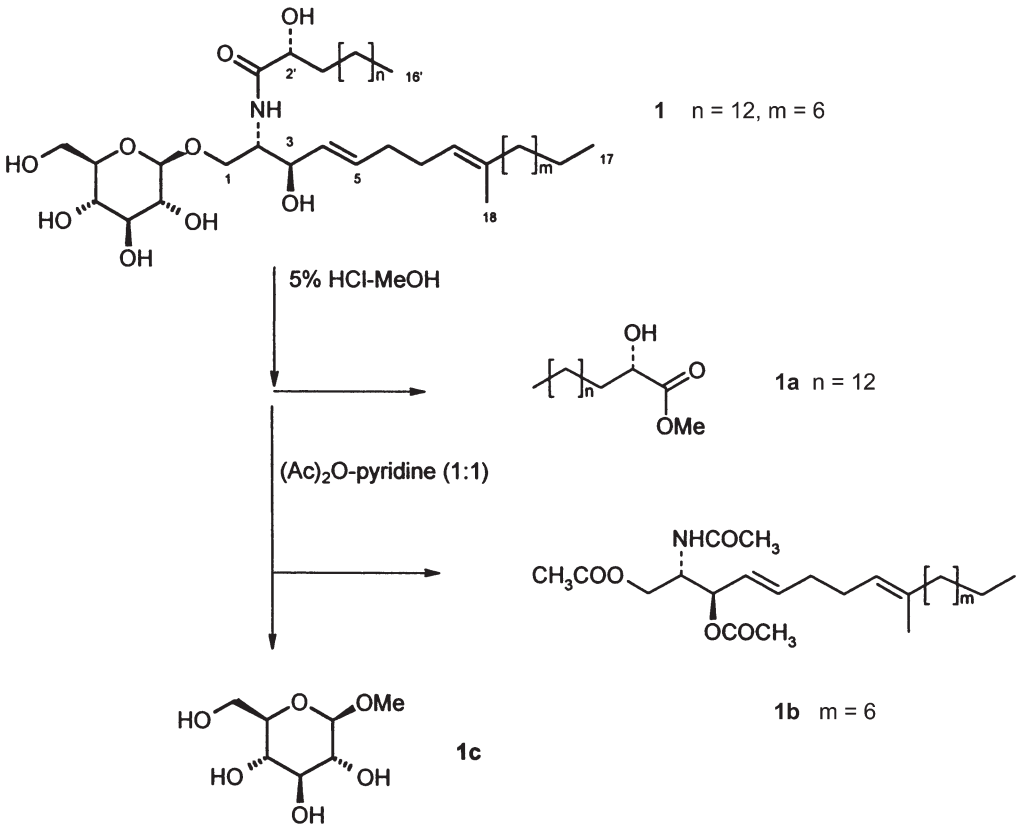
RESULTS AND DISCUSSION

The $CHCl_3$-soluble fraction of MeOH and $CHCl_3$/MeOH extract from the fruiting bodies of *C. umidicola* was subjected to repeated CC to yield compounds **1–6**. The structures of new compounds **1**, and **4–6** were elucidated as follows.

Compound **1** was isolated as an optically active amorphous solid ($[\alpha]_D^{20}$ +3.5°; *c* 0.15, $CHCl_3$). The IR spectrum displayed absorption bands of hydroxyl groups (3400 cm^{-1}, br.) and amide carbonyl (1652 cm^{-1}). Negative FABMS exhibited a molecular ion peak at *m/z* 713 ($[M]^-$) and the characteristic ion peak at *m/z* 550 ($[M - H - 162]^-$) with the loss of a hexose residue from the quasi-molecular ion. Its ^1H NMR spectrum presented an amide NH doublet at δ 8.33 (1H, *d*, *J* = 8.7 Hz; exchangeable with D_2O). The ^{13}C NMR (DEPT) spectrum of **1** showed the signals at δ 175.5 (C-1′) of a carbonyl carbon and δ 54.6 (C-2) of a methine carbon; both carbons were connected to amide nitrogen. The correlation peaks between NH and H-2 (δ 4.78, *m*) in ^1H-^1H COSY, and cross-peaks between NH and C-1′, H-2 and C-1′ in heteronuclear multiple bond correlation (HMBC) were observed. The existence of a methyl side chain and two long aliphatic chains in the molecule was provided by the presence of signals of three methyl groups [δ_C 14.1 (C-17), 14.4 (C-16′), 16.2 (CH_3-18); δ_H 0.86 (6H, *t*, CH_3-17, 16′), 1.61 (3H, *s*, CH_3-18)] and a complex region with overlapping signals characteristic of methylenes of long alkyl chain in the NMR spectra and with absorptions at 2924, 2853, and 721 cm^{-1} in the IR spectrum. All these analyses and comparisons of spectroscopic data with those of cerebrosides B and D strongly suggested the glycosphingolipid nature of **1** with the molecular formula $C_{40}H_{75}NO_9$ (11,12). From the reaction mixture from acidic methanolysis (Scheme 1), three fractions containing long-chain methyl esters (**1a**), triacetates of the sphingoid base (**1b**), and methyl glycosides (**1c**), respectively, were separated.

The sugar part of the molecular comprised a hexose unit that was attached at the C-1 position of the LCB, as indicated by the cross-peak between H-1″ (δ 4.88, 1H, *d*, *J* = 7.9 Hz) and C-1 (70.2) exhibited in the HMBC spectrum. The ^{13}C NMR spectrum displayed the signal of an anomeric carbon atom at δ 105.7, indicative of a β configuration of C-1″. The high-field chemical shift of proton H-5″ (δ 3.88, 1H, *ddd*, *J* = 9.0, 5.7, 2.5 Hz) identified the pyranose nature of the sugar moiety. The large coupling constants between H-2″ and H-3″, H-3″ and H-4″, and H-4″ and H-5″ (see Table 1), which evidenced the axial stereochemistry of all these protons, demonstrated the hexose as a glucose. The optical rotation of methyl glucoside ($[\alpha]_D^{20}$ +76.0°), separated from the methanolysis reaction mixture, was close to that of an authentic sample ($[\alpha]_D^{25}$ +77.3°), defining glucose as the D-isomer (19).

The cross-peak between H-2′ and H-3′ exhibited in ^1H-^1H COSY and correlations between NH and C-2′, H-3′, and the carbonyl carbon (C-1′) shown in the HMBC spectrum sug-



SCHEME 1

gested that the methylene of the fatty acyl chain is attached to an amide carbonyl only through an oxygenated methine carbon. Long-chain methyl α-hydroxy ester, one of the products of chemical degradation of **1**, was identified as methyl 2-hydroxyhexadecanoate (**1a**) by GC–MS analysis. It clearly revealed the length of the FA moiety of **1**. Moreover, the fragment ion at *m/z* 456 $[M - 2H - COCH(OH)C_{14}H_{29}]^+$, corresponding to the loss of the fatty acyl chain, and the ion at *m/z* 443 $[M - NHCOCH(OH)C_{14}H_{29}]^+$, corresponding to the loss of amide NH, along with the fatty acyl moiety displayed in negative FABMS, supported the GC–MS results. The optical rotation ($[\alpha]_D^{20}$ –5.7°; *c* 0.18, $CHCl_3$), which was very close to those of the methyl esters of 2-(*R*)-hydroxy FA reported earlier (20), identified the FAME as an *R*-isomer.

Apart from moieties of the sugar residue, FA, and the amide carbonyl group, the ^{13}C NMR spectrum exhibited signals at δ 72.4 (C-3) of oxygenated methine carbons and δ 70.2 (C-1) of an oxygenated methylene carbon, indicating the presence of 1,3-dihydroxy-sphinganines in LCB. This was also confirmed by the related correlation peaks shown in ^1H-^1H COSY and the HMBC spectrum. In addition, NMR showed the presence of two double bonds at δ_H 5.94 (1H, *dt*, *J* = 15.3 Hz, H-4), 5.97 (1H, *dd*, *J* = 15.3, 6.8 Hz, H-5), and 5.25 (1H, *m*, H-8) and δ_C 132.4 (C-4), 132.0 (C-5), 135.6 (C-8), and 124.2 (C-9). The correlation peaks between H-3 and H-4, H-4 and H-5, H-5 and H-6, and H-7 and H-8 in ^1H-^1H COSY, and the cross-peaks between H-2 and C-4, H-6 and C-4, H-3 and C-5, H-7 and C-5, H-8 and CH_3-18, H-10 and C-8, and H-7 and C-9 in the HMBC spectrum established the position of two double bonds at C-4 and C-8. It also indicated that C-9 was attached with a methyl side chain. The 4,5-alkene bond was found to be *trans*, as evidenced by the large vicinal coupling constants (*J* = 15.3 Hz). The *trans* geometry of 8,9 was established by the chemical shift of methyl (δ 16.2) attached to C-9 since the chemical shift of methyl carbons attached to a *trans* double bond appears at δ 15.4 ppm, whereas those of *cis* appear at 22.7 ppm (21). Thus, the sphingoid base of **1** was established to be (4*E*,8*E*)-9-methyl-4,8-sphingadienine (22). The EI-MS spectrum of **1b** exhibited characteristic ion peaks at *m/z* 423 $[M]^+$, 381 $[M - Ac]^+$, 363 $[M - CH_3COOH]^+$, 305 $[M - 2CH_3COOH]^+$, and 144 $[AcOCH_2CHNHAc + H]^+$, establishing **1b** as 2-acetoamino-6-methyl-1,3,4-triacetoxyheptadecane. The rotation of **1** ($[\alpha]_D^{20}$ +3.5°) is in accordance with those of natural (23) and synthetic analogs (9,24). This suggests that the absolute configuration of **1** at chiral centers C-2, -3, -2′ were *S, R*, and *R,* respectively, as for those of the analogs. Based on all the above facts, compound **1** was established as (2*S*,3*R*,4*E*,8*E*)-1-(β-D-glucopyranosyl)-3-hydroxy-2-[(*R*)-2′-hydroxyhexadecanoyl]amino-9-methyl-4,8-heptadecadiene.

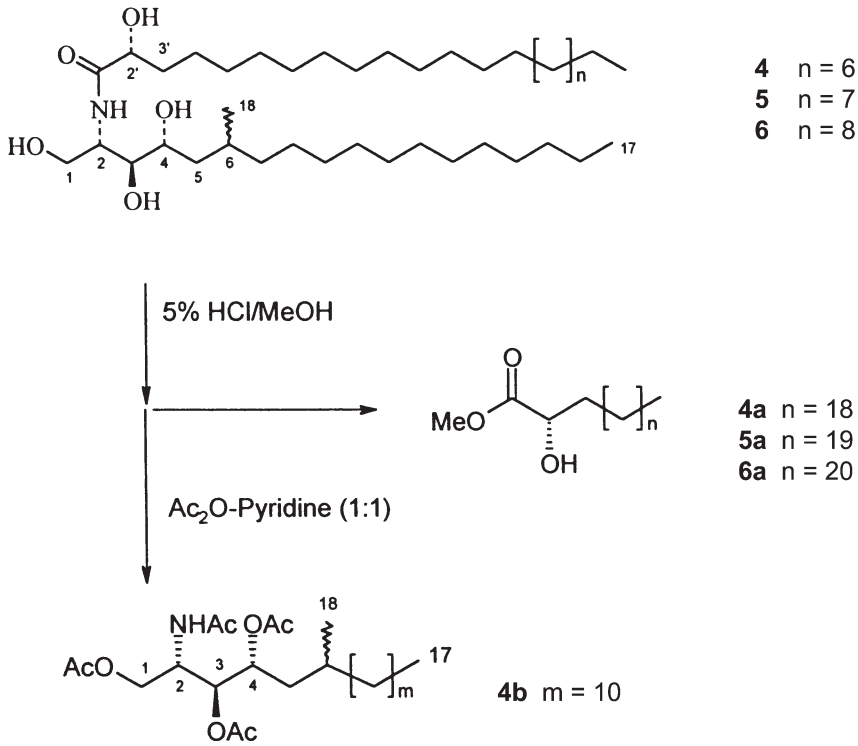
Several pseudomolecular ion peaks at *m/z* 655, 669, and 683 displayed in the negative ion fast atom bombardment mass spectra of compounds **4–6** proved to be a mixture of three congeners, in accordance with the molecular formula $C_{40}H_{81}NO_5 + nCH_2$ (*n* = 0–2). Its IR spectrum exhibited strong absorption bands for hydroxyl (3402 cm^{-1}, *br*), amide carbonyls (1644, 1635, 1628 cm^{-1}), and a long aliphatic chain (721 cm^{-1}). Resonance for two terminal methyl groups at δ 0.86 (6H, *t, J* = 6.7 Hz) and a branched methyl group at δ 0.90 (3H, *d*, *J* = 7.3 Hz, CH_3-18), together with the overlapped signals of methylenes at δ 1.24–1.41, indicated the existence of two long aliphatic chains and a methyl side chain. In addition, the ^1H NMR spectrum also presented a characteristic amide NH doublet at δ 8.58 (1H, *d*, *J* = 8.8 Hz; exchangeable with D_2O) and the ^{13}C NMR (DEPT) spectrum of **1** showed the presence of an amide functionality at δ 175.3 (C-1′) and 53.0 (C-2). This evidence led to the conclusion that compounds **4–6** were ceramides.

To determine the length of the FA and LCB of **4–6**, the mixture was treated as shown in Scheme 2 to afford long-chain methyl esters and peracetyl LCB. The long-chain methyl esters were identified as containing methyl 2-hydroxydocosanoate (**4a**), methyl 2-hydroxytricosanoate (**5a**), and methyl 2-hydroxytetracosanoate (**6a**) by the GC–MS analysis. The optical rotation ($[\alpha]_D^{20}$ –3.2°; *c* 0.09, C_5H_5N) of the mixture **4a–6a** is very close ($[\alpha]_D^{25}$ –3.0°; *c* 0.02, CHC_{13}) to that of the mixture of homologs reported earlier (25) with *R*-configuration at C-2, indicative that the stereochemistry of C-2 in **4a–6a** is *R*.

The sphingoid base was deduced to be 4-hydroxyl-6-methyl-sphinganine on the basis of ^1H-^1H COSY (see Table 2) and the HMBC spectrum, which showed correlation peaks between NH and C-1, NH and C-3, and the CH_3-18 group and C-6. EI–MS of peracetyl LCB displayed the molecular ion at *m/z* 485 $[M]^+$ and prominent fragment ions at *m/z* 425 $[M - CH_3COOH]^+$, 365 $[M - 2CH_3COOH]^+$, 305 $[M - 3CH_3COOH]^+$, 245 $[M - 4CH_3COOH]^+$, suggesting that the sphingosine part of **4–6** is 2-amino-1,3,4-trihydroxyl-6-methyl-heptadecane (**4b**).

In comparing the chemical shift of C-1, C-2, C-3, and C-4 with those of phytosphingosine-type LCB possessing (2*S*,3*S*,4*R*)-configurations (10,26,27), the relative configurations of C-2, C-3, and C-4 were predicted to be *S*, *R*, and *R*, respectively. The stereochemistry of position C-6 has not yet been determined. Based on the above facts, compounds **4–6** were established as (2*S*,3*S*,4*R*,2′*R*)-2-(2′-hydroxydocosanoylamino)heptadecane-6-methyl-1,3,4-triol, (2*S*,3*S*,4*R*,2′*R*)-2-(2′-hydroxytricosanoylamino)heptadecane-6-methyl-1,3,4-triol, and (2*S*,3*S*,4*R*,2′*R*)-(2′-hydroxytetracosanoylamino)-heptadecane-6-methyl-1,3,4-triol, respectively.

In nature, the most widely occurring sphingoid base is D-*erythro*-4*E*-sphingenine, whereas 6- and 9-branched methyls in the hydrocarbon chain are minor sphingoid bases. The present investigation demonstrated the presence in *C. umidicola* of a previously unrecognized sphingolipid and three ceramides, consisting of 9-methyl-4,8-sphingadienine in an amide linkage with a hydroxy FA; 9-methyl-4,8-sphingadienine in a β-glycosidic bond with glucose; and 6-methyl-4-hydroxysphingosine in amide linkage with different hydroxy FA, respectively. The natural occurrence of similar molecules has been found in a unique marine protist *Thraustochytrium globosum* (28), an imperfect fungus *Pachbasium* sp. (18), a



SCHEME 2

pathogenic fungus *Fusicoccum amygdali* (29), a sea anemone *Metridium senile* (30), and a basidiomycete *Polyporus ellisii* (8) and is presumed to be a characteristic component of lower organisms.

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