

# New Cerebrosides from the Basidiomycete *Cortinarius tenuipes*

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**ABSTRACT:** Five cerebrosides (**1**–**5**), including three new ones named cortenuamide A (**1**), cortenuamide B (**2**), and cortenuamide C (**3**), were isolated from the fruiting bodies of the basidiomycete *Cortinarius tenuipes*. The structures of those compounds were elucidated as (4*E*,8*E*)-*N*-D-2'-hydroxytetracosanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine (**1**), (4*E*,8*E*)-*N*-D-2'-hydroxytricosanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine (**2**), (4*E*,8*E*)-*N*-D-2'-hydroxydocosanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine (**3**), (4*E*,8*E*)-*N*-D-2'-hydroxyoctadecanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine (**4**), and (4*E*,8*E*)-*N*-D-2'-hydroxypalmitoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine (**5**) by spectral and chemical methods.

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*Cortinarius* (Cortinariaceae) is one of the largest genera in the subdivision Basidiomycotina in kingdom of fungi, comprising hundreds of species and widely distribute in the world (1). From the fruiting bodies of *Cortinarius* spp., a large number of toxins and/or pigments, including cyclic polypeptides (2), bipyridyl compounds (3), several types of anthraquinone derivatives, and chromogenic triterpenoids (4,5), have been isolated and characterized. Most investigations into the chemical constituents of *Cortinarius* have focused on toadstools in Europe and Australia. In continuing our studies on basidiomycete-derived bioactive secondary metabolites, we investigated chemical constituents of the mushroom *C. tenuipes*. This report describes the structural elucidation of five cerebrosides, including three new ones: (4*E*,8*E*)-*N*-D-2'-hydroxytetracosanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine (**1**), (4*E*,8*E*)-*N*-D-2'-hydroxytricosanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine (**2**) and (4*E*,8*E*)-*N*-D-2'-hydroxydocosanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine (**3**).

## EXPERIMENTAL PROCEDURES

**Instrumentation.** Melting points were obtained on an XRC-1 apparatus (Sichuan, People's Republic of China) and are uncorrected. Optical rotations were measured on a Horiba Sepa-300 polarimeter (Horiba, Tokyo, Japan). <sup>1</sup>H, and <sup>13</sup>C NMR and 2-D NMR spectra were recorded on Bruker AM-400 and

DRX-500 spectrometers (Karlsruhe, Germany), with chemical shifts ( $\delta$ ) in ppm relative to trimethylsilane (TMS) as internal standard and coupling constants in hertz (Hz). MS spectra were measured with a VG Autospec3000 mass spectrometer (VG, Manchester, England). IR spectra were obtained on a Bio-Rad FTS-135 IR spectrophotometer (Bio-Rad, Richmond, CA) with KBr pellets. GC–MS was performed on a Finnigan 4510 GC–MS spectrometer (San Jose, CA) employing the EI mode (ionizing potential 70 eV) and a capillary column (30 m  $\times$  0.25 mm) packed with 5% phenyl and 95% methylsilicone on 5% phenyl–dimethylsilicone (HP-5) (Hewlett-Packard, Palo Alto, CA). Helium was used as carrier gas, and the column temperature was increased from 160 to 240°C at a rate of 5°C/min.

**Materials.** Column chromatography was carried out on silica gel (200–300 mesh). TLC was carried out on plates pre-coated with silica gel F<sub>254</sub> (Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China). Reversed-phase chromatography was with RP-8 (LiChroprep, 40–63  $\mu$ m; Merck, Darmstadt, Germany).

**Fungal material.** Fresh fruiting bodies of *C. tenuipes* were collected at Tenchong, Yunnan Province, People's Republic of China, in July 1999 and identified by Prof. P.G. Liu, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, People's Republic of China. A voucher specimen is deposited at the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences, People's Republic of China.

**Extraction and isolation.** Fresh fruiting bodies of *C. tenuipes* (340 g) were extracted with 95% ethanol (5 L  $\times$  3), followed by extraction with chloroform/methanol (1:1, vol/vol) at 20°C. The combined extracts were concentrated *in vacuo* to give a crude extract, which was partitioned between water and chloroform to provide a chloroform-soluble extract (6.5 g) and a water-soluble fraction. The chloroform-soluble fraction was subjected to column chromatography by eluting with a solvent mixture of chloroform/methanol from 100:0 (vol/vol) to 70:30 (vol/vol) to give several fractions. The fraction eluted by chloroform/methanol (82:18, vol/vol) was concentrated to a small volume, and then a residue (0.32 g) was precipitated from the solution (one spot in normal TLC test). The components of the mixture were purified by reversed-phase chromatography (RP-8) by eluting with methanol/water from 50:50 (vol/vol) to 100% (vol/vol). Five cerebrosides—**1** (18.2 mg), **2** (5.5 mg), **3** (6.4 mg), **4** (13 mg), and **5** (32 mg)—were obtained as white powders.

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Abbreviations: DEPT, distortionless enhancement by polarization transfer; HR, high-resolution; LCB, long-chain base; TMS, trimethylsilane.

**Acidic methanolysis.** Compound **1** (12.1 mg) was refluxed with 1.5 mL of 0.9 M HCl in 82% (vol/vol) aqueous methanol at 80°C for 16 h. The reaction mixture was extracted with petroleum ether, and the petroleum ether layer was concentrated and chromatographed on TLC using silica gel (petroleum/ethyl acetate 9:1–7:3, vol/vol) to yield a FAME (**1b**) as a white solid, which was subjected to GC–MS. The result showed that **1b** was a methyl 2-hydroxytetracosanoate, which displayed major ion peaks at  $m/z$  398  $[M]^+$ , and 339  $[M - 59]^+$ ; its retention time was 35 min.  $[\alpha]_D = -4.5^\circ$  ( $c = 0.83$ ,  $CHCl_3$ ); EI–MS (70 eV)  $m/z$  398  $[M]^+$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ , in ppm)  $\delta$  4.19 (1H, *dd*,  $J = 4.2, 7.4$  Hz, H-2), 3.79 (3H, *s*,  $OCH_3$ ), 2.74 (1H, *bs*, OH), 1.76 (1H, *m*), 1.63 (1H, *m*), 1.10–1.25 (40H, *m*), and 0.88 (3H, *t*,  $J = 7.0$  Hz,  $CH_3$ ).

**Cortenuamide A (1).** High-resolution (HR) FABMS  $m/z$  838.6772  $[(M - 1)^-]$ , calcd. for  $C_{49}H_{92}NO_9$  838.6775; IR (KBr)  $\nu$  3441 (OH), 2925, 2857, 2840, 1646, 1536, 1465, 1079, 1036, 962, 720  $cm^{-1}$ ; EI–MS (70 eV)  $m/z$  (relative

intensity, %) 822  $[M - OH]^+$  (1), 660  $[M - Glu - OH]^+$  (3), 481 (4), 427 (8), 410 (20), 385 (11), 321 (7), 292 (4), 276 (8), 262 (7), 222 (12), 180 (4);  $^{13}C$  NMR ( $CD_3OD$ ), see Table 1.

**Cortenuamide B (2).** HR FABMS  $m/z$  824.6619  $[(M - 1)^-]$ , calcd. for  $C_{48}H_{90}NO_9$  824.6612; IR (KBr)  $\nu$  3439 (OH), 2924, 2859, 2844, 1645, 1537, 1468, 1080, 1033, 960, 723  $cm^{-1}$ ; EI–MS (70 eV)  $m/z$  (relative intensity, %) 808  $[M - OH]^+$  (1), 646  $[M - Glu - OH]^+$  (3), 466 (3), 412 (6), 395 (17), 370 (11), 292 (6), 276 (8), 262 (6), 222 (13), 180 (7);  $^1H$  NMR (500 MHz,  $CD_3OD$ ):  $\delta$  5.75 (1H, *dt*,  $J = 15.4, 5.8$  Hz, H-5), 5.51 (1H, *dd*,  $J = 15.4, 6.8$  Hz, H-4), 5.11 (1H, *bt*,  $J = 6.8$  Hz, H-8), 4.28 (1H, *d*,  $J = 7.8$  Hz, H-1''), 4.14 (1H, *t*,  $J = 6.8$  Hz, H-3), 4.11 (1H, *dd*,  $J = 10.4, 5.6$  Hz, H-1a), 4.00 (1H, *m*, H-2'), 3.75 (1H, *dd*,  $J = 10.4, 3.6$  Hz, H-1b), 3.34 (2H, *m*, H-6''), 3.30–3.27 (5H, *m*, H-2'', 3'', 4'', 5''), 2.10 (4H, *m*,  $H_2$ -10,  $H_2$ -7), 1.98 (2H, *m*,  $H_2$ -6), 1.61 (3H, *s*,  $H_3$ -19), 1.33 (2H, *m*, H-3'), 1.31–1.41 (aliphatic- $CH_2$ -), 0.92 (6H, *t*,  $J = 7.0$  Hz, Me-18, Me-24').  $^{13}C$  NMR ( $CD_3OD$ ), see Table 1.

**TABLE 1**  
 **$^{13}C$  NMR ( $CD_3OD$ ) Data of Cerebrosides 1–5<sup>a</sup>**

C (DEPT) <sup>b</sup>	$\delta_C$ (1)	$\delta_C$ (2)	$\delta_C$ (3)	$\delta_C$ (4)	$\delta_C$ (5)
1( $CH_2$ )	69.8	69.8	69.7	69.8	69.7
2(CH)	54.7	54.6	54.6	54.7	54.6
3(CH)	73.0	72.9	72.9	72.9	72.9
4(CH)	134.6	134.7	134.6	134.6	134.6
5(CH)	131.1	131.1	131.1	131.1	131.1
6( $CH_2$ )	33.1	33.1	33.1	33.1	33.0
7( $CH_2$ )	33.8	33.8	33.8	33.8	33.8
8(CH)	124.8	124.8	124.8	124.8	124.8
9(CH)	136.8	136.8	136.8	136.8	136.8
10( $CH_2$ )	40.8	40.8	40.8	40.8	40.7
18( $CH_3$ )	14.4	14.5	14.5	14.4	14.4
19( $CH_3$ )	16.2	16.2	16.2	16.2	16.2
1'(C)	177.2	177.2	177.2	177.2	177.1
2'(CH)	73.2	73.1	73.1	73.2	73.1
3'(CH)	35.9	35.9	35.9	35.9	35.9
16'( $CH_3$ )	—	—	—	—	14.4
18'( $CH_3$ )	—	—	—	14.4	—
22'( $CH_3$ )	—	—	14.5	—	—
23'( $CH_3$ )	—	14.5	—	—	—
24'( $CH_3$ )	14.4	—	—	—	—
1''(CH)	104.8	104.7	104.7	104.7	104.6
2''(CH)	75.0	75.0	75.0	75.0	75.0
3''(CH)	78.0	78.0	78.0	78.0	77.9
4''(CH)	71.7	71.6	71.6	71.6	71.6
5''(CH)	78.0	78.0	78.0	78.0	77.9
6''( $CH_2$ )	62.8	62.7	62.7	62.7	62.7
Aliphatic $-CH_2-$	33.8	33.8	33.8	33.8	33.8
	33.1	33.1	33.1	33.1	33.0
	30.7	30.8	30.8	30.8	30.8
	30.4	30.5	30.5	30.5	30.4
	29.1	29.1	29.1	29.1	29.1
	28.7	28.7	28.7	28.7	28.7
	26.1	26.2	26.2	26.1	26.1
	23.7	23.8	23.7	23.7	23.7

<sup>a</sup>Compound **1**, (4*E*,8*E*)-*N*-D-2'-hydroxytetracosanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine; **2**, (4*E*,8*E*)-*N*-D-2'-hydroxytricosanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine; **3**, (4*E*,8*E*)-*N*-D-2'-hydroxydocosanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine; **4**, (4*E*,8*E*)-*N*-D-2'-hydroxyoctadecanoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine; **5**, (4*E*,8*E*)-*N*-D-2'-hydroxypalmitoyl-1-*O*- $\beta$ -D-glycopyranosyl-9-methyl-4,8-sphingadienine.

<sup>b</sup>DEPT, distortionless enhancement by polarization transfer.

**Cortenuamide C (3).** HR FABMS  $m/z$  810.6459 [ $(M - 1)^-$ , calcd. for  $C_{47}H_{88}NO_9$  810.6449]; IR (KBr)  $\nu$  3444 (OH), 2927, 2863, 2843, 1647, 1537, 1466, 1081, 1035, 962, 721  $cm^{-1}$ ; EI-MS (70 eV)  $m/z$  (relative intensity, %) 794 [ $M - OH]^+$  (1), 632 [ $M - Glu - OH]^+$  (3), 452 (3), 398 (20), 381 (15), 356 (24), 292 (6), 276 (8), 262 (6), 222 (13), 180 (7);  $^1H$  NMR (500 MHz,  $CD_3OD$ ):  $\delta$  5.76 (1H, *dt*,  $J = 15.4, 5.8$  Hz, H-5), 5.52 (1H, *dd*,  $J = 15.6, 6.6$  Hz, H-4), 5.16 (1H, *bt*,  $J = 7.0$  Hz, H-8), 4.29 (1H, *d*,  $J = 8.0$  Hz, H-1''), 4.15 (1H, *t*,  $J = 7.0$  Hz, H-3), 4.14 (1H, *dd*,  $J = 10.2, 5.6$  Hz, H-1a), 4.02 (1H, *m*, H-2'), 3.77 (1H, *dd*,  $J = 10.2, 3.6$  Hz, H-1b), 3.36 (2H, *m*, H-6''), 3.34–3.33 (5H, *m*, H-2'', 3'', 4'', 5''), 2.12 (4H, *m*, H<sub>2</sub>-10, H<sub>2</sub>-7), 2.02 (2H, *m*, H<sub>2</sub>-6), 1.63 (3H, *s*, H<sub>3</sub>-19), 1.35 (2H, *m*, H-3'), 1.32–1.42 (aliphatic-CH<sub>2</sub>-), 0.94 (6H, *t*,  $J = 6.4$  Hz, Me-18, Me-24').  $^{13}C$  NMR ( $CD_3OD$ ), see Table 1.

(4*E*, 8*E*)-*N*-*D*-2'-Hydroxyoctadecanoyl-1-*O*- $\beta$ -*D*-glucopyranosyl-9-methyl-4,8-sphingadienine (4). FABMS (negative)  $m/z$  755 [ $M]^+$ ; EI-MS (70 eV)  $m/z$  (relative intensity, %) 755 [ $M]^+$  (1), 593 [ $M - Glu - OH]^+$  (2), 397 (11), 356 (9), 292 (8), 276 (5), 262 (6), 222 (10), 180 (12);  $^1H$  NMR (400 MHz,  $CD_3OD$ ):  $\delta$  5.72 (1H, *dt*,  $J = 15.2, 5.4$  Hz, H-5), 5.48 (1H, *dd*,  $J = 15.2, 7.0$  Hz, H-4), 5.07 (1H, *bt*,  $J = 7.0$  Hz, H-8), 4.26 (1H, *d*,  $J = 7.6$  Hz, H-1''), 4.10 (1H, *t*,  $J = 7.0$  Hz, H-3), 4.06 (1H, *dd*,  $J = 10.2, 5.4$  Hz, H-1a), 3.98 (1H, *m*, H-2'), 3.72 (1H, *dd*,  $J = 10.2, 3.6$  Hz, H-1b), 3.31 (2H, *m*, H-6''), 3.17–3.25 (5H, *m*, H-2'', 3'', 4'', 5''), 2.06 (4H, *m*, H<sub>2</sub>-10, H<sub>2</sub>-7), 1.95 (2H, *m*, H<sub>2</sub>-6), 1.55 (3H, *s*, H<sub>3</sub>-19), 1.31 (2H, *m*, H-3'), 1.28–1.40 (aliphatic-CH<sub>2</sub>-), 0.89 (6H, *t*,  $J = 6.6$  Hz, Me-18, Me-24').  $^{13}C$  NMR ( $CD_3OD$ ) see Table 1.

(4*E*, 8*E*)-*N*-*D*-2'-Hydroxypalmitoyl-1-*O*- $\beta$ -*D*-glucopyranosyl-9-methyl-4,8-sphingadienine (5). FABMS (negative)  $m/z$  727 [ $M]^+$ ; EI-MS (70 eV)  $m/z$  (relative intensity, %) 727 [ $M]^+$  (1), 709 [ $M - OH]^+$  (3), 548 [ $M - Glu - OH]^+$  (5), 508 (10), 397 (20), 372 (18), 262 (12), 222 (23), 180 (5);  $^1H$  NMR (400 MHz,  $CD_3OD$ ):  $\delta$  5.74 (1H, *dt*,  $J = 15.4, 5.4$  Hz, H-5), 5.51 (1H, *dd*,  $J = 15.4, 6.8$  Hz, H-4), 5.09 (1H, *bt*,  $J = 7.0$  Hz, H-8), 4.29 (1H, *d*,  $J = 7.8$  Hz, H-1''), 4.12 (1H, *t*,  $J = 6.8$  Hz, H-3), 4.07 (1H, *dd*,  $J = 10.2, 5.6$  Hz, H-1a), 4.01 (1H, *m*, H-2'), 3.73 (1H, *dd*,  $J = 10.2, 3.6$  Hz, H-1b), 3.33 (2H, *m*, H-6''), 3.19–3.25 (5H, *m*, H-2'', 3'', 4'', 5''), 2.08 (4H, *m*, H<sub>2</sub>-10, H<sub>2</sub>-7), 1.98 (2H, *m*, H<sub>2</sub>-6), 1.57 (3H, *s*, H<sub>3</sub>-19), 1.35 (2H, *m*, H-3'), 1.31–1.43 (aliphatic-CH<sub>2</sub>-), 0.93 (6H, *t*,  $J = 6.4$  Hz, Me-18, Me-24').  $^{13}C$  NMR ( $CD_3OD$ ), see Table 1.

2-Amino-1,3-dihydroxy-9-methyl-4,8-octadecanediene (1c). HR FAB. MS (positive)  $m/z$ : 438.3219 [ $(M + H)$ , calcd. for  $C_{47}H_{88}NO_9$  438.3235]; EI-MS  $m/z$ : 438 [ $M + 1]^+$  (5), 378 [ $M + 1 - HOAc]^+$  (45), 318 [ $M + 1 - 2 \times HOAc]^+$  (33), 257 [ $M - 3 \times HOAc]^+$  (3).  $^1H$  NMR (400 MHz,  $CDCl_3$ , in ppm):  $\delta$  5.38 (1H, *dt*,  $J = 15.4, 6.6$  Hz, H-5), 5.45 (1H, *dd*,  $J = 15.4, 6.8$  Hz, H-4), 4.25 (1H, *t*,  $J = 6.8$  Hz, H-3), 4.07 (1H, *m*, H-2), 2.16 (3H, *s*,  $COCH_3$ ), 2.08 (3H, *s*,  $COCH_3$ ), 2.01 (3H, *s*,  $COCH_3$ ), 1.98 (3H, *s*, Me-19), 1.85–1.97 (6H, *m*, CH<sub>2</sub>-6, CH<sub>2</sub>-7, CH<sub>2</sub>-10), 1.22 (aliphatic-CH<sub>2</sub>-), and 0.96 (3H, *t*,  $J = 7.0$  Hz, Me-18).

GC-MS data of 1b–4b. 1b: (48.25 min), EI-MS  $m/z$ : 398 (100), 366 (5), 339 (85), 320 (17); 2b: (40.89 min), EI-MS  $m/z$ : 384 (65), 352 (4), 325 (52), 306 (12); 3b: (37.12 min),

EI-MS  $m/z$ : 370 (75), 338 (3), 311 (62), 292 (18); 4b: (24.14 min), EI-MS  $m/z$ : 314 (80), 282 (7), 255 (71), 236 (15).

## RESULTS AND DISCUSSION

Cortenuamide A (1),  $C_{49}H_{93}O_9N$  (HR FABMS showed [ $M - 1]^+$   $m/z$  838.6772, calcd. for  $C_{49}H_{92}NO_9$  838.6775) was obtained as a white powder. Its IR spectrum exhibited strong hydroxyl absorption bands at 3440  $cm^{-1}$  and bands at 1646 and 1537  $cm^{-1}$  due to the amide group. EI-MS showed characteristic fragments ions at  $m/z$  660 [ $M - Glu - OH]^+$ , 367 [ $C_{24}H_{47}O_2]^+$ , 339 [ $C_{24}H_{47}O_2 - CO]^+$ , and 293 [ $M - Glu - OH - C_{24}H_{47}O_2]^+$ . In its  $^{13}C$  NMR spectrum, derived through distortionless enhancement by polarization transfer (DEPT), a *D*-glucopyranose moiety was indicated by the signals at  $\delta$  104.8 (CH), 75.0 (CH), 78.0 (CH), 71.7 (CH), 78.0 (CH), and 62.8 (CH<sub>2</sub>). The signal at  $\delta$  177.2 was assigned to an amide carbon, and the signals at  $\delta$  136.8 (C), 134.6 (CH), 131.1 (CH), and 124.8 (CH) showed the presence of two double bond groups, which were also revealed by the signals at  $\delta$  5.78 (1H, *m*), 5.52 (1H, *dd*,  $J = 15.5, 7.0$  Hz), and 5.18 (1H, *m*) in its  $^1H$  NMR spectrum. The signals at  $\delta_C$  16.2 (CH<sub>3</sub>),  $\delta_H$  1.62 (3H, *s*) were contributed by the methyl group vicinal to the quaternary double bond carbon, and the signals at  $\delta_C$  14.4 (CH<sub>3</sub>  $\times$  2),  $\delta_H$  0.92 (6H, *br*,  $J = 6.8$  Hz) were assigned to the two normal long-chain terminal methyl groups. Those data and literature precedents (6–8) led us to establish the structure of compound 1 as (4*E*, 8*E*)-*N*-*D*-2'-hydroxytetracosanoyl-1-*O*- $\beta$ -*D*-glucopyranosyl-9-methyl-4,8-sphingadienine. This conclusion could be further confirmed as follows. The coupling constant between C-4 and C-5 olefinic protons ( $J_{4,5} = 15.5$  Hz), and the chemical shift of the methyl carbon at  $\delta_C$  14.3 ppm (CH<sub>3</sub>-19) showed that the double bonds at positions 4 and 8 were all *E* geometry (7). The  $\beta$ -*D*-glucopyranoside linkage was assigned based on the coupling constant of the anomeric proton (4.30,  $J = 7.8$  Hz, H-1'') (7). Assembly of NMR data revealed that the absolute configuration of 1 should be the same as those of literature precedents (6), and the length of the two side chains was determined by means of acidic methanolysis (9) in which 1 gave a characteristic long-chain FAME (1a), a characteristic long-chain base (LCB), and a methyl glucoside. The structure of 1a, (2*R*)-2-hydroxy-tetracosanoyl, was confirmed by GC-MS analysis, which showed the molecular ion at  $m/z$  398 [ $C_{25}H_{50}O_3]^+$  and the characteristic fragment ion at 339 [ $M - COOCH_3]^+$ , and by its optical rotation value,  $[\alpha]_D^{15} = -3.8$  ( $c = 0.23$ ,  $CHCl_3$ ); these values were consistent with literature data (10–12). The LCB was determined as 2-amido-1,3-dihydroxy-9-methyl-4,8-octadecanediene (b) whose peracetylated derivative, 2-acetoamido-1,3-diacetoxy-9-methyl-4,8-octadecanediene (c), gave characteristic ion peaks at  $m/z$  438 [ $M + 1]^+$ , 378 [ $M + 1 - HOAc]^+$ , 318 [ $M + 1 - 2 \times HAc]^+$ , and 257 [ $M - 3 \times HAc]^+$ . That evidence as well as the 2-D NMR data (Fig. 1) further supported the structure of 1 as shown in Figure 2.

Cortenuamide B (2),  $C_{48}H_{91}O_9N$  (HR FABMS showed [ $M - 1]^+$   $m/z$  824.6619, calcd. for  $C_{49}H_{92}NO_9$  824.6612) and

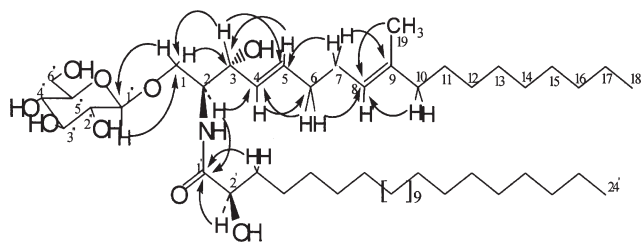


FIG. 1. Key heteronuclear multiple bond correlations of compound **1**.

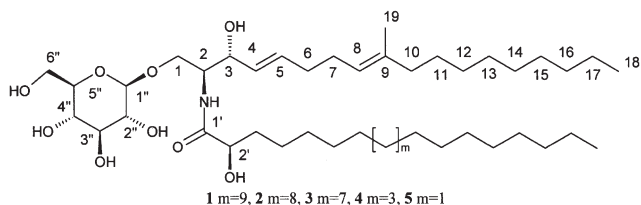


FIG. 2. Structures of five cerebrosides (**1**–**5**)

cortenuamide C (**3**),  $C_{47}H_{89}O_9N$  (HR FABMS showed  $[M - 1]^-$ ,  $m/z$  810.6459, calcd. for  $C_{49}H_{88}NO_9$  810.6449) were obtained as white powders. By comprehensive comparison, we found that the  $^1H$  and  $^{13}C$  NMR (DEPT) data for **2** and **3** were almost the same as those of cortenuamide A (**1**). This suggested that cortenuamide B and C were cerebrosides with the same basic skeleton and absolute chemical configuration of cortenuamide A. The difference between **2**, **3**, and **1** was that **2** contained (2*R*)-2-hydroxytricosanoyl (**2a**) and **3** contained (2*R*)-2-hydroxydocosanoyl (**3a**) instead of (2*R*)-2-hydroxytetracosanoyl (**1a**) in the FA moiety of compound **1**. This was indicated by GC–MS analysis of **2a** (major ion peaks:  $m/z$  398  $[M]^+$ , 339  $[M - 59]^+$ ), and **3a** (major ion peaks:  $m/z$  384  $[M]^+$ , 325  $[M - 59]^+$ ). Acidic methanolysis experiments with **2** and **3** yielded the same LCB as that of **1**, as confirmed by the EI–MS and  $^1H$  NMR of its peracetylated derivatives. The overall structures of cortenuamide B and C could then be established as (4*E*,8*E*)-*N*-D-2'-hydroxytricosanoyl-1-*O*-β-D-glycopyranosyl-9-methyl-4,8-sphingadienine (**2**) and (4*E*,8*E*)-*N*-D-2'-hydroxydocosanoyl-1-*O*-β-D-glycopyranosyl-9-methyl-4,8-sphingadienine (**3**).

Cerebrosides **4** and **5** were two known compounds whose structures were characterized as (4*E*,8*E*)-*N*-D-2'-hydroxyoctadecanoyl-1-*O*-β-D-glycopyranosyl-9-methyl-4,8-sphingadi-

enine (**4**) and (4*E*,8*E*)-*N*-D-2'-hydroxypalmitoyl-1-*O*-β-D-glycopyranosyl-9-methyl-4,8-sphingadienine (**5**) based on comparison spectroscopic (MS, IR,  $^1H$ , and  $^{13}C$  NMR) and physical data with **1**–**3** and the literature (6,7).

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