Dong Jin-Yan[a]
Li Ru[a]
He Hong-Ping[b]
Zhang Ke-Qin[a]

[a] Key Laboratory for
Conservation and Utilization of
Bioresources,
Yunnan University,
Kunming, Yunnan,
People's Republic of China
[b] Laboratory of Phytochemistry,
Kunming Institute of Botany,
Chinese Academy of Sciences,
Kunming, Yunnan,
People's Republic of China

Nematicidal sphingolipids from the freshwater fungus *Paraniesslia* sp. YMF1.01400

A new (**1**) and a known (**2**) sphingolipid were isolated from mycelial cultures of *Paraniesslia* sp. YMF1.01400. The structures of these two isolates were elucidated as (2*S*,2'*R*,3*R*,3'*E*,4*E*,8*E*)-1-O-(β-D-glucopyranosyl)-3-hydroxyl-2-[N-2'-hydroxyl-3'-eicosadecenoyl]amino-9-methyl-4,8-octadecadiene (**1**) and (2*S*,2'*R*,3*R*,3'*E*,4*E*,8*E*)-1-O-(β-D-glucopyranosyl)-3-hydroxyl-2-[N-2'-hydroxyl-3'-octadecenoyl]amino-9-methyl-4,8-octadecadiene (**2**) by spectroscopic and chemical methods. In *in vitro* tests, both compounds showed moderate nematicidal activities against *Bursaphelenchus xylophilus*. This is the first report of secondary metabolites in a genus *Paraniesslia* aquatic fungus.

Keywords: Sphingolipid, *Paraniesslia*, fungus, *Bursaphelenchus xylophilus*, cerebroside.

1 Introduction

Since pine wilt disease was discovered in Nanjing in 1982 [1], it has spread rapidly in China and become a serious problem for pine forests in China. There has been no effective means to control the disease. Although there are several registered nematicides in Japan, they are used only to protect valuable trees in parks because of their high cost [2]. There is an increasing need to find more acceptable alternatives. Fungi appear to be a source of effective pesticidal compounds and may come to be regarded as an inexhaustible source of harmless pesticides having low plant and human toxicity and being easily biodegradable [3]. Consequently, several groups of investigators have surveyed the toxicity of culture filtrates of fungi against *Bursaphelenchus xylophilus*, *Heterodera glycines*, *Meloidogyne incognita*, *M. javanica*, *Caenorhabditis elegans*, *Panagrellus redivivus*, and *Rotylenchulus reniformis* by means of immersion tests, and isolated some nematicidal metabolites such as alkaloids, peptides, terpenes, fatty acids *etc.* [4–10]. Among the selective nematicides reported lately, the most interesting is omphalotin A, a cyclic dodecapeptide isolated from *Omphalotus olearius*. Omphalotin A is very potent against the plant parasite *M. incognita* (LD$_{50}$: 0.75 μg/mL). It is considerably more active than the commercially available nematicide ivermectin, but is 50 times less active against the saprophytic *C. elegans* [11, 12].

During an ongoing screening for biologically active aquatic fungi, we have found that mycelial cultures of the fungal strain YMF1.01400 exhibit potent nematicidal activity against the pine wood nematode *B. xylophilus* [13]. The fungus was isolated from a submerged woody substrate collected in the freshwater habitat, and was identified as the genus *Paraniesslia* of the subfamily Niessliaceae of the Sordariomycetidae [14]. For the genus *Paraniesslia*, only one species, *Paraniesslia tuberculata*, was reported but no detailed phytochemical studies have appeared in the literature to the present day [15]. This prompted us to investigate its secondary metabolites. One new glycosphingolipid, (2*S*,2'*R*,3*R*,3'*E*,4*E*,8*E*)-1-O-(β-D-glucopyranosyl)-3-hydroxyl-2-[N-2'-hydroxyl-3'-eicosadecenoyl]amino-9-methyl-4,8-octadecadiene (**1**), was isolated along with one known glycosphingolipid, (2*S*,2'*R*,3*R*,3'*E*,4*E*,8*E*)-1-O-(β-D-glucopyranosyl)-3-hydroxyl-2-[N-2'-hydroxyl-3'-octadecenoyl]amino-9-methyl-4,8-octadecadiene (**2**). This paper reports the isolation, structure elucidation, and nematicidal activities of these two compounds.

2 Materials and methods

2.1 General

Optical rotations were measured on a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). The nuclear magnetic resonance (NMR) spectra were recorded on Bruker AM-400 (Bruker, Rheinstetten, Germany) and DRX-500 (Bruker, Karlsruhe, Germany) NMR spectrometers, with TMS as internal standard, and coupling constants were represented in Hz. Infrared (IR) spectra were obtained in KBr

Correspondence: Zhang Ke-Qin, Key Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Kunming, Yunnan 650091, People's Republic of China. Phone: +86–871–5032538, Fax: +86–871–5034838, e-mail: zhangkq-1@hotmail.com

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pellets with a Bio-Rad FTS-135 spectrophotometer (Bio-Rad, Richmond, CA, USA). EI-MS and FAB-MS data were taken on a VG Auto Spec-3000 mass spectrometer (VG, Manchester, UK), and GC-MS was performed with a HP 6890 GC-MS Spectrometer (Finnigan, San Jose, CA, USA) employing the electron impact (EI) mode (ionizing potential 70 eV) and a capillary column (30 m × 0.25 mm) packed with 5% phenyl-dimethylsilicone on HP-5 (Hewlett-Packard, Palo Alto, CA, USA). Helium was used as carrier gas, column temperature was 50–180 °C (rate of temperature increase: 15 °C/min), 180–240 °C (rate of temperature increase: 10 °C/min).

2.2 Materials

Column chromatography was performed on silica gel (200–300 mesh) (Qingdao Marine Chemical Ltd., Qingdao, China), Sephadex LH-20 gel (Pharmacia, Uppsala, Sweden) or Lichroprep RP C-8 gel (40–63 μm) (Merck, Darmstadt, Germany). Thin-layer chromatography was carried out on plates precoated with RP C-18 (Merck) and silica gel F_{254} (Qingdao Marine Chemical Ltd.), with compounds visualized by spraying with 5% (vol/vol) H_2SO_4 in ethanol.

2.3 Culture and fermentation of *Paraniesslia* sp. YMF1.01400

The fungal strain of *Paraniesslia* sp. YMF1.01400 was isolated from a submerged woody substrate collected in a freshwater habitat [14] and stored in the Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Yunnan Province, People's Republic of China. The strain was maintained on PDA medium (potato 200 g, sucrose 20 g, agar 18 g, water 1000 mL) and fermented with solid Sabouraud's medium (peptone 10 g, glucose 40 g, water 1000 mL) for 20 days at 26 °C.

2.4 Extraction and isolation

The mycelial cultures of *Paraniesslia* sp. YMF1.01400 were extracted successively with MeOH four times at room temperature. The combined extracts were concentrated to dryness *in vacuo* to give the residue (123 g), which was subjected to silica gel column chromatography using a solvent mixture of $CHCl_3/CH_3COCH_3$ with increasing concentrations of CH_3COCH_3 in $CHCl_3$ as eluent. The resulting fractions, eluted with $CHCl_3/CH_3COCH_3$ (5:5), were chromatographed over a RP C-8 column using CH_3OH/H_2O (9:1 to 7.5:2.5), followed by purification through Sephadex LH-20 column chromatography using CH_3OH to afford compounds **1** (30 mg) and **2** (56 mg).

(2S,2'R,3R,3'E,4E,8E)-1-O-(β-D-glucopyranosyl)-3-hydroxyl-2-[N-2'-'hydroxyl-3'-eicosadecenoyl]amino-9-methyl-4,8-octadecadiene (**1**): white amorphous powder; $[\alpha]_D^{21.3}$ –4.21 (c 0.14, CH_3OH); EI-MS (relative intensities, %): m/z 602 [M–179+1]$^+$ (4.0), 421 (9), 352 (33), 111 (55), 57 (73), 55 (100); (–)-FAB-MS (relative intensities, %): m/z 781 [M–1]$^+$ (100), 618 [M–162–2]$^+$ (14), (–)-HRFAB-MS: m/z 780.5989 (calcd. for $C_{45}H_{82}NO_9$, 780.5985); IR (KBr) ν_{max} 3401, 2945, 2852, 1643, 1535, 1469, 1382, 1230, 1083, 976, 720 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (400 MHz, CD_3OD) (see Tab. 1).

(2S,2'R,3R,3'E,4E,8E)-1-O-(β-D-glucopyranosyl)-3-hydroxyl-2-[N-2'-hydroxyl-3'-octadecenoyl]amino-9-methyl-4,8-octadecadiene (**2**): white amorphous powder; $[\alpha]_D^{21.9}$ –2.03° (c 0.24, CH_3OH); EI-MS (relative intensities, %): m/z 574 [M–179+1]$^+$ (5.0), 393 (13), 324 (35), 111 (59), 57 (70), 55 (100); (–)-FAB-MS (relative intensities, %): m/z 753 [M–1]$^+$ (100), 590 [M–162–2]$^+$ (17), (–)-HRFAB-MS: m/z 752.5679 (calcd. for $C_{43}H_{78}NO_9$, 752.5676); IR (KBr) ν_{max} 3407, 2921, 2850, 1641, 1535, 1467, 1380, 1232, 1085, 974, 722 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (400 MHz, CD_3OD) (see Tab. 1).

2.5 Methanolysis of compounds 1 and 2 and acetylation of the long-chain base

Compounds **1** (10 mg) and **2** (20 mg) were refluxed with 4 mL 1 M HCl in 82% aqueous methanol at 80 °C for 18 h. After adding H_2O, the resultant reaction mixture was extracted with *n*-hexane, and the *n*-hexane layer was concentrated and chromatographed on a silica gel column using *n*-hexane/ethyl acetate (8:2 to 7:3) to give the corresponding methyl ester of a fatty acid identified by GC-MS.

Methyl 2-hydroxyl-eicosadec-3-enoate, retention time 21.3 min, $[\alpha]_D^{18.2}$ –31.5° (c 0.13, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94 (1H, dt, J = 15.4, 6.5 Hz, H-4), 5.54 (1H, dd, J = 6.3, 15.4 Hz, H-3), 4.62 (1H, t, J = 6.3 Hz, H-2), 3.79 (3H, s, $COOCH_3$), 2.83 (1H, br s, OH), 2.08 (2H, m, H-5), 1.26–1.38 (24H, br s, methylenes), 0.94 (3H, t, J = 7.6 Hz, terminal methyl); EI-MS m/z (relative intensities, %): 340 [M]$^+$ (2.0), 281 [M–$COOCH_3$]$^+$ (100); IR (KBr) ν_{max} 3352, 2930, 1762, 1741, 1261, 1205, 1143, and 972 cm^{-1}.

Methyl 2-hydroxyl-octadec-3-enoate, retention time 18.6 min, $[\alpha]_D^{16.7}$ –41.8° (c 0.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 (1H, dt, J = 15.4, 6.5 Hz, H-4), 5.53 (1H, dd, J = 6.3, 15.4 Hz, H-3), 4.60 (1H, t, J = 6.3 Hz, H-2), 3.79 (3H, s, $COOCH_3$), 2.87 (1H, br s, OH), 2.08 (2H, m, H-5), 1.26–1.38 (24H, br s, methylenes), 0.94 (3H, t, J = 7.6 Hz, terminal methyl); EI-MS m/z (rela-

Tab. 1. NMR spectral data for compounds **1** and **2** (δ in ppm; J in Hz, CD$_3$OD).†

Position	1		2	
	^{13}C (DEPT)	^{1}H	^{13}C (DEPT)	^{1}H
1	69.7t	4.16 (dd, 10.5, 5.3)	69.5t	4.16 (dd, 10.5, 5.3)
	–	3.72 (m, 1H)	–	3.72 (m)
2	54.8d	3.98 (br s, 1H)	54.6d	3.97 (br s)
3	72.7d	4.11 (dd, 10.7, 7.6)	72.8d	4.09 (dd, 10.7, 7.6)
4	131.3d	5.46 (dd, 15.5, 6.6)	131.0d	5.42 (dd, 15.5, 6.6)
5	134.8d	5.73 (dt, 15.1)	134.5d	5.75 (dt, 15.1)
6	33.3t	2.04 (m)	33.1t	2.04 (m)
7	29.4t	2.04 (m)	29.2t	2.04 (m)
8	125.2d	5.14 (br)	124.9d	5.14 (br)
9	136.9s		136.7s	
10	40.5t	2.00 (t, 7.4)	40.8t	1.98 (t, 7.4)
11	34.0t	2.04 (m)	33.8t	2.04 (m)
12–16	29.4–31.0	1.28 (br s)	29.2–30.9	1.28 (br s)
17	24.0t	1.28 (m)	23.8t	1.28 (m)
18-CH$_3$	14.3q	0.91 (t, 6.9)	14.6q	0.91 (t, 6.9)
19-CH$_3$	16.3q	1.59 (s)	16.0q	1.59 (s)
NH		7.78 (d, 9.3)		7.73 (d, 9.3)
1'	175.8s		175.5s	
2'	74.2d	4.45 (d, 5.6)	74.0d	4.45 (d, 5.6)
3'	129.4d	5.50 (dd, 15.3, 6.0)	129.0d	5.56 (dd, 15.3, 6.0)
4'	134.8d	5.81 (dt, 15.3)	134.6d	5.86 (dt, 15.3)
5'	33.3t	2.04 (m)	33.1t	2.04 (m)
6'–18'/16'	29.4–31.0	1.28 (br s)	29.2–30.9	1.28 (br s)
19'/17'-CH$_3$	24.0t	1.28 (m)	23.8t	1.28 (m)
20'/18'-CH$_3$	14.3q	0.91 (t, 6.9)	14.6q	0.91 (t, 6.9)
1"	104.8d	4.28 (d, 7.7)	104.6d	4.28 (d, 7.7)
2"	75.0d	3.22 (t, 8.4)	74.9d	3.22 (t, 8.4)
3"	78.0d	3.36 (t, 8.8)	77.8d	3.36 (t, 8.8)
4"	71.8d	3.30 (m)	71.5d	3.30 (m)
5"	78.1d	3.30 (m)	77.9d	3.30 (m)
6"	62.3t	3.87 (d, 11.6)	62.6t	3.87 (d, 11.6)
	–	3.69 (dd, 11.6, 3.0)	–	3.66 (dd, 11.6, 3.0)

† Assignments were made by distortionless enhancement by polarization transfer (DEPT) and heteronuclear multiple quantum coherence (HMQC) analysis. HMBC-heteronuclear multiple bond coherence, ROESY, COSY-correlation spectroscopy.

tive intensities, %): 312 [M]$^+$ (1.0), 253 [M-COOCH$_3$]$^+$ (100); IR (KBr) v_{max} 3350(OH), 2934, 1760 (C = O), 1740, 1260, 1200, 1140, and 970 cm^{-1}.

The aqueous methanol layer was neutralized with sat. Na$_2$CO$_3$, concentrated to dryness and then heated with Ac$_2$O/pyridine (1:1) for 1.5 h at 70 °C. The reaction mixture was diluted with H$_2$O and extracted with EtOAc. The residue of the EtOAc fraction was chromatographed over silica gel using *n*-hexane/EtOAc (8:2) to yield a peracetate of the long-chain base, 2-acetamino-1,3-diacetoxy-9-methyl-4,8-octadecanediene. ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.75 (1H, m, H-5), 5.62 (1H, d, J = 9.2 Hz, NHAc), 5.40 (1H, m, H-4), 5.25 (1H, m, H-3), 5.01 (1H, m, H-8), 4.40 (1H, m, H-2), 4.25 (1H, dd, J = 11.5, 5.8 Hz, H-1a), 4.02

(1H, dd, J = 11.5, 3.4 Hz, H-1b), 2.04, 2.07 (each 3H, s, 2 × OAc), 2.01 (3H, s, HNAc), 1.94–2.16 (6H, m, H-6, H-7 and H-10), 1.59 (3H, s, H-19), 1.21–1.63 (12H, m), 0.90 (3H, t, J = 6.5 Hz, CH$_3$); EI-MS (70 eV) (relative intensities, %) m/z: 437 [M]$^+$ (3), 396 [M+1-Ac]$^+$ (4), 378 [M+1-HOAc]$^+$ (7), 318 [M+1-2 × HOAc]+ (11), 284 (5), 268 (35), 185 (22), 144 [AcOCH$_2$CHNHAc+H]$^+$ (46), 102 [144-Ac+1]+ (43), 84 [144-HOAc]$^+$ (96), 69 (70), 55 (100).

2.6 Methyl D-glucopyranosides

The remaining water layer was evaporated *in vacuo*. The residue was then subjected to silica gel column chromatography using CHCl$_3$/MeOH/H$_2$O (7:3:0.5) to give a mix-

ture of α- and β-anomers of methyl glucopyranoside. $[\alpha]_D^{16.7}$ +74.4° (c 0.13, MeOH), $[[\alpha]_D^{25.0}$ +77.3° (c 0.10, MeOH)] [16]; (−)-FAB-MS *m/z* 193 [M–1]⁻.

2.7 Nematicidal assay

Cultures of the nematode *B. xylophilus* were maintained in the Key Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Kunming, Yunnan, People's Republic of China. *B. xylophilus* was cultured on PDB agar medium containing a strain of *Botrytis cinerea* in disposable petri dishes. The cultures were stored at room temperature and subcultured prior to the assay. Nematicidal activity was performed in a 48-well microtiter plate as described in the literature [17]. Each compound (2 mg) was dissolved in DMSO (100 μL) and serially diluted with water to the concentrations of 2000, 200, and 20 μg/mL. Then, 50 μL of each serial solution was added to a well containing 30–50 nematodes in 50 μL water. Aqueous DMSO 5% was used as control. After incubation at 25 °C for 24 h in the dark, the numbers of live and dead nematodes were counted under a binocular microscope. The average mortality of three replications at each concentration and control was calculated, and LC_{50}, which was defined as the concentration causing 50% mortality, was determined. The LC_{50} values determined for both compound **1** and **2** were identical at 110 μg/mL.

3 Results and discussion

3.1 Identification of structures of compounds

The CH_3OH extract of mycelia cultures of *Paraniesslia* sp. YMF1.01400 was chromatographed successively on silica gel, RP C-8, and Sephadex LH-20 columns to lead to compounds **1** and **2**. Their structures were established by spectroscopic analysis and chemical transformation, as well as comparison with the data in the literature.

Compound **1** was assigned a molecular formula of $C_{45}H_{83}NO_9$ determined by negative-ion HRFAB-MS ([M–H]⁻ at *m/z* 780.5989 for $C_{45}H_{82}NO_9$, calcd. for $C_{45}H_{82}NO_9$, 780.5985), as well as from its ^{13}C NMR data. An IR absorption band at 3401 cm⁻¹ indicated the presence of OH and amide groups. The typical IR absorptions at 1535, 1643 cm–1 again suggested a secondary amide, as supported by the presence of a nitrogen-attached carbon signal at δ 54.8 and a carbonyl signal at δ 175.8 in the ^{13}C NMR (Tab. 1). In the ^1H NMR spectrum, one olefinic signal at δ 5.14 (1H, br s, H-8), assignable to a trisubstituted C = C bond, and four olefinic signals (δ 5.46 (1H, dd, J = 15.5, 6.6 Hz, H-4), 5.50 (1H, dd, J = 15.3, 6.0 Hz, H-3'), 5.73 (1H, dt, J = 15.1 Hz, H-5), 5.81 (1H, dt,

J = 15.3Hz, H-4')), attributable to the presence of two disubstituted C = C bonds, were evident. The ^1H NMR spectrum also showed the presence of two terminal methyl groups at δ 14.6 and an allylic methyl at δ 16.0, indicating the presence of either two long aliphatic chains or a single branched aliphatic chain. Comparison of the ^1H and ^{13}C NMR data of **1** with those of cerebrosides A–D [18] indicated that compound **1** is most likely a glycosphingolipid analogue. The amino alcohol moiety could be identified as a sphingosine unit by the characteristic signals that appeared in the ^1H and ^{13}C NMR spectra, especially due to the presence of typical Δ^4 and Δ^8 double bonds and a Me-19 group [19]. A glucopyranose moiety could be distinguished in the structure of **1** on the basis of ^1H and ^{13}C NMR spectra. Methanolysis [20] of compound **1** afforded 2-hydroxyeicos-3-enoic acid methyl ester, which was identified by GC-MS. The double bond was located in between C-3' and C-4' by HMBC experiments with compound **1** (see Fig. 1).

The linkages of the three parts of the molecule of **1** were resolved from the ^1H-^1H COSY, HMQC, HMBC and ROESY spectrum (see Fig. 1). A correlation from δ 7.78 (NH) to δ 3.98 (1H, br s, H-2) and correlations from δ 7.78 (NH) to δ 175.8 (C-1'), 54.8 (C-2), and 74.2 (C-2') were observed in the ^1H-^1H COSY and HMBC spectra of 1, respectively. The correlation between H-1/H-2, H-2/H-3, H-3/H-4, H-4/H-5, H-5/H-6, H-6/H-7, H-7/H-8 was observed. The above correlation analysis unambiguously assigned the positions of the two double bonds at C-4 and C-8, respectively. This was further supported by the HMBC spectrum of **1**, which displayed the correlation between H-1 and C-3, H-2 and C-4, H-3 and C-5, H-4 and C-6, H-5 and C-7, H-6 and C-4, H-7 and C-9, H-8 and C-10. On the other hand, the presence of a C-19 allylic methyl group in the branched long-chain base was also confirmed by the HMBC spectrum in which the correlation between H-8 and C-19 was observed [19]. HMBC correlation between H-1''/C-1 suggested that the glucopyranose unit was attached at the C-1 position of the amide acyl chain [21, 22]. This was also supported by the
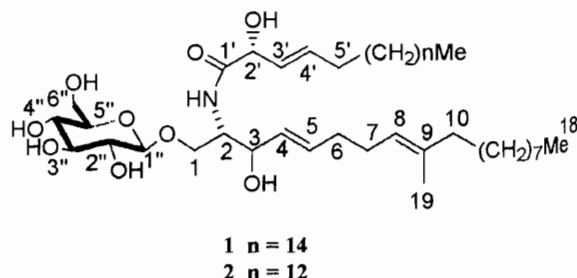

Fig. 1. 1H-1H COSY and key HMBC correlations for compound **1**.

cross-peaks between H-1a/H-1" and H-1b/H-1" in the ROESY spectrum [22]. The planar structure of **1** was thus formulated. The assignments (Tab. 1) of the ^{1}H NMR and ^{13}C NMR data of **1** were achieved unambiguously from the ^{1}H-^{1}H COSY, HMQC, HMBC, ROESY spectra.

The large vicinal coupling constant J (4, 5) (15 Hz), and J (3', 4') (15.3 Hz) clearly indicated two (E)-configured C = C bonds in the 4 and 3' positions. The *trans* geometry of these two double bonds was also supported by the chemical shift of C-6 (δ 33.3t) and C-5' (δ 33.3t). Usually, the signals of carbons next to a *trans* double bond appear at δ 32–33, whereas those of a *cis* double bond appear at δ 27–28 [18, 22]. The alkene bond at C-8/C-9 was also assigned an (E)-configuration on the basis of the upfield-shifted ^{13}C NMR signal for CH$_3$ [18, 22]. The carbon signals of the sugar moiety in the ^{13}C NMR spectrum suggested a β-configuration, which was confirmed by the large coupling constants of H-1" at δ 4.28 (1H, d, J = 7.7 Hz) and H-2" at δ 3.22 (1H, t, J = 8.4 Hz) [23, 24]. On the basis of the optical rotation value ([α]$_D^{167}$ +74.4°) of the methyl glucoside, afforded by methanolysis of **1**, the sugar moiety of **1** was assigned as β-D-glucopyranosyl. By considering biogenetic and steric factors, the chemical shift of H-2 and the chemical shifts of the carbon signals of C-1 to C-3, C-1', and C-2' of gluco-sphingolipids and sphingolipids may be utilized to determine their absolute stereochemistry [24–27]. The proton signal at δ 3.98 (1H, br s, H-2) and the carbon signals at δ 69.7t (C-1), 54.8d (C-2), 72.7d (C-3), 175.8s (C-1'), and 74.2d (C-2') of **1** were in good agreement with those reported for glycosphingonines (as model structures) with a 2S,3R,2'R configuration [18, 28]. The structure of **1** was assigned therefore as (2S,2'R,3R,3'E,4E,8E)-1-O-(β-D-glucopyranosyl)-3-hydroxyl-2-[N-2'-hydroxyl-3'-eicosadecenoyl]amino-9-methyl-4,8-octadecadiene, as shown in Fig. 2.

Compound **2** was assigned a molecular formula of C$_{43}$H$_{79}$NO$_9$ determined by negative-ion HRFAB-MS ([M−H]$^-$ at m/z 752.5679 for C$_{43}$H$_{78}$NO$_9$, calcd. for C$_{43}$H$_{78}$NO$_9$, 752.5676), as well as from its ^{13}C NMR data. It showed the same ^{1}H NMR and ^{13}C NMR data (Tab. 1) as **1**, ind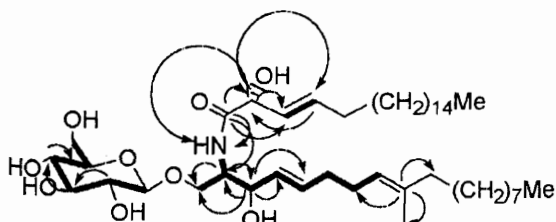icating it to be a molecular species of a 9-methyl-sphinga-4,8-dienine-type ceramide glucoside possessing a 2-hydroxy-3,4-dehydro fatty acid unit. The molecular mass of **2** was 28 (2 × CH$_2$) units lower than that of **1**. Further methanolysis gave a methyl 2-hydroxyl-octadec-3-enoate identified by GC-MS. From the above evidence and comparison of the physicochemical properties with the reported data, compound **2** was characterized as (2S,2'R,3R,3'E,4E,8E)-1-O-(β-D-glucopyranosyl)-3-hydroxyl-2-[N-2'-hydroxyl-3'-octadecenoyl]amino-9-methyl-4,8-octadecadiene, which was previously obtained from *Penicillium funiculosum* and *Pachybasium* sp. (= cere-broside C) [18, 29] and confirmed by the synthesis [30]. Thus, the structure of compound **2** was characterized as shown in Fig. 2.

The present study has demonstrated the presence in *Paraniesslia* sp. YMF1.01400 of a previously unrecognized cerebroside and a known cerebroside, consisting of 9-methyl-4,8-sphingadienine in amide linkage with an α-hydroxyl-β,γ-dehydro fatty acid and in β-glycosidic bond with glucose, respectively. In nature, the most widely occurring sphingoid base is D-erythro-4(E)-sphingenin, whereas branched (4E,8E)-sphingadienines having two double bonds in the hydrocarbon chain are minor sphingoid bases. So far, this type of cerebrosides has only been found in lower organisms such as the sea anemone *Metridium senile* [31], the marine sponge *Ircinia fasciculate* [32], yeast [33], the marine microorganism *Thraustochytrium globosum* [34], and the fungi *Catathelasma ventricosa* [19], *Fusicoccum amygdale* [35], *Ganoderma lucidum* [36], *Lactarium volemus* [37], *Leccinum extremiorientale* [38], *Mortierella alpine* [39], *Polyporus ellisii* [40], *Pachybasium* sp. [18], *Tuber indicum* [21], *etc*. Thus, the branched nonadecasphingadienine is presumed to be a characteristic component in cerebrosides from lower organisms. From the viewpoint of comparative biochemistry, it will be of considerable interest to elucidate fully its distribution and also to investigate the physiological significance of the 9-methyl branch, as well as the biosynthetic pathway. It should be noted that the occurrence of structurally closely related sphingolipid derivatives in taxonomically remote species is very intriguing and may indicate the connection with a common producer, probably symbiotic microorganisms.

The α-hydroxyl-β,γ-dehydro fatty acid contained in compound **1** and **2** comes, to our knowledge, seldom from natural sources, although many ceramides of α-hydroxy fatty acid have been obtained from marine invertebrates, invertebrates, algae, and fungi *etc*. [41–44]. Thus, this is the first report of secondary metabolites in a genus *Paraniesslia* fungus, and also the first report on the cerebrosides present in an aquatic fungus.

Fig. 2. Structures of two glycosphingolipids.

3.2 Nematicidal activity

In an assessment of nematicidal activity against *B. xylophilus*, compounds **1** and **2** showed identical moderate activities, both with LC_{50} values at 110 µg/mL.

Cerebroside C has been reported as an inducer of cell differentiation in the fungus *Schizophyllum commune*, stimulating the formation of the fruiting body [29], and potentiated the antifungal activity of aculeacin against *Candida albicans* [18]. A number of reports have described physiological activities for cerebrosides [36, 45–49]. Sphingolipids have been shown to be potent and specific inhibitors of protein kinase C, Na- and K-ATPase, calmodulin kinase, and eukaryotic DNA polymerases of the α-type, and activate phospholipase C, phospholipase D, casein kinase α, tyrosine kinase, and DG kinase [50–52]. It has also been reported that sphingolipids can induce Ca^{2+} release [53], proliferate cells [54], protect human keratinocytes from apoptosis [55], inhibit colon carcinogenesis in mice [56] and enhance apoptosis of radiation-resistant prostate cancer cells [57]. But according to our knowledge, this is the first report on the nematicidal activity of cerebrosides.

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