Morinagadepsin, a Depsipeptide from the Fungus Morinagamyces vermicularis gen. et comb. nov.

Karen Harms 1,2, Frank Surup 1,2,*, Marc Stadler 1,2, Alberto Miguel Stchigel 3 and Yasmina Marin-Felix 1,*

1 Department Microbial Drugs, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany; Karen.Harms@helmholtz-hzi.de (K.H.); Marc.Stadler@helmholtz-hzi.de (M.S.)
2 Institute of Microbiology, Technische Universität Braunschweig, Spielmannstrasse 7, 38106 Braunschweig, Germany
3 Mycology Unit, Medical School and IISPV, Universitat Rovira i Virgili, C/ Sant Llorenç 21, 43201 Reus, Tarragona, Spain; albertomiguel.stchigel@urv.cat
* Correspondence: Frank.Surup@helmholtz-hzi.de (F.S.); Yasmina.MarinFelix@helmholtz-hzi.de (Y.M.-F.)

Abstract: The new genus Morinagamyces is introduced herein to accommodate the fungus Apiosordaria vermicularis as inferred from a phylogenetic study based on sequences of the internal transcribed spacer region (ITS), the nuclear rDNA large subunit (LSU), and partial fragments of ribosomal polymerase II subunit 2 (rpb2) and ß-tubulin (tub2) genes. Morinagamyces vermicularis was analyzed for the production of secondary metabolites, resulting in the isolation of a new depsipeptide named morinagadepsin (1), and the already known chaetone B (3). While the planar structure of 1 was elucidated by extensive 1D- and 2D-NMR analysis and high-resolution mass spectrometry, the absolute configuration of the building blocks Ala, Val, and Leu was determined as -l by Marfey’s method. The configuration of the 3-hydroxy-2-methyldecanyl unit was assigned as 22R,23R by j-based configuration analysis and Mosher’s method after partial hydrolysis of the morinagadepsin to the linear derivative compound 2. Compound 1 showed cytotoxic activity against the mammalian cell lines KB3.1 and L929, but no antimicrobial activity against the fungi and bacteria tested was observed, while 2 was inactive. Compound 3 was weakly cytotoxic against the cell line L929, but did not show any antimicrobial activity.

Keywords: cytotoxicity; depsipeptide; Morinagamyces; morinagadepsin; Schizotheciaceae; secondary metabolites; Sordariales

1. Introduction

The genus Apiosordaria was introduced by von Arx and Gams in 1967 to accommodate Pleurago verruculosa, which differs from the other species of the genus by its ornamented ascospores [1]. Apiosordaria included species with two-celled ascospores with an ellipsoidal to subglobose ornamented upper cell, and with a triangular to cylindrical mostly smooth-walled lower cell [1–3]. The genera Apiosordaria and Triangularia were traditionally segregated by the shape of the upper cell of their ascospores, which are more or less conical in Triangularia fide Guarro and Cano [2]. However, in a recent phylogenetic study, the type strains of both genera were placed in the same monophyletic clade of the family Podosporaceae, resulting in the synonymization of Apiosordaria with Triangularia [4]. However, most species of Apiosordaria remained with an uncertain taxonomic placement. Subsequently, A. sacchari and A. striatipora were transferred to Triangularia, and A. globosa, A. hispanica, and A. vestita to Jugulospora, based on phylogenetic and morphological data [5]. In the present phylogenetic study, based on analysis of sequences of the internal transcribed spacer region (ITS), the nuclear rDNA large subunit (LSU), and fragments of ribosomal polymerase II subunit 2 (rpb2) and ß-tubulin (tub2) genes, the new genus Morinagamyces is introduced to accommodate A. vermicularis, with phylogenetic affiliation to the recently established family Schizotheciaceae [5].
The order Sordariales includes producers of a great diversity of biologically active secondary metabolites [6–9], with potential uses as drugs. In this context, the ex-type strain of Morinagamyces vermicularis was tested for the production of bioactive compounds, leading to the isolation of a new depsipeptide named morinagadepsin (1), whose structure elucidation, antimicrobial activity, and cytotoxicity are presented herein. Chaetone B (3) was also produced, and its antimicrobial and cytotoxic activity tested.

2. Materials and Methods

2.1. Molecular Study

DNA of the ex-type strain of Apiosordaria vermicularis was extracted and purified directly from colonies according to the Fast DNA Kit protocol (MP Biomedicals, Solon, Ohio). The amplification of the ITS, D1–D3 domains of the LSU, rpb2, and tub2 was performed according to White et al. [10] (ITS), Vilgalys and Hester [11] (LSU), and Miller and Huhndorf [12] (rpb2 and tub2). PCR products were purified and sequenced at Macrogen Europe (Amsterdam, The Netherlands) with a 3730XL DNA analyzer (Applied Biosystems). Consensus sequences were obtained using SeqMan (version 7.0.0; DNASTAR, Madison, WI, USA). The phylogenetic analysis was carried out based on the combination of the four loci (ITS, LSU, rpb2, and tub2) sequences of the ex-type strain of A. vermicularis and selected members of the Sordariales, with Camarops amorpha SMH 1450 as outgroup. Each locus was aligned separately using MAFFT v. 7 [13] and manually adjusted in MEGA v. 10.2.4 [14]. Individual gene phylogenies were checked for conflicts before the four loci datasets were concatenated [15,16]. The Maximum Likelihood (ML) and Bayesian Inference (BI) analysis including the four loci were performed as described by Harms et al. [9]. The best evolutionary model for each sequence dataset was calculated using MrModeltest v. 2.3 [17]. Bootstrap support (bs) ≥ 70% and posterior probability values (pp) ≥ 0.95 were considered significant [18]. The sequences generated in this study are deposited in GenBank (Table 1) and the alignment used in the phylogenetic analysis is deposited in TreeBASE (S28234).

Table 1. Strains of the order Sordariales included in the phylogenetic study. GenBank accession numbers in bold correspond to the newly generated sequences. Taxonomic novelty is indicated in italic bold.

| Taxa                          | Strain      | GenBank Accession Number | Source |
|-------------------------------|-------------|--------------------------|--------|
|                               |             | LSU | ITS | rpb2 | tub2 |        |
| Anopodium ampullaceum*        | MJR 40/07   | KF557662 - - KF557701    |        |
| Apiosordaria microcarpa*       | CBS 692.82T | MK926841 MK926841 MK876803 - |        |
| Arotheca ambiguia             | CBS 215.60  | AY999114 AY999137 - -     | [20]   |
| Arotheca areolata             | UAMH 7495   | AY587936 AY587911 AY600275 AY600252 |        |
| Arnium cirriferum*            | CBS 120041  | KF557673 - - KF557709     |        |
| Arnium hirtum*                | E00204950   | KF557675 - - KF557711     |        |
| Belloisia rhynchostoma*       | CBS 118484  | EU999217 - -             | [22]   |
| Camarops amorpha              | SMH 1450    | AY780854 AY780938 AY780903 - |        |
| Cercophora mirabilis          | CBS 120402  | KF91429 MT784128 KP981611 KP981556 |        |
| Cercophora sparsa*            | JF 0029     | AY587937 AY587912 - AY600253 |        |
| Cercophora sulphurella*       | SMH 2531    | AY587938 AY587913 AY600276 AY600254 |        |
| Chaetomium globosum           | CBS 160.62T | MH899713 KT214565 KT214666 - | [23,24]|
| Cladorrhinum fuscundissimum   | CBS 180.66T | MK926856 MK926856 MK926856 MK926856 |        |
| Cladorrhinum hyalocarpyum     | CBS 322.70T | MK926857 MK926857 MK926857 MK926857 |        |
| Cladorrhinum intermedium      | CBS 433.96T | MK926859 MK926859 MK926859 MK926859 |        |
| Corylomyces selenosporus*     | CBS 113930T | DQ327607 MT784130 KP981612 KP981557 |        |
| Corynascus sepedonium*        | CBS 111.69T | MH871003 MH859271 FJ666394 - | [23,26]|
| Corynascus humicola           | CBS 337.72T | MH872209 MH860493 - -     | [23]   |
| Diplogelasinospora grovesii   | CBS 340.73T | MH872401 MH860693 - -     | [23]   |
| Diplogelasinospora madensis   | CBS 136018T | KP981430 HG514152 KP981613 KP981558 |        |
| Diplogelasinospora princeps   | FMR 13415   | KP981432 KP981432 KP981432 KP981432 |        |
| Echria gigantospora           | F77-1       | KS57674 - - KS57710       | [19]   |
| Echria macrotheca             | Lundqvist 2311 | KS57684 - - KS57715       | [19]   |
| Taxa                                      | Strain     | GenBank Accession Number | Source                           |
|-------------------------------------------|------------|--------------------------|----------------------------------|
| Immersiella caudata                       | SMH 3298   | AY436407 -              | AY780161 AY780101 [12,28]        |
| Immersiella inermis                       | SMH 4104   | AY436409 -              | AY780181 AY780123 [12,28]        |
| Jugulospora antarctica                    | IMI 381338 | KP981433 -              | KP981616 KP981561 [5]            |
| Jugulospora carbonaria                    | ATCC 34567 | AY436302 -              | AY780196 AY780141 [12,29]        |
| Jugulospora rotula                        | CBS 110112 | KP981434 -              | KP981617 KP981562 [5]            |
|                                         | CBS 110113 | KP981435 -              | KP981618 KP981563 [5]            |
|                                          | FMR 12690  | KP981437 MT784133 -     | KP981620 KP981565 [5]            |
|                                          | FMR 12781  | KP981438 MT784134 -     | KP981621 KP981566 [5]            |
| Jugulospora vestita                       | CBS 135.91T| MT785872 MT784135 MT783824 MT783825 | [5] |
| Lasiosphaeria globatibiza                 | TL 4529    | AY436410 AY587914 -     | AY600277 AY600255 [21,28]        |
| Lasiosphaeria ovina                       | SMH 1538   | AFO6463 AY587926 -      | AY600287 FA66046 [21,30,31]      |
| Lasiosphaeria rugulosos                   | SMH 1518   | AY436414 AY587933 -     | AY600294 AY600272 [21,28]        |
| Lundquistomyces karahicoesi               | CBS 657.74 | KP981447 MK926850 KP981630 KP981478 [4,5] |
| Lundquistomyces tanzaniaesi               | TRTC 51981T| AY780081 MH862260 AY780197 AY780143 | [12,29] |
| Morinagamys verrucularis                  | CBS 303.81T| KP981427 MT904879 KP981609 KP981554 | Present study |
| Nahiculaeus terrestris                    | CBS 137295T| KP981439 MT784136 KP981622 KP981567 [5] |
| Neurospora panonica                       | TRTC 51327 | AY800070 -              | AY80185 AY80126 [12]            |
| Podospora duda-myae                       | CBS 232.78 | AY999100 AY999127 -     | - [20]                          |
| Podospora funicola                        | CBS 482.64ET| KP981440 MK926862 KP981623 KP981568 [4,5] |
| Podospora sacchari                        | CBS 713.70T| KP981425 MH859915 KP981607 KP981552 [5,23] |
| Podospora striatissora                    | CBS 154.77T| KP981426 MT784137 KP981608 KP981553 [5] |
| Pseudoechia curvicolli                    | CBS 259.69 | MH8571036 MH859302 -   | [23]                            |
| Pseudoechia decipiens                     | CBS 254.71T| MK926842 MK926842 MK876804 - [4] |
| Pseudoechia. prolifica                    | CBS 250.71T| MK926848 MK926848 MK876810 - [5] |
| Pseudoneurospora canariensis              | FMR 12156T | MH877580 -              | - [23,27]                       |
| Pseudoschlomophila mangetoni              | CBS 419.67T| KP981444 MT784143 KP981627 KP981571 [5] |
| Pseudoschlomophila marina                 | CBS 155.77T| MK926851 MK926851 MK876813 - [4] |
| Pseudoschlomophila philippica             | CBS 698.96T| MK926853 MK926853 MK876815 - [4] |
| Pseudoschlomophila atropturpuricem        | CBS 143.73T| MK926852 MK926852 MK876814 - [4] |
| Rinaldiella pentagonospora                | CBS 132344T| KP981442 MH866007 KP981625 KP981570 [5,23] |
| Rhopophila coehleariformis                | CBS 249.71 | AY999098 AY999123 -     | - [20]                          |
| Rhopophila decipiens                      | CBS 258.69 | AY780073 KX171946 AY780187 AY780130 [12, Miller [unpubl. data] |
| Rhopophila pleispora                      | TNM F16889 | - EF197084 -              | [32]                            |
| Schizochleumum embriatum                  | CBS 1314.54| AY800705 AY999115 AY780189 AY780132 [12,28] |
| Schizochleumum inaequale                  | CBS 356.49T| MK926846 MK926846 MK876808 - [4] |
| Schizochleumum selenoporeum               | CBS 109403T| MK926849 MK926849 MK876811 - [4] |
| Sordaria finicolica                       | SMH 4106   | AY780079 -              | AY80194 AY80138 [12]            |
| Triangularia allahabadensis               | CBS 724.68T| MK926865 MK926865 MK876827 - [4] |
| Triangularia backusii                     | CBS 539.89SOT| MK926866 MK926866 MK876828 - [4] |
| Triangularia backusii                     | FMR 12439  | KP981423 MT784138 KP981605 KP981550 [5] |
| Triangularia backusii                     | FMR 13591  | KP981424 MT784139 KP981606 KP981551 [5] |
| Triangularia bumbusae                     | CBS 352.33T| MK926868 MK926868 MK876830 - [4] |
| Triangularia batistae                     | CBS 381.68T| KP981413 MT784140 KP981626 KP981577 [5] |
| Triangularia longicaudata                 | CBS 252.57T| MK926871 MK926871 MK876833 - [4] |
| Triangularia setosa                       | FMR 12787  | KP981441 MT784144 KP981624 KP981569 [5] |
| Triangularia tetraspera                   | FMR 5770   | AY999130 AY999108 -     | - [5]                            |
| Triangularia verruculosa                  | CBS 148.77 | MK926874 MK926874 MK876836 - [4] |
| Zopfilla tabulata                         | CBS 230.78 | MK926854 MK926854 MK876816 - [4] |
| Zopfilla tardipecis*                      | CBS 670.82T| MK926855 MK926855 MK876817 - [4] |
| Zygopleurage zygosspora                   | SMH 4219   | AY436306 -              | - AY780147 [12,29]              |

**Table 1.** Continued.

[unpubl. data]
2.2. Fermentation and Extraction

The fungus was grown in yeast malt extract agar (YM agar; malt extract 10 g/L, yeast extract 4 g/L, D-glucose 4 g/L, agar 20 g/L, pH 6.3 before autoclaving [33]) at 23 °C. Later, the colonies were cut into small pieces using a cork borer (1 cm × 1 cm) and 8 pieces were placed into a 200 mL Erlenmeyer flask containing 100 mL of yeast malt extract broth (YM broth; malt extract 10 g/L, yeast extract 4 g/L, D-glucose 4 g/L, pH 6.3 before autoclaving) at 23 °C and under shake condition at 140 rpm. After 7 days, 6 mL of this seed culture were transferred to 40 conical flasks of 500 mL containing solid rice medium (BRFT, brown rice 28 g as well as 0.1 L of base liquid (yeast extract 1 g/L, di-sodium tartrate di-hydrate 0.5 g/L, KH₂PO₄ 0.5 g/L [34])) per flask. The rice cultures were incubated for 15 days at 23 °C.

For compound extraction, the mycelia in BRFT were covered with acetone, and sonicated in an ultrasonic bath for 30 min at 40 °C. The acetone extract was separated from the mycelium by filtration throughout a cellulose filter paper (MN 615 1/4 Ø 185 mm, Macherey-Nagel GmbH & Co. KG, Düren, Germany), and the mycelium was sonicated and extracted again. Both extracts were combined, and the acetone was evaporated to an aqueous residue in vacuo at 40 °C. The resulting aqueous phase was extracted twice with an equal amount of ethyl acetate in a separatory funnel. The ethyl acetate fraction was evaporated to dryness in vacuo (evaporator: Heidolph Instruments GmbH & Co. KG, Germany; pump: Vacuubrand GmbH & Co. KG, Wertheim am Main, Germany) at 40 °C. Afterwards, the ethyl acetate extract was dissolved in methanol. This was followed by extraction with an equal amount of heptane in a separatory funnel. This later step was repeated with the methanol phase obtained, which was evaporated to dryness in vacuo at 40 °C. The extracts were combined, dried in vacuo at 40 °C and weighed. Methanol extract yield was 1345 mg.

2.3. Compound Isolation

For compound isolation, the methanol extract dissolved in MeOH was portioned to 5 × 269 mg and separated using a PLC 2250 preparative HPLC system (Gilson, Middleton, WI, USA) with a Nucleodur® C18ec column (125 × 40 mm, 7 µm; Macherey-Nagel, Düren, Germany) as stationary phase and the following conditions: solvent A: H₂O + 0.1% formic acid, solvent B: Acetonitrile (ACN) + 0.1% formic acid; flow: 45 mL/min, fractionation: 15 mL, gradient: isocratic conditions at 20% B for 2 min, followed by an increase to 32% B in 8 min, then increase to 65% B in 25 min, followed by an increase to 100% B in 10 min, followed by isocratic conditions of 100% B for 10 min. This yielded compound 1 (48.4 mg, t_R = 43.5–44 min) and compound 3 (6.2 mg, t_R = 37–37.5 min).

2.4. Chromatography and Spectral Methods

Crude extract and pure compounds were dissolved to a concentration of 4.5 and 1 mg/mL, respectively, in an acetone and methanol solution (1:1). Then they were analyzed in an UltiMate® 3000 Series UHPLC system (Thermo Fisher Scientific, Waltman, MA, USA) connected to an ion trap mass spectrometer (ESI-Ion Trap-MS, amazon speed, Bruker, Billerica, MA, USA), utilizing a C18 Acquity® UPLC BEH column (2.1 × 50 mm, 1.7 m; Waters, Milford, MA, USA) to obtain the electrospray ionization mass spectra (ESI-MS). Solvent A was H₂O + 0.1% formic acid and solvent B was ACN + 0.1% formic acid. The gradient started with 5% of solvent B for 0.5 min, followed by an increase to 100% B in 19.5 min, and maintained in 100% B for 5 min more, with a flow rate of 0.6 mL/min. The UV/vis spectra were recorded by diode array detection (DAD) in a range from 190–600 nm.

High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded with an Agilent 1200 Infinity Series HPLC-UV system (Agilent Technologies, Santa Clara, CA, USA) connected to a time-of-flight mass spectrometer (ESI-TOF-MS, Maxis, Bruker, Billerica, MA, USA) (scan range 100–2500 m/z, rate 2 Hz, capillary voltage 4500 V, dry temperature 200 °C), using the same HPLC conditions described in ESI-MS measurements.
The 1D- and 2D- nuclear magnetic resonance (NMR) spectra of compounds 1 and 2 were recorded with an Avance III 700 spectrometer with a 5 mm TXI cryoprobe (Bruker, $^1$H NMR: 700 MHz, $^{13}$C: 175 MHz, Billerica, MA, USA) and an Avance III 500 (Bruker, $^1$H NMR: 500 MHz, $^{13}$C: 125 MHz, Billerica, MA, USA) spectrometer, respectively. The chemical shifts $\delta$ were referenced to the solvents DMSO-$d_6$ ($^1$H, $\delta = 2.50$; $^{13}$C, $\delta = 39.51$), and pyridine-$d_5$ ($^1$H, $\delta = 7.22$; $^{13}$C, $\delta = 123.87$).

Optical rotations were measured with an MCP 150 circular polarimeter at 20 $^\circ$C (Anton Paar, Graz, Austria) and UV/Vis spectra with a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). The optical rotation was obtained in MeOH and the UV/Vis spectra were measured in ACN.

2.5. Spectral Data

2.5.1. Morinagadepsin (1)

White powder; $[\alpha]^{20}_D = -93^\circ$ (c 0.001, MeOH); UV (ACN) $\lambda_{\text{max}}$ (log $\varepsilon$) 194 (4.2); $^1$H-NMR and $^{13}$C-NMR see Table 2; ESI-MS: $m/z$ 579 (M − H)$^-$ and 581 (M + H)$^+$; HR-ESI-MS: $m/z$ 581.4275 (M + H)$^+$ (calculated for C$_{31}$H$_{57}$N$_4$O$_6$, 581.4278).

Table 2. NMR data ($^1$H 700 MHz, $^{13}$C 175 MHz) of morinagadepsin 1 in DMSO-$d_6$.

| Atom# | Atom# | C Shift | H Shift | Atom# | Atom# | C Shift | H Shift |
|-------|-------|---------|---------|-------|-------|---------|---------|
| Leu1  | 1     | 170.9, C |         | Val   | 16    | 172.4, C |         |
|       | 2     | 51.1, CH | 4.39, ddd (9.5,9.0,5.8) | 17    | 58.0, CH | 4.01, t (7.4) |         |
|       | 2NH   | 7.80, br d (7.5) |         | 17NH  | 6.91, d (7.4) |         |
|       | 3     | 39.6, CH$_2$ | 1.74, m | 18    | 30.5, CH | 1.83, m |         |
|       | 3     | 1.67, m   |         | 19    | 18.74, CH$_3$ | 0.87, m$^*$ |         |
|       | 4     | 24.1, CH | 1.67, m | 20    | 18.77, CH$_3$ | 0.87, m$^*$ |         |
|       | 5     | 22.5, CH$_3$ | 0.91, d (6.5) | 21    | 172.5, C |         |         |
|       | 6     | 21.7, CH$_3$ | 0.86, m$^*$ | 22    | 45.8, CH | 2.47, qd (7.3, 4.8) |         |
| Ala1  | 7     | 171.5, C |         | 23    | 75.1, CH | 4.92, ddd (8.7, 4.8, 4.0) |         |
|       | 8     | 49.8, CH | 3.96, qd (7.5, 6.0) | 24    | 32.1, CH$_2$ | 1.44, m$^*$ |         |
|       | 8NH   | 7.98, d (6.0) |         | 24    | 1.35, m$^*$ |         |         |
|       | 9     | 16.1, CH$_3$ | 1.37, d | 25    | 24.5, CH$_2$ | 1.05, m$^*$ |         |
| Leu2  | 10    | 170.7, C |         | 26    | 28.59, CH$_2$ | 1.19, m$^*$ |         |
|       | 11    | 53.7, CH | 3.53, m | 27    | 28.61, CH$_2$ | 1.19, m$^*$ |         |
|       | 11NH  | 9.30, d (7.0) |         | 28    | 31.1, CH$_2$ | 1.19, m$^*$ |         |
|       | 12    | 36.6, CH$_2$ | 1.54, m$^*$ | 29    | 22.1, CH$_2$ | 1.24, m$^*$ |         |
|       | 12    | 1.99, m   |         | 30    | 13.9, CH$_3$ | 0.84, t (7.1) |         |
|       | 13    | 24.7, CH | 1.57, m$^*$ | 31    | 14.8, CH$_3$ | 1.05, d (7.3) |         |
|       | 14    | 23.6, CH$_3$ | 0.91, d (6.5) |         |         |         |         |
|       | 15    | 22.5, CH$_3$ | 0.87, m$^*$ |         |         |         |         |

* Signals overlapping in the $^1$H-NMR spectrum.

2.5.2. Chaetone B (3)

White to yellow oil; $^1$H-NMR and $^{13}$C-NMR were in good agreement with the literature [35]; ESI-MS: $m/z$ 301 (M + H)$^+$; HR-ESI-MS: $m/z$ 301.1068 (M + H)$^+$ (calculated for C$_{17}$H$_{17}$O$_5$, 301.1076).

2.6. Determination of Amino Acid Stereochemistry

Determination of amino acid stereochemistry of 1 was performed with Marfey’s reagent (1-fluoro-2,4-dinitrophenyl-5-L-valinamide (FDAA) (Sigma-Aldrich, Deisenhofen, Germany)) following the protocol described by Viehrig et al. [36] with slight modifications. Compound 1 (1 mg) was hydrolyzed with 6 N HCl at 90 $^\circ$C for 18 h. The hydrolysate was evaporated to dryness and redissolved in water (200 µL). Then, 1 N NaHCO$_3$ (20 µL) and 1% FDAA (100 µL in acetone) were added. The solution was heated at 40 $^\circ$C for 40 min. After cooling down, the solution was neutralized with 2 N HCl using pH paper and the sample was dried. The amino acids found in 1 were used as standards (D-L-Val (Sigma-
Aldrich, Deisenhofen, Germany), L-Val (Sigma-Aldrich, Deisenhofen, Germany), D-L-Leu (Sigma-Aldrich, Deisenhofen, Germany), L-Leu (Sigma-Aldrich, Deisenhofen, Germany), L-Ala (Merck KGaA, Darmstadt, Germany), and D-Ala (Sigma-Aldrich, Deisenhofen, Germany) and treated as explained above for the hydrolysate of 1. All the resulting products were dissolved in 1 mL MeOH and analyzed with the UHPLC system connected to an ion trap mass spectrometer described above. The retention times in minutes of the FDAA-derivatized amino acids were Ala 5.1; Leu 7.4; and Val 6.5. Retention time of the FDAA-derivatized standards were L-Ala 5.0; D-Ala 5.8 m/z 340 (M - H)−; L-Leu 7.4; D-Leu 8.4 m/z 382 (M - H)−; L-Val 6.5; and D-Val 7.5 m/z 368 (M - H)−. The retention times showed that compound 1 was built with L-amino acids.

2.7. Partial Hydrolysis of Marinagadepsin to Compound 2

For the hydrolysis of 1, the protocol described by Kwon et al. [37] was followed with slight modification. A portion of compound 1 (14.5 mg) was dissolved in 1 mL of 5% NaOMe (dissolved in methanol) and stirred for 20 h at 40 °C. Afterward, the reaction was neutralized with 1 N HCl using pH paper and evaporated to dryness. Preparative HPLC used an Agilent 1100 series system (Santa Clara, CA, USA) with a Gemini® C18ec column (250×21.2 mm, 7 μm; Phenomenex, Torrance, CA, USA) as stationary phase and the following conditions: solvent A: H2O + 0.1% formic acid, solvent B: ACN + 0.1% formic acid; flow: for 2 min at 17 to 20 mL/min and afterwards at 20 mL/min; fractionation: 10 mL/min; and gradient: isocratic conditions at 5% B for 2 min, followed by an increase to 55% B in 8 min, then increase to 65% B in 30 min, followed by an increase to 100% B in 10 min, followed by isocratic conditions of 100% B for 10 min. This yielded the pure compound 2 (7.9 mg, tR = 23.8–24.8 min).

2.7.1. Spectral Data of the Linear Peptide (2)

White powder; [α]20D −30° (c 0.001, MeOH); UV (ACN) λmax (log ε) 192 (4.1); 1H-NMR δH 9.54 (br d, J = 8.4 Hz, 11-NH), 9.43 (br d, J = 7.3 Hz, 8-NH), 9.23 (br d, J = 5.6 Hz, 2-NH), 8.91 (br d, J = 9.0 Hz, 17-NH), 5.28–5.20 (m, 2-H, 8-H, 11-H), 5.09 (t, J = 8.3 Hz, 17-H), 4.09 (m, 23-H), 2.91 (qd, J = 7.1 Hz, 5.9 Hz, 22-H), 2.44 (ddsp, J = 8.2 Hz, 6.9 Hz, 18-H), 2.06–1.82 (m, 3-H2, 4-H, 12-H, 13-H), 1.79 (m, 24-H2), 1.73 (m, 25-H4), 1.61 (d, J = 7.1 Hz, 9-H3), 1.57 (m, 25-H3), 1.44 (d, J = 7.1 Hz, 31-H3), 1.29–1.18 (m, 26-H2 – 29-H2), 1.17 (d, J = 6.9 Hz, 20-H), 1.00 (d, J = 6.5 Hz, 5-H3), 0.91 (d, J = 6.5 Hz, 6-H3), 0.84 (m, 14-H3), 0.83 (t, J = 6.9 Hz, 30-H3), 0.81 (d, J = 6.5 Hz, 15-H3); ESI-MS: m/z 597 (M − H)− and 599 (M + H)+; HR-ESI-MS: m/z 599.4370 (M + H)+ (calculated for C31H59N4O7, 599.4384).

2.7.2. Derivatization with MTPA

Compound 2 (1 mg) was dissolved in pyridine-d5 (0.6 mL), transferred into a NMR tube and then (R)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (10 μL) was added. The mixture was incubated for 2 h at room temperature before the measurement of 1H, COSY, TOCSY, HSQC and HMBC NMR spectra was taken. This resulted in a (S)-MTPA ester derivative: 1H NMR (700 MHz, pyridine-d5): similar to 2, but δH 9.05 (m, 17-NH), 5.90 (m, 23-H), 4.85 (m, 17-H), 3.30 (m, 22-H), 2.35 (m, 18-H), 2.01 (m, 24-H3), 1.78 (m, 24-H4), 1.47 (m, 25-H2), 1.27 (m, 31-H3), 1.27 (m, 26-H2), 1.21 (m, 29-H2), 1.20 (m, 27-H2), 1.18 (m, 28-H2), 1.14 (m, 19-H3), 1.03 (m, 20-H3), 0.83 (t, J = 7.3 Hz, 30-H3). The (R)-MTPA ester derivative was yielded analogously with (S)-(-)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (10 μL). The reaction was incubated in pyridine-d5 (0.6 mL) for 65 h at room temperature: 1H NMR (700 MHz, pyridine-d5): similar to 2, but δH 9.22 (m, 17-NH), 5.87 (m, 23-H), 4.90 (m, 17-H), 3.28 (m, 22-H), 2.42 (m, 18-H), 1.97 (m, 24-H3), 1.67 (m, 24-H4), 1.32 (m, 31-H3), 1.21 (m, 25-H2, 29-H2), 1.19 (m, 19-H3), 1.18 (m, 26-H3), 1.13 (m, 28-H2), 1.12 (m, 27-H2), 1.10 (m, 20-H3), 0.84 (m, 30-H3).
2.8. Antimicrobial and Cytotoxic Activity Assays

The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) in 96-well round-bottom plates. Compounds 1, 2, and 3 were tested against five fungi (i.e., *Candida albicans*, *Mucor hiemalis*, *Rhodotorula glutinis*, *Schizosaccharomyces pombe*, and *Wickerhamomyces anomalus*) and against bacteria (*Bacillus subtilis*, *Mycolicibacterium smegmatis*, and *Staphylococcus aureus* (Gram-positive), as well as *Acinetobacter baumannii*, *Chromobacterium violaceum*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Gram-negative)). The cell suspension of most bacteria was done in Mueller–Hinton Broth (SN X927.1, Carl Roth GmbH, Karlsruhe, Germany) and was adjusted at OD$_{600}$ nm to 0.01. *Mycolicibacterium smegmatis* was cultured in 27H9 + ADC (Middlebrook 7H9 Broth Base + Middlebrook ADC Growth Supplement (SN M0678 + M0553, Merck, Darmstadt, Germany)) and adjusted at OD$_{548}$ nm to 0.1. The fungi were grown in MYC (1% bactopeptone, 1% yeast extract, 2% glycerol, pH 6.3) and adjusted at OD$_{548}$ nm to 0.1. Then, 150 µL of the adjusted suspension was added to all wells of a 96-well microtiter plate. In row A, an additional 130 µL of suspensions plus 20 µL of the test compounds (1 mg/mL in methanol) were added. MeOH (20 µL) was used as negative control, while different positive controls were used depending on the test organisms. Nystatin (1 mg/mL) was used as positive control against the fungi. Oxytetracycline (0.1 mg/mL, *B. subtilis* 1 mg/mL) was used for the bacteria, except for *Ac. baumannii*, *M. smegmatis*, and *P. aeruginosa*, against which cibrobay (0.25 mg/mL), kanamycin (0.1 mg/mL), and gentamycin (0.1 mg/mL) were used, respectively. Then, starting from row A, 150 µL of the suspension were transferred to the next row, and 150 µL transferred to the following row. The remaining 150 µL after row H were discarded. This resulted in a serial dilution of the test compounds, ranging from 66.7 µg/mL in row A to 0.52 µg/mL in row H. The assay was incubated overnight at 800 rpm on a microplate shaker. The temperature was chosen due to the microorganisms. They were grown at 30 °C, except *M. smegmatis*, *E. coli*, and *P. aeruginosa* which were grown at 37 °C. The lowest concentration of the compounds preventing visible growth of the test organism was recorded as the MIC.

The cytotoxicity of compounds 1, 2, and 3 were tested against the two mammalian cell lines KB 3.1 (human endo-cervical adenocarcinoma) and L929 (mouse fibroblasts) in a 96-well plate. The compounds were dissolved as described in the previous section and epothilone B was used as the positive control. The cell lines were incubated with a serial dilution assay of the compounds (final range: 37 to 0.6 × 10$^{-3}$ µg/mL) at 37 °C with 10% CO$_2$ in Gibco™ Dulbecco’s Modified Eagle Medium (SN 61965026, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% Fetal Bovine Serum (SN 10500064, Thermo Fisher Scientific). After five days the cells were stained with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, (M2128, Sigma-Aldrich, Deisenhofen, Germany)). The dye is converted to its purple derivative by living cells. The intensity of the purple derivative in the relation to the cells without additive (100% viability) was quantified for each concentration of the test compound. For the quantification, a microplate reader with 595 nm was used to calculate the percentage of the cell viability. From these results, the half-maximum inhibitory concentration (IC$_{50}$) in µM was calculated.

3. Results

3.1. Molecular Phylogeny and Taxonomy

The lengths of the individual alignments used in the combined dataset were 681 bp (ITS), 894 bp (LSU), 984 bp (*rpb2*), and 618 bp (*tub2*), being the final total alignment of 3177 bp. The Maximum Likelihood tree obtained from the RAxML analysis of the combined dataset, including RAxML bootstrap support (BS) and Bayesian posterior probability at the nodes, is shown in Figure 1. For the BI analysis, the GTR + I + G model was selected for all partitions. The RAxML tree topology agreed with the topology of the tree generated by the BI analysis. The combined phylogenetic tree (Figure 1) showed seven main clades representing the families Chaetomiaceae, Diplogelasinosporaceae, Lasiosphaeriaceae, Naviculisporaceae, Podosporaceae, Schizotheciaceae, and Sordariaceae. The ex-type strain of
*Apiosordaria vermicularis* was located in the family Schizotheciaceae, far from the *Triangularia* clade in Podosporaceae, where the type species of *Apiosordaria* (now *T. verruculosa*) is placed. *Apiosordaria vermicularis* formed a well-supported clade (79% bs/0.98 pp) with *Echria* spp. and *Rinaldiella pentagonospora*, but showed enough phylogenetic distance to propose it as the type species of the new genus *Morinagamyces*, as *Morinagamyces vermicularis*.

**Morinagamyces Y. Marin and Stchigel, gen. nov.** MycoBank MB839453.

Type species: *Morinagamyces vermicularis* (Morinaga, Minoura and Udagawa) Y. Marin and Stchigel.

Etymology: Named in honor of the mycologist Tsutomu Morinaga, who collected and isolated the ex-type strain and introduced the basionym.

Ascomata non-ostiolate, scattered, semi-immersed to immersed, brownish black to black, opaque, globose to subglobose, glabrous or covered on upper exposed part with long, straight or flexuous, brown, septate, unbranched or branched, smooth-walled, hypha-like hairs; ascomatal wall brown to dark brown, membranaceous to coriaceous, three-layered; outer layer of textura intricata; middle layer composed of thin-walled, yellow brown to brown angular cells; inner layer composed of hyaline, flattened cells. Paraphyses filiform to ventricose, hyaline. Asci unitunicate, eight-spored, long cylindrical, often curved or sinuous, disposed in a basal fascicle, rounded apex, apical ring indistinct in the apex, non-amyloid, long-stipitate. Ascospores uniseriate, at first one-celled, hyaline, cylindrical-ellipsoidal, later becoming transversely uniseptate; upper cells dark olivaceous brown to dark brown, ovate to broadly ellipsoidal, truncate at base, rounded or slightly acuminate at apex, with walls ornamented by numerous, stiff warts, with a germ pore in apex; lower cell hyaline to pale brown, cylindrical to long triangular, frequently 1-septate, smooth-walled. Asexual morph of two types: (1) Conidiophores indistinguishable from the hyphae. Conidiogenous cells phialidic, integrated to hyphae, cylindrical, with a terminal collarette. Conidia hyaline, subglobose to ovate, smooth-walled, gathering in a globose, slimy mass; (2) Conidia holoblastic, borne along the sides of hyphae, sessile or short-stipitate, hyaline, pyriform to clavate, truncate at base, rounded apex, smooth-walled.

Notes: *Echinopodospora vermicularis* was introduced by Morinaga et al. to accommodate a fungus from soil in Hong Kong characterized by the production of non-ostiolate ascomata and ascospores with a warted upper cell [38]. Subsequently, it was transferred to *Apiosordaria*, when the genus *Echinopodospora* was synonymized with *Apiosordaria* based on their morphological similarities [39]. However, the phylogenetic data demonstrated that this species represents a new lineage in the recently introduced family Schizotheciaceae, and consequently the genus *Morinagamyces* was introduced. The main distinctive feature of this new genus is the presence of two different kinds of asexual morphs, i.e., cladorrhinum- and chrysosporium-like. This particular feature has only been reported in another species of the Sordariales, *A. effusa* [38], which has never been studied with molecular data, and its taxonomic position remains unresolved. Surprisingly, the cladorrhinum-like asexual morph is distinctive of the three genera belonging to the family Podosporaceae, i.e., *Cladorrhinum*, *Podospora*, and *Triangularia*, as it is not observed in any other families of the Sordariales.
Figure 1. RAxML phylogram obtained from the combined sequences of the internal transcribed spacer region (ITS), the nuclear rDNA large subunit (LSU), and fragments of ribosomal polymerase II subunit 2 (rpb2) and β-tubulin (tub2) genes of selected strains belonging to the families Chaetomiaceae, Diplogelasinosporaceae, Lasiosphaeriaceae, Naviculisporaceae, Podosporaceae, Schizotheciaceae, and Sordariaceae. *Camarops amorpha* was used as outgroup. Bootstrap support values ≥ 70/ Bayesian posterior probability scores ≥ 0.95 are indicated along branches. Branch lengths are proportional to distance. Novelty is indicated in **bold**. Ex-epitype, ex-isotype, and ex-type strains of the different species are indicated with ET, IsoT and T, respectively.
The closest related genera to *Morinagamyces* are *Echria* and *Rinaldiella*. However, the later genera have not been reported to produce asexual morphs and they are characterized by the production of ostiolate ascomata, while *Morinagamyces* produces non-ostiolate ones. *Echria* can be easily distinguished from the other two genera by production of one-celled roughened or smooth-walled ascospores (two-celled and warted in the other two genera) [19]. *Morinagamyces* and *Rinaldiella* produce two-celled warded ascospores, but the upper cell is five-angled in side view in *Rinaldiella* [27], while it is ovate to broadly ellipsoidal in *Morinagamyces*.

*Morinagamyces vermicularis* (Morinaga, Minoura and Udagawa) Y. Marín and Stchigel, comb. nov. MycoBank MB839454.

**Basionym:** *Echinopodospora vermicularis* Morinaga, Minoura and Udagawa, Trans. Mycol. Soc. Japan 19: 138. 1978.

**Synonym:** *Apiosordaria vermicularis* (Morinaga, Minoura and Udagawa) J.C. Krug, Udagawa and Jeng, Mycotaxon 17: 547. 1983.

3.2. Isolation and Structure Elucidation of Secondary Metabolites

Morinagadepsin (1) was isolated as a white powder. Its molecular formula of C_{31}H_{56}N_{4}O_{6} was derived from its HR-ESI-MS peak observed at m/z 581.4271, implying six degrees of unsaturation. $^1$H and HSQC (Heteronuclear single-quantum correlation spectroscopy) NMR spectra measured in DMSO-$d_6$ specified the presence of nine methyls, seven methylenes, and nine methines, of which four were bound to nitrogen and one bound to oxygen, in addition to four exchangeable protons bound to heteroatoms. The $^{13}$C-NMR spectrum indicated the presence of five carbonyls. By COSY (correlation spectroscopy), TOCSY (total correlation spectroscopy) and intra-residue HMBC (heteronuclear multiple-bond correlation spectroscopy) correlations, alanine (Ala), valine (Val), and two leucine (Leu-1 and Leu-2), as well as 3-hydroxy-2-methyldecanoic acid (HMDA) residues were assembled (see Figure 2b). The sequence of entities was assigned by inter-residue $^1$H,$^{13}$C HMBC correlations (Figure 2b). The low field shift of 23H ($\delta_{1}H$ 4.92) indicated an ester linkage at this position, which was confirmed by the $^1$H,$^{13}$C HMBC correlation of 23-H to C-1, establishing the planar depsipeptidal structure of 1.

![Figure 2. (a) Structure of morinagadepsin 1. (b) selected $^1$H,$^1$H COSY (bold lines) and $^1$H,$^{13}$C HMBC (black arrows) correlations of 1.](image)

After complete hydrolysis and derivatization with FDAA, we observed L-Leu, L-Val, and L-Ala according to Marfey’s method [36]. Thus, C-2, C-8, C-11, and C-17 are $S$-configured. The relative configurations of the chiral centers C-22/C-23 in HMDA was determined by $J$-based configurational analysis using $^3$J$_{HH}$, $^2$J$_{CH}$, $^3$J$_{CH}$ and ROESY correlations (Figure 3) as 22$R_1$,23$R_2$. Necessary proton-carbon coupling constants were obtained from a HSQC-Hecade NMR spectrum (Figure S10), except $^3$J(H23,C21) = 6.6 Hz, which was extracted from a J-HMBC NMR experiment (Figure S11) [40].
Finally, the absolute configuration of the HMDA subunit was determined by the derivatization of the methanolsysis product with S- and R-MTPA according to Mosher’s method. The pattern of $\Delta \delta^{SR}$ values (see Figure 4) with negative values for 17-NH, 17-H, 18-H, 19-H$_2$, 20-H$_3$, and 31-H$_3$, and positive ones for 24-H$_2$ to 29-H$_2$, is diagnostic for a 23R configuration [41].

Figure 3. J-based analysis of six hypothetical rotamers with 22S,23S (A–C) and 22S,23R (D–F) configuration to determine the stereochemistry of 1. Expected couplings contrary (shown in red) to the observed ones ($^2$J(H22,H23) = 4.8 Hz; $^2$J(H22,C23) = 2.2 Hz; $^3$J(H22,C24) = 1.0 Hz; $^3$J(H23,C31) = 2.6 Hz; $^3$J(H23,C21) = 6.6 Hz) exclude all configurations except A. This rotamer is confirmed by ROESY correlations between 23-H/22-H, 23-H/31-H, 24-H$_{2b}$/22-H, and 31-H$_3$/3-H$_6$.

Figure 4. $\Delta \delta^{SR}$ values in ppm for the C-23-MTPA esters of 2 in pyridin-d$_5$.

3.3. Biological Activities

Compounds 1 and 2 were not active against the microorganisms tested in the serial dilution assay. Compound 3 showed weak activity against B. subtilis and Mu. hiemalis (Table 3).
Table 3. Minimum inhibitory concentration (MIC, µg/mL) of 1–3 against bacterial and fungal test organisms.

| Test Organism Strain Number | 1  | 2  | 3  | Positive Control |
|-----------------------------|----|----|----|------------------|
| *Bacillus subtilis* DSM 10  | –  | –  | 66.6 | 8.30 ^O          |
| *Mycolicibacterium smegmatis* ATCC 700084 | – | – | – | 1.70 ^K          |
| *Staphylococcus aureus* DSM 346 | – | – | – | 0.83 ^O          |
| *Acinetobacter baumanii* DSM 3008 | – | – | – | 0.53 ^C          |
| *Chromobacterium violaceum* DSM 30191 | – | – | – | 0.83 ^O          |
| *Escherichia coli* DSM 1116 | – | – | – | 1.70 ^O          |
| *Pseudomonas aeruginosa* DSM 19882 | – | – | – | 0.42 ^G          |
| *Mucor hiemalis* DSM 2656 | – | – | 66.6 | 2.10 ^N          |
| *Candida albicans* DSM 1665 | – | – | – | 4.20 ^N          |
| *Rhodotorula glutinis* DSM 10134 | – | – | – | 1.00 ^N          |
| *Schizosaccharomyces pombe* DSM 70572 | – | – | – | 4.20 ^N          |
| Wickerhamomyces anomalae DSM 6766 | – | – | – | 4.20 ^N          |

ATCC: American Type Culture Collection, Manassas, VA, USA; DSM: Leibniz-Institut DSMZ—German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany. ^c: ciprofloxacin, ^g: gentamicin, ^k: kanamycin, ^n: nystatin, and ^o: oxytetracycline. –: no inhibition observed under test conditions.

Only compound 1 showed weak cytotoxic activity against the mammalian cell lines tested, while compound 3 was only weakly cytotoxic against the L929 cell line, and compound 2 did not have any activity (Table 4).

Table 4. Cytotoxicity of 1–3 against mammalian cell lines (half maximal inhibitory concentrations (IC₅₀): µM).

| Cell Line                  | Number ¹ | IC₅₀ [µM] | 1   | 2   | 3   | ⁵Epothilone B* |
|----------------------------|-----------|-----------|-----|-----|-----|---------------|
| HeLa KB 3.1                | ACC 158   | 37.2      | –   | –   | –   | 0.00003       |
| Mouse fibroblast L929      | ACC 2     | 13.7      | –   | 34.9| –   | 0.00051       |

–: no inhibition observed under test conditions. ¹ ACC: Leibniz-Institut DSMZ—German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany. * positive control (1 mg/mL).

4. Discussion

The genus *Apiosordaria*, as well as other lasiosphaeriaceous genera, such as *Cercophora*, *Podospora*, and *Zopfiella*, resulted in a polyphyletic clade. Encompassing species scattered among the order Sordariales [4,5,12,20,28,32]. The main problem is that the traditional classification of the lasiosphaeriaceous taxa was based predominantly on the ascospore morphology, but this was found to be an extremely homoplastic character [12]. *Apiosordaria* was recently synonymized with *Triangularia*, located in the Podosporaceae, as the type species *A. verruculosa* formed a monophyletic clade with the type species of this later genus, *T. backusii*, being consequently proposed the new combination *T. backusii* and *T. verruculosa* [4]. Subsequently, *A. sacchari* and *A. striatispora* were also transferred to *Triangularia*, whereas *A. antarctica*, *A. globosa*, *A. hispanica*, and *A. vestita* have been transferred to *Jugulospora*, phylogenetically located in the Schizotheciaceae [5]. However, a high number of *Apiosordaria* ssp. remain in an uncertain taxonomic placement, as in the case of *A. microcarpa* (see Figure 1). In order to get a more natural classification of these species, the new genus *Morinagamyces* is introduced to accommodate *A. vermicularis*, which has proven to be an independent lineage in the Schizotheciaceae. This genus differs from the other taxa of the family, and even the order Sordariales, by the production of two types of asexual morphs, i.e., cladorrhinum- and chrysosporium-like. This characteristic was also observed in *A. effusa* [38]. Therefore, further studies will be needed to verify if this species also belongs to the genus *Morinagamyces*.

Morinagadepsin (1) belongs to the large class of depsipeptides, compounds containing both amide and ester bonds, which are widely distributed in nature. They have been
isolated from plants, sponges and lower animals, cyanobacteria, bacteria, and fungi, with bioactivities ranging from antimicrobial, nematicidal, antiviral, and cytotoxic/cytostatic, to immunosuppressive and other pharmacologically important properties [42]. Fungal depsipeptides have been reported from many fungal genera, and it would lead too far to mention them all here. Some prominent examples are shown in Figure S23 (Supplementary Information). The nematicidal cyclodepsipeptide PF1022-A (Figure S23d), which has given rise to the marketed antiparasitic agent emodepside, had originally been discovered from an endophytic fungus associated with the tea plant, and only recently the affinities of the producer strain to the genus *Rosellinia* (Xylariaceae) were established [43]. The related cyclic hexadepsipeptide beauvericin (Figure S23a) probably acts as pathogenicity factor in the insect pathogenic *Beauveria* and *Isaria* species, and was also found in the genus *Fusarium* [44,45]. Verlamelin (Figure S23g) is another known depsipeptide with antifungal properties, produced by *Simplicillium lamellicola* (syn. *Verticillium lamellicola*) [46] and was eventually under development as antymycotic. Morinagadepsin belongs to the subgroup of cyclic pentadepsipeptides, which have been isolated from the genera *Acremonium*, *Alternaria*, *Fusarium*, *Hapidospora*, and *Penicillium* [47]. Its hallmark is the presence of a 3-hydroxy-2-methyldecanoic acid (HMDA) moiety. HMDA has previously been detected as part of emericellamides C and D (Figure S23b) produced by *Aspergillus nidulans* [48], the lipopeptoid trichopolyn V (Figure S23f) from *Trichoderma polysporum* [49], hapalosin (Figure S23c) from the cyanobacterium *Hapalasiphon wetwitschii* [50], and the globomycin derivative SF-1902A 4a (Figure S23e) from the bacterium *Streptomyces hygroscopicus* [51]. In the case of globomycin and its derivatives, the β-hydroxy-α-methyl carboxylic acid greatly contributes to the antibacterial activity [52]. Compound 1 did not show any activity against any microorganisms tested in the present study, but it was weakly cytotoxic against the two cell lines tested, while compound 2, which is the linear peptide obtained from the partial hydrolysis of 1, did not show antimicrobial or cytotoxic activity.

The other compound isolated from *M. vermicularis* was chaetone B (3). This compound was previously isolated from a strain of *Chaetomium* isolated from submerged woody substrate in fresh water [35], which is another member of the order Sordariales. Shen et al. [35] observed moderate activity of this compound against *S. aureus* in a standard disk assay. However, this compound did not show activity against this bacterium in our serial dilution assay. It showed weak bioactivity against the Gram-positive bacterium *B. subtilis*, and the fungus *Mu. hiemalis*. Compound 3 was also weakly cytotoxic against the L929 cell line.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/microorganisms9061191/s1, Figure S1: HPLC-ESI-MS spectrum of morinagadepsin (1) in positive and negative; Figure S2: HPLC-HR-ESI-MS spectrum of morinagadepsin (1) in positive mode; Figure S3: 1H NMR spectrum (700 MHz, DMSO-d6) of morinagadepsin (1); Figure S4: 13C NMR spectrum (175 MHz, DMSO-d6) of morinagadepsin (1); Figure S5: COSY NMR spectrum (700 MHz, DMSO-d6) of morinagadepsin (1); Figure S6: HSQC NMR spectrum (700 MHz, DMSO-d6) of morinagadepsin (1); Figure S7: HMBC NMR spectrum (700 MHz, DMSO-d6) of morinagadepsin (1); Figure S8: ROESY NMR spectrum (700 MHz, DMSO-d6) of morinagadepsin (1); Figure S9: J-HMBC NMR spectrum (700 MHz, DMSO-d6) of morinagadepsin (1); Figure S10: Sections from the HSQC-Hedade NMR spectrum (700 MHz, DMSO-d6) of morinagadepsin (1); Figure S11: J-HHMBC NMR spectrum (700 MHz, DMSO-d6) of morinagadepsin (1); Figure S12: HPLC-ESI-MS spectrum of 2 in positive and negative mode; Figure S13: HPLC-HR-ESI-MS spectrum of 2 in positive mode; Figure S14: 1H NMR spectrum (700 MHz, pyrindine-d5) of 2; Figure S15: 13C NMR spectrum (175 MHz, pyridine-d5) of 2; Figure S16: HSQC NMR spectrum (700 MHz, pyridine-d5) of 2; Figure S17: COSY NMR spectrum (700 MHz, pyridine-d5) of 2; Figure S18: HMBC NMR spectrum (700 MHz, pyridine-d5) of 2; Figure S19: HSQC NMR spectrum (700 MHz, pyridine-d5) of the S-MTPA-ester of 2; Figure S20: HSQC NMR spectrum (700 MHz, pyridine-d5) of the R-MTPA-ester of 2; Figure S21: HPLC-ESI-MS spectrum of chaetone B (3) in positive and negative mode; Figure S22: HPLC-HR-ESI-MS spectrum of chaetone B (3) in positive mode; and Figure S23: Chemical structures of some known cyclodepsipeptides.
Author Contributions: Conceptualization, F.S. and Y.M.-F.; methodology, F.S., K.H., and Y.M.-F.; software, M.S.; formal analysis, F.S., K.H., and Y.M.-F.; investigation, F.S., K.H., and Y.M.-F.; resources, A.M.S. and M.S.; data curation, F.S. and Y.M.-F.; writing—original draft preparation, F.S., K.H., and Y.M.-F.; writing—review and editing, A.M.S. and M.S.; visualization, F.S., K.H., and Y.M.-F. All authors have read and agreed to the published version of the manuscript.

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