HETEROCYCLES, Vol. 104, No. 10, 2022, pp. 1822 - 1835. © 2022 The Japan Institute of Heterocyclic Chemistry
Received, 26th July, 2022, Accepted, 18th August, 2022, Published online, 24th August, 2022
DOI: 10.3987/COM-22-14728

STEREOCHEMISTRIES OF MARIANNAMIDES C AND D, TWO LIPOHEXAPEPTIDES, ISOLATED FROM *MARIANNAEA ELEGANS* NBRC102301

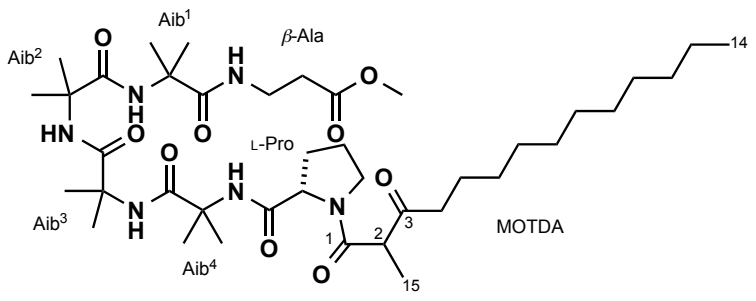
Kan'ichiro Ishiuchi,[a,*] Akiho Nagumo,[a] Mitsuyasu Kawaguchi,[b] Honoka Furuyashiki,[b] Hidehiko Nakagawa,[b] and Dai Hirose[c]

[a]Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Aichi, Japan. [b]Department of Organic and Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Aichi, Japan. [c]School of Pharmacy, Nihon University, 7-7-1, Narashinodai, Funabashi, 274-8555, Chiba, Japan. E-mail: ishiuchi@phar.nagoya-cu.ac.jp

Abstract – Two lipohexapeptides, mariannamides C (**1**) and D (**2**), were isolated from *Mariannaea elegans* NBRC102301, a filamentous fungus isolated from a decayed *Pinus densiflora* needle. The stereochemistries of **1** and **2** were fully elucidated based on Marfey's method and modified phenylglycine methyl ester method. Mariannamides C (**1**) and D (**2**) significantly and selectively promoted the defatty-acylase activity of sirtuin 3.

INTRODUCTION

The genus *Mariannaea* belongs to the Nectriaceae family, and the species of this genus can be isolated from soil, dead plant materials, and insect larva.[1,2] Currently, only 20 species[3] of this genus are accepted, and chemical investigation into this genus is not very active. So far, three polyketides, marianins A and B



1 : 2*S*
2 : 2*R*

from *M. camptospora* TAMA 118[4] and mariannaeapyrone from *M. elegans* UR 742,[5] one glycosphingolipid, Glc1-6Gal*β*1-Cer from *M. elegans* JCM12789,[6] two terpenoids, terpestacin[7] and 19-acetyl-4-hydroxydictyodiol[7] from *M. humicola* IG100, and one meroterpenoid, sesquicillin F[8] from *M. macrochlamydospora* FKI-4735 have been reported. In our continuing efforts to find structurally and biologically attractive natural products from plant-inhabiting fungi,[9] we have recently surveyed the chemical constituents of *M. elegans* NBRC102301 and isolated two cyclic octapeptides, mariannamides A and B.[10] Interestingly, mariannamide A promoted mRNA expression of sirtuin 1 (SIRT1) in C2C12 cells, a mouse skeletal muscle myoblast cell lines, and the mRNA expression activity of mariannamide A was at the same level as that of resveratrol. In this study, we further investigated NBRC102301 strain cultured ten times the scale of a previous report[10] and isolated two lipohexapeptides, mariannamides C (**1**) and D (**2**).

SIRTs (SIRT1–7), mammalian orthologues of yeast Sir2, are a family of NAD^+-dependent histone deacetylases, and their activities are closely correlated with cellular energy levels.[11] SIRTs are involved in metabolic regulation, stabilization of genomic DNA, stress responses, and even aging.[12] SIRT modulators are attractive therapeutic targets.[13] It was previously assumed that SIRTs catalyze deacetylation reactions of histones and that some SIRTs also catalyze ADP ribosylation.[14] However, some SIRTs exhibit weak deacetylase activity but relatively strong defatty-acylase activities.[15] While establishing methodologies to detect SIRT activity using radioisotopes,[16] antibodies,[17] HPLC,[18] and fluorescent probes,[19] the FRET-based SIRT fluorescent probe SFP3 was designed and synthesized,[20] each consisting of a nonapeptide derived from histone H3K9 and bearing a Dabcyl quencher dye on the *ε*-amino group of the lysine residue, along with a *C*-terminal fluorophore. The probe SFP3 was used as a one-step procedure to detect SIRT defatty-acylase activity.[20] Herein, we describe the isolation and structural elucidation of **1** and **2** and evaluate the modulating activities of SIRT1, 2, 3, and 6 using an assay system with the fluorescence probe, SFP3.

RESULTS AND DISCUSSION

The ethyl acetate (EtOAc)-soluble fraction from the culture solution of the NBRC102301 strain was subjected to a Sephadex LH-20 column, a silica gel column, and C_{18} HPLC to obtain mariannamides C (**1**) and D (**2**). The molecular formula of mariannamide C (**1**) was elucidated to be $C_{40}H_{70}N_6O_9$ based on HRESIMS [*m/z* 801.5101 $(M+Na)^+$, *Δ*-0.1 mmu], and the ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra resembled a known lipopeptide, lipohexin.[21] Heinze *et al.*, isolated lipohexin from *Moeszia lindtneri* HKI-0054 and *Paecilomyces* spp. HKI-0055 and HKI-0096, where HKI-0055 and HKI-0096

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** in $CDCl_3$

Position	δ_H	δ_C	Position	δ_H	δ_C
β-Ala			Pro		
α	2.63 (2H, m)	33.9	α	4.35 (1H, dd 8.5, 5.0 Hz)	61.8
β	3.53 (2H, m)	35.5	β	2.30 (1H, m)	29.2
CO		172.5		2.05 (1H, m)	
NH	7.49 (1H, t 6.0 Hz)		γ	2.03 (2H, m)	25.1
O-Me	3.64 (3H, s)	51.4	δ	3.53 (1H, m)	47.3
Aib[1]				3.32 (1H, m)	
α		57.1	CO		171.6
β1	1.49 (3H, s)	27.5	MOTDA		
β2	1.57 (3H, s)	24.0	1		170.2
CO		175.7	2	3.68 (1H, q 7.0 Hz)	52.0
NH	7.33 (1H, s)		3		211.0
Aib[2]			4	2.66 (1H, m)	40.7
α		56.9		2.58 (1H, m)	
β1	1.45 (3H, s)	26.8	5	1.61 (2H, m)	23.6
β2	1.47 (3H, s)	23.4	6−11	1.31−1.25 (12H)	29.7−29.2
CO		174.2	12	1.25 (2H, m)	31.9
NH	7.29 (1H, s)		13	1.28 (2H, m)	22.7
Aib[3]			14	0.88 (3H, t 6.5 Hz)	14.1
α		56.7	15	1.47 (3H, d 7.0 Hz)	12.9
β1	1.43[a] (3H, s)	26.4			
β2	1.43 (3H, s)	23.2			
CO		175.1			
NH	7.44 (1H, s)				
Aib[4]					
α		56.8			
β1	1.44[a] (3H, s)	26.6			
β2	1.51 (3H, s)	23.5			
CO		174.3			
NH	7.53 (1H, s)				

[a]These signals may be interchangeable.

were reidentified as *M. elegans* CBS 120677 and CBS 120687, respectively. However, the stereochemistry of lipohexin has not been elucidated yet. ^{1}H and ^{13}C NMR data (Table 1) and the HSQC spectrum of **1** revealed 40 carbon signals because of 8 carbonyl carbons, 4 nonprotonated sp^3 carbons, 2 sp^3 methine carbons, 15 sp^3 methylene carbons, and 11 sp^3 methyl carbons, and 5 amide protons. Among them, 4 nonprotonated sp^3 carbons (δ_C 57.1, 56.9, 56.8, and 56.7), 1 sp^3 methine carbon (δ_C 61.8), and 2 sp^3 methylene carbons (δ_C 47.3 and 35.5) were ascribed to those attached to a nitrogen atom.

Two-dimensional (2D) NMR correlations of ^{1}H-^{1}H COSY and HMBC spectra revealed that **1** has one *β*-alanine, four *α*-aminoisobutyric acids (Aib), one proline, and one 2-methyl-3-oxotetradecanoic acid (MOTDA) residues (Figure 1). The connectivities of amino acid and *β*-oxofatty acid residues were elucidated by HMBC spectral analysis. HMBC correlations for *β*-Ala-Hβ (δ_H 3.53) to Aib^1-CO (δ_C 175.7), Aib^1-NH (δ_H 7.33) to Aib^2-CO (δ_C 174.2), Aib^2-NH (δ_H 7.29) to Aib^3-CO (δ_C 175.1), Aib^3-NH (δ_H 7.44) to Aib^4-CO (δ_C 174.3), and Aib^4-NH (δ_H 7.53) to Pro-CO (δ_C 171.6) revealed amide linkages of *β*-Ala-Aib^1-Aib^2-Aib^3-Aib^4-Pro. Furthermore, HMBC cross-peaks of *O*-Me (δ_H 3.64) to *β*-Ala-CO (δ_C 172.5) indicated that the *C*-terminus of *β*-Ala was methyl esterified. Finally, the molecular formula and chemical shifts of **1** suggested that MOTDA residue (C-1) was attached to a nitrogen atom of proline residue through an amide bond. Thus, the planar structure of **1** was elucidated to be the same as a methyl ester of lipohexin[21] (Figure 1). **1** was the methyl ester form of lipohexin, which indicated the possibility that **1** was an artifact. However, **1** was isolated from the culture broth under rapid and mild conditions using as little methanol as possible, which suggested that **1** was not an artifact but a natural product. The stereochemistries of lipohexin and its methyl ester have not been determined, and we further attempted to elucidate the structural details of **1**.

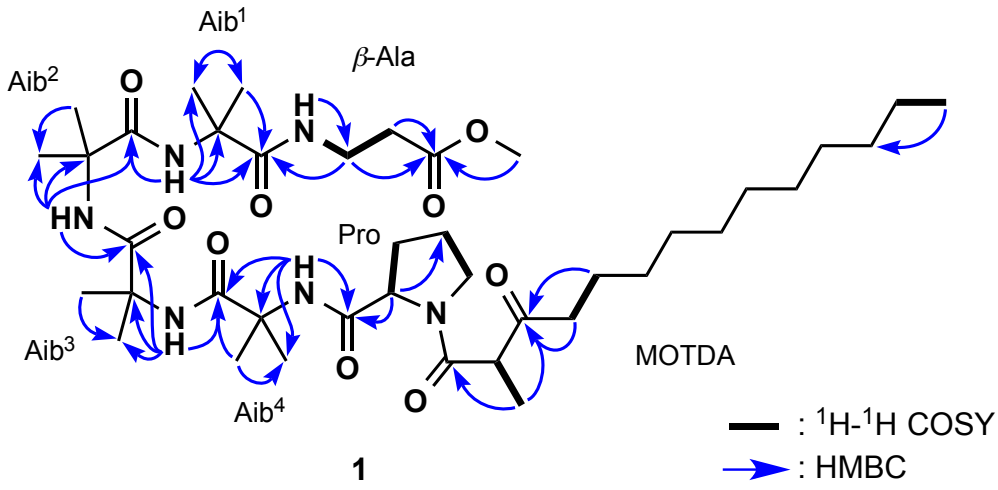


Figure 1. Selected 2D NMR correlations for **1**

The geometry of the amide bond of Pro-MOTDA moiety in **1** was empirically assigned from the $\delta_{C\gamma}$ and $\Delta\delta_{C\beta\text{-}C\gamma}$ values of proline.[22] These values (Pro $\delta_{C\gamma}$ 25.1 ppm, $\Delta\delta_{C\beta\text{-}C\gamma}$ 4.1 ppm) indicated that the geometry is *trans*. Marfey's analysis[23] of the hydrolysates of **1** revealed that the absolute configuration of the proline residue was L-form. Lipohexin, texenomycins A and B,[24] SCH 466456,[25] SCH 466457,[25] SCH 643432,[26] and sphaerostilbellins A and B[27] are a few examples of lipopeptides with MOTDA moiety. Although the stereochemistry of MOTDA at C-2 has been elucidated only in texenomycins,[24] the detail of this elucidation remains hidden from view. Then, we newly tried to establish the elucidation method of the stereochemistry of MOTDA. C-2 was at the *α*-position of carboxylic acid, and we intended to apply

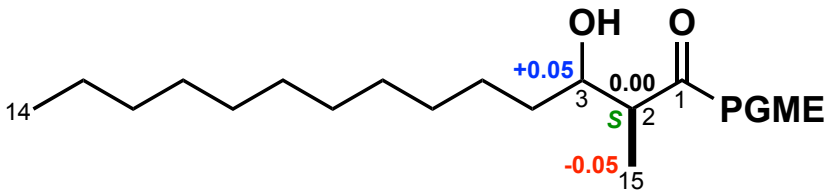


Figure 2. $\Delta\delta$ Values [$\Delta\delta$ (in ppm) = δ_S–δ_R] obtained for (*S*)- and (*R*)-PGME amides-1 (**1a** and **1c**) of MHTDA derived from **1**

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **2** in $CDCl_3$

Position	δ_H	δ_C	Position	δ_H	δ_C
β-Ala			Pro		
α	2.63 (2H, m)	33.9	α	4.20 (1H, dd 7.5, 5.5 Hz)	61.4
β	3.53 (2H, m)	35.5	β	2.22 (1H, m)	29.1
CO		172.5		2.01 (1H, m)	
NH	7.43 (1H, t 5.5 Hz)		γ	2.16 (2H, m)	25.2
O-Me	3.65 (3H, s)	51.4		2.01 (2H, m)	
Aib[1]			δ	3.59 (2H, m)	48.1
α		57.1	CO		172.1
β1	1.48 (3H, s)	26.9	MOTDA		
β2	1.54 (3H, s)	24.5	1		170.4
CO		175.5	2	3.63 (1H, m)	52.7
NH	7.23 (1H, s)		3		206.7
Aib[2]			4	2.49 (2H, m)	40.4
α		56.9	5	1.57 (2H, m)	23.5
β1	1.39 (3H, s)	26.3	6–11	1.29–1.21 (12H)	29.7–29.1
β2	1.43 (3H, s)	24.1	12	1.25 (2H, m)	31.9
CO		173.9	13	1.28 (2H, m)	22.7
NH	7.37 (1H, s)		14	0.88 (3H, t 6.5 Hz)	14.1
Aib[3]			15	1.38 (3H, d 7.5 Hz)	13.2
α		56.9			
β1	1.41 (3H, s)	25.9			
β2	1.46 (3H, s)	23.8			
CO		175.0			
NH	7.11 (1H, s)				
Aib[4]					
α		56.8			
β1	1.45 (3H, s)	26.4			
β2	1.46 (3H, s)	23.8			
CO		173.6			
NH	6.48 (1H, s)				

the phenylglycine methyl ester (PGME) method[28] to MOTDA. However, when **1** was directly hydrolyzed, MOTDA was not obtained because MOTDA is a *β*-ketoacid, and decarboxylation has occurred because of heating. Then, **1** was reduced by $NaBH_4$ before the hydrolysis to afford 2-methyl-3-hydroxytetradecanoic acid (MHTDA). After that, MHTDA was treated with (*S*)- and (*R*)-PGME hydrochlorides to obtain (*S*)- and (*R*)-PGME amide diastereomers of MHTDA at C-3, (*S*)-PGME amide-1 (**1a**), (*S*)-PGME amide-2 (**1b**), (*R*)-PGME amide-1 (**1c**), and (*R*)-PGME amide-2 (**1d**). Comparing the differences in chemical shifts between **1a** and **1b** and between **1c** and **1d** at C-3, the stereochemistries of **1a** and **1c** at C-3 were the same. The values of $\Delta\delta$[δ(*S*-PGME amide-1 (**1a**))−δ(*R*-PGME amide-1 (**1c**))] of MHTDA from **1** are presented in Figure 2. The $\Delta\delta$ value for H-3 was positive, whereas that for H-15 was negative. These data indicate that the absolute configuration at C-2 of MHTDA was *S*, implying that the absolute configuration at C-2 of **1** was also *S*.

The molecular formula of mariannamide D (**2**) was elucidated to be $C_{40}H_{70}N_6O_9$ based on HRESIMS [*m/z* 801.5099 $(M+Na)^+$, *Δ*-0.3 mmu]. 2D NMR analysis revealed that the planar structure of **2** was the same as **1** (Figure S11). The geometry of the amide bond of Pro-MOTDA in **2** was assigned to be *trans* from the $\delta_{C\gamma}$ (25.2 ppm) and $\Delta\delta_{C\beta\text{-}C\gamma}$ (3.9 ppm) values of proline.[22] The absolute configuration of the proline residue was elucidated to be the L-form using Marfey's method.[23] The absolute configuration of MOTDA of **2** at C-2 was elucidated in the same way as described above. The values of $\Delta\delta$[δ(*S*-PGME amide-1 (**2a**))−δ(*R*-PGME amide-1 (**2c**))] of MHTDA from **2** are depicted in Figure 3. The $\Delta\delta$ value for H-3 was negative, whereas that for H-15 was positive. These data indicate that the absolute configuration of MHTDA at C-2 was *R*. Thus, the structure of **2** was assigned to be the stereoisomer of **1** at C-2. The *α*-methyl-*β*-ketocarboxamide moieties of **1** and **2** are likely to be stereochemically labile, which indicated the possibility that these compounds epimerize. However, it has been confirmed that **1** and **2** did not epimerize after purification and that they also nearly unepimerized by HPLC analyses when they were heated in MeOH at 60 °C for 1 h, which suggested that these compounds do not epimerize with each other under conditions of the isolation procedures.

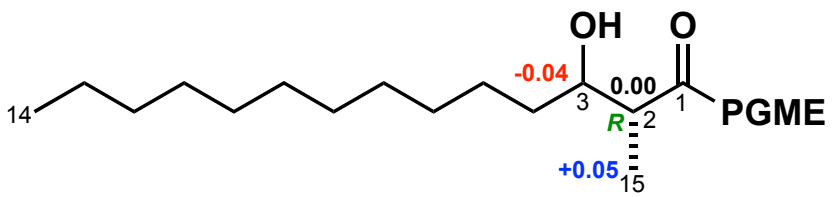


Figure 3. $\Delta\delta$ Values [$\Delta\delta$ (in ppm) = δ_S−δ_R] obtained for (*S*)- and (*R*)-PGME amides-1 (**2a** and **2c**) of MHTDA derived from **2**

We investigated the effect of mariannamides A, B, C (**1**), and D (**2**) on the modulating activities of SIRT1, 2, 3, and 6 using the assay system using the fluorescence probe, SFP3. 50 μM of mariannamides C (**1**) and D (**2**) significantly and selectively promoted SIRT3 (Figure S12). On the one hand, mariannamides A and B neither inhibited nor promoted SIRT activity (Figure S12). The SIRT3 protein is widely expressed in mitochondria-rich tissues, such as the kidney, heart, brain, and liver.[29] The acetylation modifications regulated by SIRT3 are essential for maintaining the mitochondrial function in these tissues. SIRT3 can regulate aging, neurodegeneration, liver disease, kidney disease, heart disease, and other metabolic diseases. Therefore, SIRT3 has been proposed to be a promising therapeutic target, and several SIRT3-based small-molecule compounds have been reported, such as honokiol, silybin, resveratrol, polydatin, dihydromyricetin, pyrroloquinoline quinone, metformin, adjudin, melatonin, and 7-hydroxy-3-(4'-methoxyphenylcoumarin) (C12).[30] Among them, only C12 has been proven to bind directly to the SIRT3 and promote the deacetylation. There were no positive controls for the promoting assay system of SIRT using SFP3, and we further evaluated SIRT3 defatty-acylase activity of **1** and **2** with synthesized C12. C12 activated SIRT3 by 6.25 μM, and mariannamides C (**1**) and D (**2**) also significantly promoted it by 100 μM, 6.25 μM, respectively (Figure 4). The results of C12 by 50.0 and 100 μM were unreliable because C12 was insoluble in the buffer at these concentrations (Figure 4). Mariannamides C (**1**) and D (**2**) are the first examples that selectively promoted SIRT3 activity, namely, defatty-acylation.

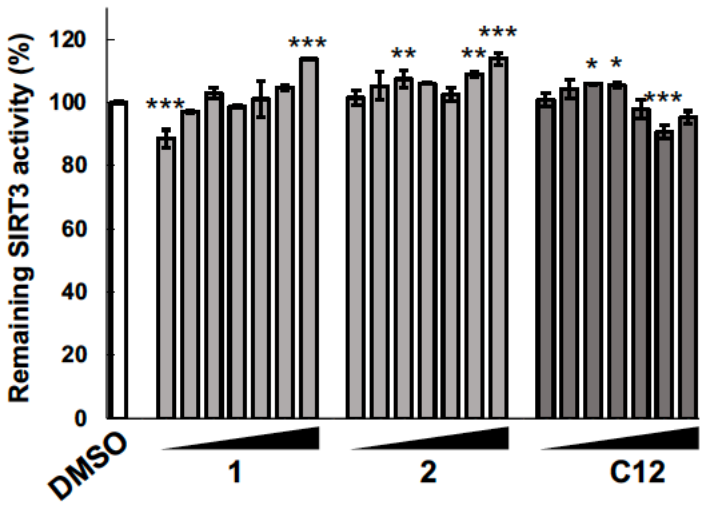


Figure 4. SIRT3 (20 nM) promoting assay of **1**, **2**, and C12 (1.56, 3.13, 6.25, 12.5, 25.0, 50.0, 100 μM) using a peptide substrate, SFP3 (2.5 μM). Values are expressed as percentages relative to the DMSO-treated well. Data are presented as mean ± S.D. (n = 3). Statistical significance was examined using the Bonferroni-type multiple *t*-test by using GraphPad Prism6: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the DMSO-treated well.

EXPERIMENTAL

General procedures

Optical rotations were recorded on a JASCO P-2100 polarimeter. IR spectra were recorded on a Shimadzu IR Affinity-1 spectrometer (Kyoto, Japan). NMR spectra were recorded on an Agilent Varian VNS500 spectrometer (Santa Clara, CA, USA). Chemical shifts (ppm) were referenced to the residual solvent peaks ($CDCl_3$; δ_H 7.26 and δ_C 77.0, CD_3OD; δ_H 3.31 and δ_C 49.0). Positive-mode ESITOFMS was obtained on a JEOL JMS-T100LP AccuTOF LC-plus 4G spectrometer (Tokyo, Japan).

Fungal Material

Mariannaea elegans NBRC102301 was isolated from decayed pine needles from the *Pinus densiflora* forest at the Sugadaira Montane Research Center at the University of Tsukuba by Dr. Seiji Tokumasu.

Extraction and isolation procedure of mariannamides C (1) and D (2)

Mariannaea elegans NBRC102301 was cultured on a PDA plate at 25 °C for 4 days. Ten 0.3-cm squared pieces of the grown mycelia were inoculated in 20 Erlenmeyer flasks (2 L) containing 1 L of potato dextrose broth medium and shaken at 180 rpm at 25 °C for 7 days. The filtrate of the culture broth (1 L) was extracted with EtOAc (1 L × 3) to give EtOAc-soluble materials. This procedure was repeated twenty times, and the combined EtOAc-soluble fraction (2.8 g) was subjected to a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to afford 22 fractions (Fr. 0−Fr. 21). Fr. 6 and Fr. 7 were combined and separated by a silica gel column ($CHCl_3$/MeOH/H_2O, 1:0:0 → 6:4:1). A fraction (21 mg) eluted with $CHCl_3$/MeOH (30:1) was further purified using a C_{18} HPLC (COSMOSIL 5C_{18}-AR-II, 4.6 mm I.D. × 250 mm, Nacalai Tesque, Kyoto, Japan, the solvent of MeCN/H_2O, 83:17, a flow rate of 0.6 mL/min, detection at 220 nm) to afford mariannamides C (**1**, 5.8 mg) and D (**2**, 1.7 mg). While, the mycelia were extracted with MeOH to give the MeOH extracts. The extracts were partitioned between EtOAc and H_2O, and the obtained EtOAc-soluble fraction (210 mg) was subjected to a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to afford 16 fractions (Fr. 1−Fr. 16). Fr. 7 and Fr. 8 were combined and separated by a silica gel column ($CHCl_3$/MeOH/H_2O, 1:0:0 → 6:4:1). A fraction (14 mg) eluted with $CHCl_3$/MeOH (30:1) was further purified using a C_{18} HPLC (COSMOSIL 5C_{18}-AR-II, 20 mm I.D. × 250 mm, Nacalai Tesque, Kyoto, Japan, the solvent of MeCN/H_2O, 83:17, a flow rate of 8.0 mL/min, detection at 220 nm) to afford mariannamides C (**1**, 2.4 mg) and D (**2**, 1.0 mg).

Mariannamide C: a white amorphous solid; $[\alpha]_D^{23}$ +20 (*c* 1.0, $CHCl_3$); IR (ATR) ν_{max} 3287, 2932, 2855, 1721, 1651, 1535, and 1443 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see

Table 1; ESITOFMS *m/z* 801 $[M+Na]^+$; HRESITOFMS *m/z* 801.5101 $[M+Na]^+$ (calcd for $C_{40}H_{70}N_6O_9Na$, 801.5102).

Mariannamide D: a white amorphous solid; $[\alpha]_D^{22}$ -30 (*c* 0.3, $CHCl_3$); IR (ATR) ν_{max} 3371, 2924, 2854, 1728, 1643, 1550, and 1450 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; ESITOFMS *m/z* 801 $[M+Na]^+$; HRESITOFMS *m/z* 801.5099 $[M+Na]^+$ (calcd for $C_{40}H_{70}N_6O_9Na$, 801.5102).

Absolute stereochemistry of the amino acid residues

Mariannamide C (**1**, 0.1 mg) was hydrolyzed in 6 N HCl (500 μL) at 110 °C for 24 h. After drying with N_2 gas, the hydrolysate was dissolved in H_2O (25 μL), and treated with sat. $NaHCO_3$ aq. (40 μL) and 1% N^{α}-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide (FDAA) in acetone (50 μL) was mixed at 40 °C for 1 h. The reaction was stopped by adding 1 N HCl (30 μL). FDAA derivatives of amino acids from mariannamide D (**2**, 0.1 mg) and standard amino acids (0.1 mg of each) were prepared using the same procedure. The FDAA derivatives were analyzed using C_{18} HPLC (COSMOSIL Packed Column 5C_{18}-AR-II, 4.6 mm I.D. × 250 mm, the solvent of MeCN/H_2O/TFA, 28:72:0.1, a flow rate of 0.6 mL/min, detection at 340 nm). The retention times (min) of the FDAA derivatives of standard amino acids: L-Pro (23.9) and D-Pro (27.4). The retention times (min) of the FDAA derivatives of hydrolysate of **1**: L-Pro (24.0). The retention times (min) of the FDAA derivatives of standard amino acids: L-Pro (25.0), D-Pro (28.6), and the hydrolysate of **2**: L-Pro (24.9).

Derivatization of 1 to (*S*)- and (*R*)-PGME amides of MHTDA

A solution of **1** (1.0 mg) in ethanol (EtOH) (100 μL) was prepared. To this, sodium borohydride (0.9 mg) was added. The reaction mixture was stirred at 0 °C for 2 h and then at room temperature for 2 h, to which 5 M HCl aq. was dropped and evaporated in vacuo. The reactant was partitioned between EtOAc and H_2O, and the EtOAc layer was hydrolyzed in 6 N HCl (500 μL) at 110 °C for 24 h. The hydrolyzed solution was partitioned with *n*-hexane. To a DMF solution (500 μL) of the *n*-hexane layer and (*S*)-PGME hydrochloride (1.5 mg) were added PyBOP (3.4 mg), HOBt (1.2 mg), and *N*-methylmorpholine (100 μL), and stirred at room temperature for 3 h. To the reaction mixture, 5% HCl (100 μL) was added. The mixture was extracted with *n*-hexane. The *n*-hexane layer was washed with sat. $NaHCO_3$ aq. and sat. NaCl aq., and subjected to C_{18} HPLC (COSMOSIL Packed Column 5C_{18}-AR-II, 4.6 mm I.D. × 250 mm, the solvent of MeCN/H_2O, 80:20, a flow rate of 0.6 mL/min, detection at 220 nm) to afford (*S*)-PGME amide-1 (**1a**) (less than 0.1 mg) and (*S*)-PGME amide-2 (**1b**) (less than 0.1 mg) of MHTDA derived from **1**. The (*R*)-PGME amide-1 (**1c**) (less than 0.1 mg) and (*R*)-PGME amide-2 (**1d**)

(less than 0.1 mg) of MHTDA derived from **1** were prepared using (*R*)-PGME hydrochloride according to the same procedure as described above.

MHTDA (*S*)-PGME amide-1 (1a): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.41−7.32 (5H, Ph), 5.43 (1H, s, H-α), 3.70 (3H, s, O-Me), 3.61 (1H, m, H-3), 2.43 (1H, m, H-2), 1.14 (3H, d, J = 6.5 Hz, H-15), 0.90 (3H, t, J = 7.0 Hz, H-14); ESITOFMS *m/z* 428 $[M+Na]^+$; HRESITOFMS *m/z* 428.2779 $[M+Na]^+$ (calcd for $C_{24}H_{39}NO_4Na$, 428.2777).

MHTDA (*S*)-PGME amide-1 (1b): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.41−7.31 (5H, Ph), 5.48 (1H, s, H-α), 3.70 (3H, s, O-Me), 3.63 (1H, m, H-3), 2.48 (1H, m, H-2), 1.12 (3H, d, J = 7.0 Hz, H-15), 0.90 (3H, t, J = 7.0 Hz, H-14); ESITOFMS *m/z* 428 $[M+Na]^+$; HRESITOFMS *m/z* 428.2778 $[M+Na]^+$ (calcd for $C_{24}H_{39}NO_4Na$, 428.2777).

MHTDA (*R*)-PGME amide-1 (1c): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.41−7.31 (5H, Ph), 5.49 (1H, s, H-α), 3.71 (3H, s, O-Me), 3.56 (1H, m, H-3), 2.43 (1H, m, H-2), 1.19 (3H, d, J = 6.5 Hz, H-15), 0.90 (3H, t, J = 7.0 Hz, H-14); ESITOFMS *m/z* 428 $[M+Na]^+$; HRESITOFMS *m/z* 428.2778 $[M+Na]^+$ (calcd for $C_{24}H_{39}NO_4Na$, 428.2777).

MHTDA (*R*)-PGME amide-1 (1d): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.41−7.31 (5H, Ph), 5.48 (1H, s, H-α), 3.71 (3H, s, O-Me), 3.61 (1H, m, H-3), 2.48 (1H, m, H-2), 1.15 (3H, d, J = 7.0 Hz, H-15), 0.90 (3H, t, J = 7.0 Hz, H-14); ESITOFMS *m/z* 428 $[M+Na]^+$; HRESITOFMS *m/z* 428.2777 $[M+Na]^+$ (calcd for $C_{24}H_{39}NO_4Na$, 428.2777).

Derivatization of 2 to (*S*)- and (*R*)-PGME amides of MHTDA

The (*S*)-PGME amide-1 (**2a**) (less than 0.1 mg), (*S*)-PGME amide-2 (**2b**) (less than 0.1 mg), (*R*)-PGME amide-1 (**2c**) (less than 0.1 mg), and (*R*)-PGME amide-2 (**2d**) (less than 0.1 mg) of MHTDA derived from **2** (0.5 mg) were prepared according to the similar procedure as described above. NMR data of **2b** could not be acquired due to the trace amount.

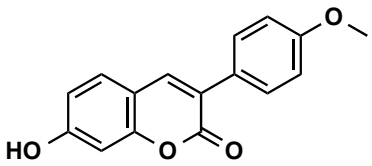
MHTDA (*S*)-PGME amide-1 (2a): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.40−7.32 (5H, Ph), 5.49 (1H, s, H-α), 3.71 (3H, s, O-Me), 3.57 (1H, m, H-3), 2.43 (1H, m, H-2), 1.19 (3H, d, J = 7.0 Hz, H-15), 0.90 (3H, t, J = 6.0 Hz, H-14); ESITOFMS *m/z* 428 $[M+Na]^+$; HRESITOFMS *m/z* 428.2776 $[M+Na]^+$ (calcd for $C_{24}H_{39}NO_4Na$, 428.2777).

MHTDA (*R*)-PGME amide-1 (2c): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.40−7.33 (5H, Ph), 5.43 (1H, s, H-α), 3.70 (3H, s, O-Me), 3.61 (1H, m, H-3), 2.43 (1H, m, H-2), 1.14 (3H, d, J = 7.0 Hz, H-15), 0.90 (3H, t, J = 6.5 Hz, H-14); ESITOFMS *m/z* 428 $[M+Na]^+$; HRESITOFMS *m/z* 428.2778 $[M+Na]^+$ (calcd for $C_{24}H_{39}NO_4Na$, 428.2777).

MHTDA (*R*)-PGME amide-2 (2d): ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.41−7.33 (5H, Ph), 5.48 (1H, s, H-α), 3.70 (3H, s, O-Me), 3.64 (1H, m, H-3), 2.48 (1H, m, H-2), 1.12 (3H, d, J = 7.0 Hz, H-15), 0.90 (3H, t, J = 6.5 Hz, H-14); ESITOFMS *m/z* 428 $[M+Na]^+$; HRESITOFMS *m/z* 428.2777 $[M+Na]^+$ (calcd for $C_{24}H_{39}NO_4Na$, 428.2777).

Synthesis of 7-hydroxy-3-(4'-methoxyphenyl)-2*H*-1-benzopyran-2-one (C12)

To a solution of 4-methoxyphenylacetic acid (1.66 g, 10 mmol, 1.0 eq.) and 2,4-dihydrobenzaldehyde (1.38 g, 10 mmol, 1.0 eq.) in Ac_2O (3.8 mL, 40 mmol, 4.0 eq.) was added NEt_3 (2.8 mL, 20 mmol, 2.0 eq.). The reaction mixture was refluxed at 110 °C for 6 h, then poured into ice-cold water (50 mL). The formed precipitate was collected and washed with H_2O. The solid was dissolved in 2 N NaOH (100 mL) and washed with EtOAc (50 mL) twice, then the aqueous solution was acidified with conc. HCl to pH 4. The formed precipitate was collected and washed with H_2O to give a crude product (1.80 g), which was recrystallized from EtOH to give C12 (1.22 g, 4.5 mmol), yield 45% as a yellow solid. C12: ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.80 (s, 3H), 6.75 (1H, d, J = 2.3 Hz), 6.82 (1H, dd, J = 2.3, 8.5 Hz), 7.00 (2H, d, J = 8.8 Hz), 7.58 (1H, d, J = 8.5 Hz), 7.65 (2H, d, J = 8.8 Hz), 8.09 (s, 1H), 10.57 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 55.2, 101.7, 112.2, 113.4, 113.7, 121.9, 127.3, 129.6, 129.8, 139.8, 154.7, 159.2, 160.3, 160.9; ESITOFMS *m/z* 291 $[M+Na]^+$; HRESITOFMS *m/z* 291.0605 $[M+Na]^+$ (calcd for $C_{16}H_{12}O_4Na$, 291.0633); HPLC purity (at 254 nm): 99.3%, t_R = 16.1 min. HPLC condition: A:B = 90:10 to 0:100 (20 min), A: 0.1% TFA MilliQ, B: 0.1% TFA MeCN.



C12

Evaluation of SIRT3-promoting activity by the SFP3 assay

Mariannamides A, B, C (**1**), D (**2**), and C12 were each dissolved in DMSO and diluted as required with SIRT assay buffer I (Tris–HCl (pH 8.0), containing 150 mM NaCl, 1 mM DTT, and 0.05% Triton X-100). SIRT3 (final 20 nM, 10 μL) was finally added to a solution of SFP3 (final 2.5 μM 10 μL), NAD^+ (final 500 μM, 10 μL), and the indicated concentrations of all compounds (10 μL, final 1% DMSO) in SIRT assay buffer I. Fluorescence intensity was measured at 5 min intervals with an ARVO X5 plate reader (filters: Ex = 485/14 nm, Em = 535/25 nm) for 60 min at 37 °C. Values are expressed as percentages relative to the DMSO-treated well. Data are presented as mean ± S.D. (n = 3). Statistical significance was

examined by means of Bonferroni-type multiple *t*-test by using GraphPad Prism6: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the DMSO-treated well.

ACKNOWLEDGEMENTS

This work was partly supported by Grant-in-Aid for Scientific Research (C) (JSPS KAKENHI Grant Number JP 20K07103) from Japan Society for the Promotion of Science (JSPS).

REFERENCES

1. T. Gräfenhan, H. J. Schroers, H. I. Nirenberg, and K. A. Seifert, *Stud. Mycol.*, 2011, **68**, 79.
2. L. Tang, M. W. Hyun, Y. H. Yun, D. Y. Suh, S. H. Kim, and G. H. Sung, *Mycobiology*, 2012, **40**, 14.
3. K. Watanabe and D. Hirose, *Phytotaxa*, 2021, **522**, 211.
4. T. Fukuda, Y. Sudoh, Y. Tsuchiya, T. Okuda, F. Fujimori, and Y. Igarashi, *J. Nat. Prod.*, 2011, **74**, 1327.
5. K. Fabian, T. Anke, and O. Sterner, *Z. Naturforsch.*, 2001, **56**, 106.
6. Y. Tani, K. Nakamura, R. Sawa, M. Nishio, S. Saito, M. Ito, S. Itonori, and H. Mihara, *Biosci. Biotechnol. Biochem.*, 2013, **77**, 754.
7. L. Botta, R. Saladino, P. Barghiini, M. Fenice, and M. Pasqualetti, *Microb. Cell Factories*, 2020, **19**, 184.
8. K. Sakai, M. Iwatsuki, T. Kaneta, A. Kimishima, Y. Asami, T. Sunazuka, R. Masuma, and K. Nonaka, *J. Antibiot.*, 2021, **74**, 817.
9. K. Ishiuchi, D. Hirose, T. Suzuki, W. Nakayama, W. P. Jiang, O. Monthakantirat, J. B. Wu, S. Kitanaka, and T. Makino, *J. Nat. Prod.*, 2018, **81**, 1143; K. Ishiuchi, D. Hirose, Y. Takahashi, R. Miyagawa, K. Watanabe, and S. Kitanaka, *Heterocycles*, 2019, **98**, 1574; K. Ishiuchi, A. A. Syed, Y. Kosuge, Y. Fujiwara, T. Makino, and D. Hirose, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127307.
10. K. Ishiuchi, D. Hirose, T. Kondo, K. Watanabe, K, Terasaka, and T. Makino, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 126946.
11. S. Imai, C. M. Armstrong, M. Kaeberlein, and L. Guarente, *Nature*, 2000, **403**, 795; R. H. Houtkooper, E. Pirinen, and J. Auwerx, *Nat. Rev. Mol. Cell Biol.*, 2012, **13**, 225.
12. M. C. Haigis and L. P. Guarente, *Genes Dev.*, 2006, **20**, 2913; V. D. Longo and B. K. Kennedy, *Cell*, 2006, **126**, 257; A. A. Sauve, C. Wolberger, V. L. Schramm, and J. D. Boeke, *Annu. Rev. Biochem.*, 2006, **75**, 435; T. Finkel, C. X. Deng, and R. Mostoslavsky, *Nature*, 2009, **460**, 587.
13. S. Lavr, O. Boss, P. J. Elliott, and P. D. Lambert, *Nat. Rev. Drug Discov.*, 2008, **7**, 841; J. C. Milne, P. D. Lambert, S. Schenk, D. P. Carney, J. J. Smith, D. J. Gagne, L. Jin, O. Boss, R. B. Perni, C. B. Vu, J. E. Bemis, R. Xie, J. S. Disch, P. Y. Ng, J. J. Nunes, A. V. Lynch, H. Yang, H. Galonek, K.

Israelian, W. Choy, A. Iffland, S. Lavu, O. Medvedik, D. A. Sinclair, J. M. Olefsky, M. R. Jirousek, P. J. Elliott, and C. H. Westphal, *Nature*, 2007, **450**, 712; L. Guarente, *N. Engl. J. Med.*, 2011, **364**, 2235; J. Hu, H. Jing, and H. Lin, *Future Med. Chem.*, 2014, **6**, 945.

14. J. C. Tanny, G. J. Dowd, J. Huang, H. Hilz, and D. Moazed, *Cell*, 1999, **99**, 735.
15. J. L. Feldman, J. Baeza, and J. M. Denu, *J. Biol. Chem.*, 2013, **288**, 31350; R. A. Mathias, T. M. Greco, A. Oberstein, H. G. Budayeva, R. Chakrabarti, E. A. Rowland, Y. Kang, T. Shenk, and I. M. Cristea, *Cell*, 2014, **159**, 1615; J. Du, Y. Zhou, X. Su, J. J. Yu, S. Khan, H. Jiang, J. Kim, J. Woo, J. H. Kim, B. H. Choi, B. He, W. Chen, S. Zhang, R. A. Cerione, J. Auwerx, Q. Hao, and H. Lin, *Science*, 2011, **334**, 806; H. Jiang, S. Khan, Y. Wang, G. Charron, B. He, C. Sebastian, J. Du, R. Kim, E. Ge, R. Mostoslavsky, H. C. Hang, Q. Hao, and H. Lin, *Nature*, 2013, **496**, 110.
16. D. Kölle, G. Brosch, T. Lechner, A. Lusser, and P. Loidl, *Methods*, 1998, **15**, 323.
17. D. Herman, K. Jenssen, R. Burnett, E. Soragni, S. L. Perlman, and J. M. Gottesfeld, *Nat. Chem. Biol.*, 2006, **2**, 551.
18. B. C. R. Dancy, S. A. Ming, R. Papazyan, C. A. Jelinek, A. Majumdar, Y. Sun, B. M. Dancy, W. J. Drury III, R. J. Cotter, S. D. Taverna, and P. A. Cole, *J. Am. Chem. Soc.*, 2012, **134**, 5138.
19. M. T. Borra, B. C. Smith, and J. M. Denu, *J. Biol. Chem.*, 2005, **280**, 17187; Y. Li, T. Liu, S. Liao, Y. Li, Y. Lan, A. Wang, Y. Wang, and B. He, *Biochem. Biophys. Res. Commun.*, 2015, **467**, 459; R. Baba, Y. Hori, S. Mizukami, and K. Kikuchi, *J. Am. Chem. Soc.*, 2012, **134**, 14310; R. Baba, Y. Hori, and K. Kikuchi, *Chemistry*, 2015, **21**, 4695.
20. M. Kawaguchi, S. Ikegawa, N. Ieda, and H. Nakagawa, *ChemBioChem*, 2016, **17**, 1961; M. Kawaguchi, N. Ieda, and H. Nakagawa, *J. Med. Chem.*, 2019, **62**, 5434.
21. S. Heinze, M. Ritzau, W. Ihn, H. Hülsmann, B. Schlegel, K. Dornberger, W. F. Fleck, M. Zerlin, C. Christner, U. Gräfe, G. Küllertz, and G. Fischer, *J. Antibiot.*, 1997, **50**, 379; C. Christner, M. Zerlin, U. Gräfe, S. Heinze, G. Küllertz, and G. Fischer, *J. Antibiot.*, 1997, **50**, 384.
22. D. E. Dorman and F. A. Bovey, *J. Org. Chem.*, 1973, **38**, 1719; D. E. Dorman and F. A. Bovey, *J. Org. Chem.* 1973, **38**, 2379; I. Z. Siemion, T. Wieland, and K. H. Pook, *Angew. Chem., Int. Ed. Engl.*, 1975, **14**, 702.
23. P. Marfey, *Carlsberg Res. Commun.*, 1984, **49**, 591.
24. H. Stump, W. Stahl, J. Wink, A. Markus, H. Kogler, and J. Backhaus, U.S. Patent 6221844, 2 June 2001.
25. V. R. Hegde, J. Silver, M. Patel, V. P. Gullo, R. Yarborough, E. Huang, P. R. Das, M. S. Puar, B. J. DiDomenico, and D. Loebenberg, *J. Antibiot.*, 2001, **54**, 74.
26. V. R. Hegde, J. Silver, M. Patel, V. P. Gullo, M. S. Puar, P. R. Das, and D. Loebenberg, *J. Antibiot.*, 2003, **56**, 437.

27. B. Perlatti, C. B. Nichools, J. A. Alspaugh, J. B. Gloer, and G. F. Bills, *Biomolecules*, 2020, **10**, 1371.
28. Y. Nagai and T. Kusumi, *Tetrahedron Lett.*, 1995, **36**, 1853.
29. L. Jin, H. Galonek, K. Israelian, W. Choy, M. Morrison, Y. Xia, X. Wang, Y. Xu, Y. Yang, J. J. Smith, E. Hoffmann, D. P. Carney, R. B. Perni, M. R. Jirousek, J. E. Bemis, J. C. Milne, D. A. Sinclair, and C. H. Westphal, *Protein Sci.*, 2009, **18**, 514.
30. J. Zhang, H. Xiang, J. Liu, Y. Chen, R. R. He, and B. Liu, *Theranostics*, 2020, **10**, 8315.