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Marianins A and B, Prenylated Phenylpropanoids from *Mariannaea camptospora*

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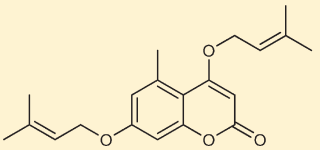
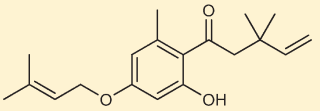
Supporting Information

ABSTRACT: Marianins A (**1**) and B (**2**), two new prenylated phenylpropanoids, were isolated from the culture extract of the fungus *Mariannaea camptospora*. Structures of marianins were elucidated by interpretation of NMR and other spectroscopic data. **1** is a 5-methylcoumarin bearing two prenyloxy groups, while **2** is an orcinol derivative substituted with a 3,3-dimethyl-4-pentenoyl chain. **2** is possibly derived from **1** through a Claisen rearrangement of the prenyl group, followed by lactone hydrolysis and decarboxylation. These compounds showed weak antibacterial activity against *Micrococcus luteus*.



Marianin A (**1**)



Marianin B (**2**)

Many of fungal species in the order Hypocreales show pathogenicity to higher organisms such as insects and plants. These pathogenic fungi are currently attracting substantial attention as a source of bioactive small molecules owing to their potential in secondary metabolite production.[1] As an example, members of the genus *Cordyceps* are host-specific entomopathogens, from which numerous structurally unique metabolites have been isolated.[1b] *Mariannaea* is also described as a pathogen to some insects[2] and reptiles,[3] and it has been recovered from soil or rotten wood, indicating its saprophytic property as well.[4] Members of this genus show high morphological similarity to the insect-pathogen *Paecilomyces*, and its teleomorph is phylogenetically close to the plant-pathogen *Nectria*.[5] Six species and one variety are included in the genus *Mariannaea*,[6] but only one metabolite, mariannaeapyrone, has been reported from this group to date.[7] In our investigation on chemically unexplored pathogenic fungi, HPLC/UV-based metabolite analysis of a *Mariannaea* strain led to the isolation of two prenylated phenylpropanoids, marianins A (**1**) and B (**2**). We herein describe the isolation and structure elucidation of these new compounds.

The producing strain *Mariannaea camptospora* TAMA 118 was isolated from a rotten wood sample collected in Tokyo, Japan. It was cultured in SGCH-X medium, and the whole culture broth was extracted with 1-butanol. The crude extract obtained after solvent removal (2.2 g from 1 L) was subjected to consecutive fractionation using silica gel and C-18 column chromatographies, followed by reversed-phase HPLC, to yield 4.0 and 1.8 mg, respectively, of marianins A (**1**) and B (**2**).

Marianin A (**1**) was obtained as a colorless, amorphous solid that gave an $[M - H]^-$ peak at m/z 327.1602 (calcd for $C_{20}H_{23}O_4$, 327.1602) in the negative ion HR-ESITOFMS, consistent with the molecular formula $C_{20}H_{24}O_4$ (nine degrees of unsaturation). The IR spectrum indicated the presence of a carbonyl functional group (1708 cm^{-1}). NMR data of **1** showed the presence of 20 carbons including four oxygenated sp^2 carbons, five olefinic or aromatic carbons, four quaternary sp^2 carbons, two oxygenated methylenes, and five methyl groups (Table 1). The $^1H-^1H$ COSY spectrum showed two cross-peaks, each connecting methylene protons and a vinyl proton to give two small fragments, H_2-11/H-12 and H_2-17/H-18. The first fragment was expanded to include a three-carbon fragment C-14/C-13/C-15 on the basis of HMBC correlations from the methyl proton singlets H_3-14 and H_3-15 to one another, to C-13, and to C-12, establishing a prenyl group. Similarly, the second COSY-defined fragment (H_2-17/H-18) and a three-carbon fragment C-20/C-19/C-21 were joined by a series of HMBC correlations from H_3-20 and H_3-21 to one another and to C-18 and C-19, to provide another prenyl unit. The aromatic part was constructed starting from the methyl protons H_3-16, which showed long-range couplings to C-10, C-5, and C-6. The *meta* relationship of C-6 and C-8 was indicated by a small coupling constant (J = 2.3 Hz) between the protons bonding to these carbons. Chemical shifts of C-6, C-8, and C-10 were relatively upfield, suggesting that these carbons were located *ortho* to the oxygenated sp^2 carbons C-7 and C-9. These data, along with

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Table 1. ^{1}H and ^{13}C NMR Data for Marianin A (1) in $CDCl_3$

position	δ_C, mult.[a]	δ_H (J in Hz)[b]	HMBC[b,c]
2	163.4, qC		
3	88.0, CH	5.51, s	2, 4, 5, 10
4	168.9, qC		
5	138.5, qC		
6	116.2, CH	6.61, d (2.3)	7, 8, 10, 16
7	161.1, qC		
8	99.4, CH	6.66, d (2.3)	4, 6, 7, 9, 10
9	156.7, qC		
10	108.0, qC		
11	66.1, CH_2	4.61, d (6.7)	4, 12, 13
12	117.5, CH	5.50, m	
13	140.0, qC		
14	25.75[d], CH_3	1.82, s	12, 13, 15
15	18.29[e], CH_3	1.76, s	12, 13, 14
16	23.6, CH_3	2.60, s	5, 6, 10
17	65.1, CH_2	4.57, d (6.8)	7, 18, 19
18	118.8, CH	5.46, m	
19	139.1, qC		
20	25.83[d], CH_3	1.80, s	18, 19, 21
21	18.34[e], CH_3	1.76, s	18, 19, 20

[a] Recorded at 100 MHz. [b] Recorded at 500 MHz. [c] HMBC correlations are from proton to the indicated carbon. [d,e] Interchangeable.

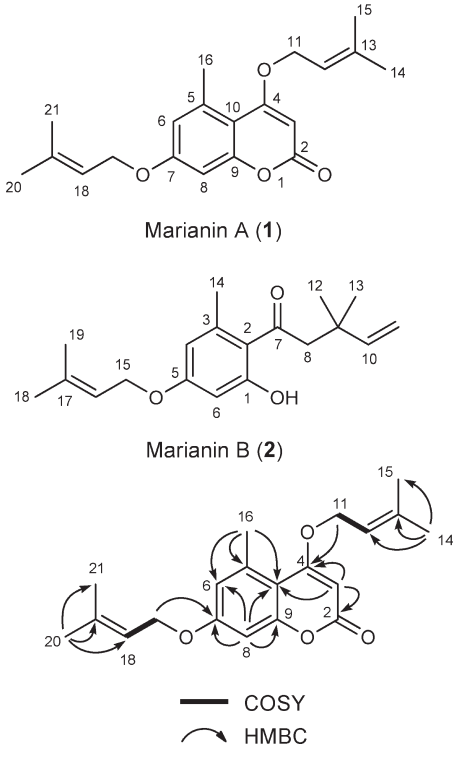

Figure 1. COSY and key HMBC correlations for **1**.

HMBC correlations from H-6 and H-8 to one another, to C-7, and to C-10, and from H-8 to C-9, established the benzenoid substructure. To this unit was connected a three-carbon fragment C-2/C-3/C-4 on the basis of HMBC correlations from H-3 to C-2, C-4, and C-10 and a four-bond correlation from H-8 to C-4. HMBC correlations from H_2-11 to C-4 and from H_2-17 to C-7 linked the prenyl groups to these carbons through ether linkages. The remaining three degrees of unsaturation were assigned to the C-2 carbonyl functionality, the C-3−C-4 double bond, and a lactone ring connected between C-2 and C-9, to complete the structure of **1** (Figure 1).

Table 2. ^{1}H and ^{13}C NMR Data for Marianin B (2) in $CDCl_3$

position	δ_C, mult.[a]	δ_H (J in Hz)[b]	HMBC[b,c]
1	165.2, qC		
2	117.1, qC		
3	140.4, qC		
4	112.3, CH	6.27, d (2.5)	2, 5, 6, 14
5	163.1, qC		
6	99.7, CH	6.30, d (2.5)	1, 2, 4, 5
7	206.2, qC		
8	54.5, CH_2	2.93, s	2, 7, 9, 10, 12, 13
9	37.7, qC		
10	147.3, CH	5.94, dd (17.5, 10.7)	9, 12, 13
11	110.5, CH_2	4.91, dd (10.7, 0.8)	9, 10
		4.95, dd (17.5, 0.8)	
12	27.4, CH_3	1.15, s	8, 9, 10, 11, 13
13	27.4, CH_3	1.15, s	8, 9, 10, 11, 12
14	25.3, CH_3	2.53, s	2, 3, 4, 6, 8
15	64.9, CH_2	4.50, d (6.5)	5, 16, 17
16	118.8, CH	5.46, m	
17	139.0, qC		
18	25.8, CH_3	1.80, s	19, 16, 17
19	18.2, CH_3	1.74, s	18, 16, 17
1-OH		12.6, s	1, 2, 5, 6

[a] Recorded at 100 MHz. [b] Recorded at 500 MHz. [c] HMBC correlations are from proton to the indicated carbon.

Marianin B (**2**) was obtained as a colorless, amorphous solid that analyzed for the molecular formula $C_{19}H_{26}O_3$ on the basis of an $[M - H]^-$ peak at m/z 301.1803 observed in the HR-ESITOFMS. The IR spectrum showed absorption bands for hydroxyl (3261 cm^{-1}) and carbonyl (1609 cm^{-1}) functionalities. ^{1}H and ^{13}C NMR analysis of **2** revealed the presence of 19 carbons including one carbonyl, two oxygenated sp^2 carbons, one sp^2 methylene, four olefinic or aromatic carbons, three quaternary sp^2 carbons, two sp^3 methylenes (one is oxygenated), one quaternary sp^3 carbon, and five methyl groups (Table 2). **2** also possessed a prenyl group, as confirmed by a COSY correlation between H_2-15 and H-16 and HMBC correlations from H_3-18 and H_3-19 to one another, to C-16, and to C-17. Typical coupling patterns for a vinyl group were recognized in the ^{1}H NMR spectrum of **2**. Specifically, deshielded protons at δ 4.91 and 4.95 bonding to a single carbon at δ 110.5 were mutually coupled with a small geminal coupling constant (J = 0.8 Hz), and these protons (H_2-11) had COSY correlations to a vinyl proton, H-10. This proton showed correlations to C-9, C-12, and C-13, and two equivalent singlet methyl protons, H_3-12 and H_3-13, in turn, showed a series of HMBC correlations to C-9, C-10, and methylene carbon C-8. Furthermore, H_2-8 was correlated to carbonyl carbon C-7 and quaternary sp^2 carbon C-2. These correlation data established a 3,3-dimethyl-4-pentenoyl chain connecting to the aromatic core. The 1,2,3,5-tetrasubstituted benzene was elucidated by HMBC correlations from an

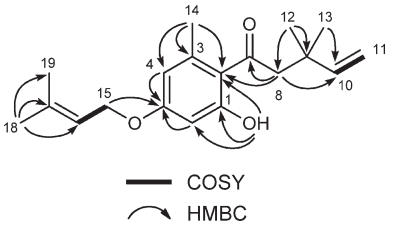

Figure 2. COSY and key HMBC correlations for **2**.

exchangeable proton at δ 12.6 to C-1, C-2, and C-6, from methyl protons H_3-14 to C-2, C-3, and C-4, and from aromatic protons H-4 and H-6 to C-5. Strong hydrogen bonding of the phenolic proton to the C-7 carbonyl was suggested by the IR absorption band at 1609 cm^{-1}, which was significantly low as a wavenumber for keto carbonyls.[8] The prenyloxy group was attached to C-5 by an HMBC correlation from H_2-15 to C-5, to complete the structure of **2** (Figure 2).

Coumarins are the phenylalanine-derived secondary metabolites widely distributed in plants and are also produced by some fungi and bacteria.[9] These aromatic lactones are often modified by prenylation,[10] but those bearing more than two prenyloxy groups are very rare. Except for **1**, only two plant-derived coumarins are known to be *O*-prenylated at two sites.[11] **2** features an unprecedented 3,3-dimethyl-4-pentenoyl chain attaching to the prenylated orcinol. This unique metabolite could be derived from **1** as illustrated in Figure 3. Migration of the 4-*O*-prenyl group to C-3 can occur by Claisen rearrangement (Figure 3, path A). Involvement of this type of rearrangement has been shown in the biogenesis of plant phenylpropanoids,[12,13] while the direct introduction of the dimethylallyl group at C-3 is also possible by reverse-prenylation (Figure 3, path B).[14] The C-2 carbonyl carbon is likely removed by lactone hydrolysis, followed by decarboxylation, as an analogous sequence of reactions has been demonstrated to proceed during alkaline hydrolysis of a plant coumarin.[15]

Marianins A (**1**) and B (**2**) showed weak antimicrobial activity against *Micrococcus luteus* with an MIC value of 15 and 30 μg/mL, respectively, while both compounds had no activity against *Eschericha coli* or *Candida albicans* at 30 μg/mL. Marianins lacked significant activity in a cancer cell cytotoxicity assay. Marianin A (**1**) was slightly active against HeLa and MCF7 cells with IC_{50} values of 34 and 39 μM, respectively, and marianin B (**2**) was inactive against these cell lines (IC_{50} >100 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were recorded on a Hitachi U-3210 spectrophotometer. IR spectra were measured on a Perkin-Elmer Spectrum 100. NMR spectra were recorded on a Bruker AVANCE 400 or a Bruker AVANCE 500 spectrometer and referenced to the signals of tetramethylsilane as an internal standard. HR-ESI-TOFMS were recorded on a Bruker microTOF focus spectrometer. Silica gel 60 (Kanto Chemical Co., Inc., 63-210 mesh) and silica gel 60-C18 (Nacalai Tesque, 250−350 mesh) were used for silica gel and ODS column chromatographies, respectively. HPLC separation was performed using a Capcell Pak C18 MGII S5 (Shiseido Co., Ltd., 20 × 150 mm) with a photodiode array detector.

Microorganism. Strain TAMA 118 was isolated from a rotten wood sample collected at Tamagawa University, Machida, Tokyo, by direct isolation under microscope. The strain was identified as *Mariannaea camptospora* Samson on the basis of morphological and cultural characteristics and 99% similarity of internal transcribed spacer (ITS) sequence (562 nucleotides; GenBank accession number AB587666) to *M. camptospora* NBRC 33106 (accession number AB112029) and 94% similarity to *M. camptospora* CBS 209.73 (accession number AY624202).

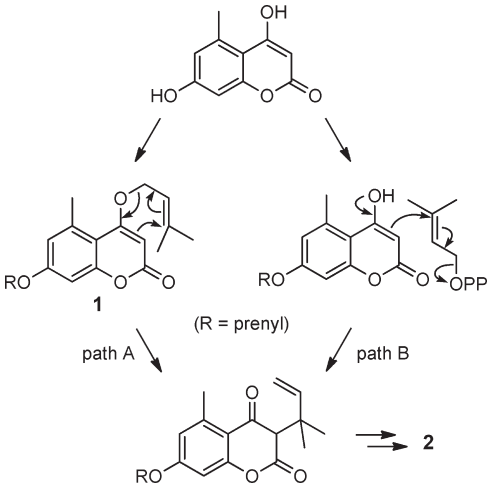

Figure 3. Proposed biogenesis of **2**.

Fermentation. Strain TAMA 118 grown on a PDA slant was inoculated into 150 mL polypropylene flasks each containing 20 mL of the SGCH-X medium [10 g of sodium glutamate, 30 g of sucrose, 0.5 g of yeast extract (Difco Laboratories), 0.4 g of KCl, 2 g of $CaCO_3$, 0.015 mg of KH_2PO_4, 0.005 mg of $MgSO_4 \cdot 7H_2O$, 2.5 mL of metal solution, and 1 L of ion exchanged water (pH was adjusted to 6.5 before addition of $CaCO_3$)], supplemented with 0.02 g of XAD1180 resin (Organo Co., Ltd.). Metal solution was prepared as containing 15 mg of $FeSO_4 \cdot 7H_2O$, 9 mg of $ZnSO_4 \cdot 7H_2O$, 4 mg of $MnSO_4 \cdot 5H_2O$, 5.5 mg of $CuSO_4 \cdot 5H_2O$, 6 mg of $Co(NO_3)_2 \cdot 6H_2O$, 2.5 mg of H_3BO_3, and 2 mg of $Na_2MoO_4 \cdot 2H_2O$ in 100 mL of 1 M H_2SO_4. After sterilization, the inoculated flasks were placed on a rotary shaker (225 rpm) at 25 °C for 21 days.

Extraction and Isolation. At the end of the fermentation period, 20 mL of 1-butanol was added to each flask, and they were allowed to shake on a rotary shaker (225 rpm) for 30 min. The mixture was centrifuged at 3000 rpm for 5 min, and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the organic solvent gave approximately 2.2 g of extract from 1 L of culture. The crude extract was subjected to silica gel column chromatography with a step gradient of $CHCl_3$/MeOH (1:0, 20:1, 10:1, 4:1, 2:1, 1:1, and 0:1 v/v). Fraction 4 was further purified by C-18 reversed-phase HPLC with MeCN/0.1% HCO_2H (80:20) to give 4.0 mg of **1**. Fractions 2 and 3 were combined and concentrated to provide semipure **2** (15 mg), which was further purified by C-18 reversed-phase HPLC with MeCN/0.1% HCO_2H (75:25) to give 1.8 mg of **2**.

*Marianin A (**1**):* colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 208 (4.52), 222 (4.30), 288 (3.91), 308 (4.07), 319 (3.99) nm; IR (ATR) ν_{max} 2913, 2855, 1708, 1594, 1155 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESITOFMS $[M - H]^-$ 327.1602 (calcd for $C_{20}H_{23}O_4$, 327.1602).

*Marianin B (**2**):* colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 220 (3.85), 275 (3.48) nm; IR (ATR) ν_{max} 3261, 2924, 2855, 1609, 1159 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HR-ESITOFMS $[M - H]^-$ 301.1803 (calcd for $C_{19}H_{25}O_3$, 301.1809).

Biological Assays. Antimicrobial assay was carried out using *Eschcerichia coli* NIH-JC2, *Micrococcus luteus* ATCC9343, and *Candida albicans* IFO1594 according to the procedures previously described.[16] An MIC value of the standard antibiotic tetracycline hydrochloride

(Sigma-Aldrich Co.) against *M. luteus* was 0.1 μg/mL. Cytotoxic assay was carried out using HeLa human cervical cancer cells and MCF7 human breast cancer cells. Cancer cells were suspended in RPMI medium containing 10% FBS (Sigma-Aldrich, Inc.) and 2 mM L-glutamine and seeded into the wells of a 96-well culture plate (1×10^4 cells/50 μL/well). Then, test compounds at various concentrations in DMSO/RPMI medium (0.8:92.2 v/v, 50 μL) were added to the wells. After incubation for 48 h in a humidified 5% CO_2 incubator at 37 °C, MTT (0.25 mg, Sigma-Aldrich, Inc.) in PBS (−) (50 μL) was added to each well, and the plates were placed in the incubator at 37 °C for 4 h. Medium in the wells was removed by suction, and DMSO (100 μL) was added to each well. After 10 min, the absorbance at 570 nm was read by a microplate reader. IC_{50} values of the positive control staurosporin (Wako Pure Chemical Industries, Ltd.) against HeLa and MCF7 cells were 4 pM and 50 nM, respectively.

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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