Massarinolins A−C: New Bioactive Sesquiterpenoids from the Aquatic Fungus *Massarina tunicata*

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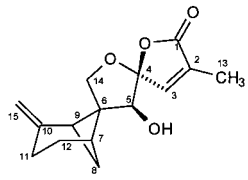
Massarinolins A−C (**1**−**3**), three new bioactive sesquiterpenoids possessing rare ring systems, have been isolated from liquid cultures of the aquatic fungus *Massarina tunicata* Shearer & Fallah. The structures were determined primarily by analysis of NMR data. Metabolites **1**−**3** are the first compounds to be reported from any member of the genus *Massarina*.

Despite the many valuable discoveries made through studies of soil fungi and the growing body of evidence suggesting that other fungal niches warrant similar investigation,[1,2] freshwater aquatic fungi remain virtually unexplored from a chemical standpoint. Antagonistic effects among competing aquatic fungi have been reported,[3,4] and similar observations of coprophilous fungi prompted us to undertake studies that have been effective in leading to a variety of new natural products with biological activity.[1,2] Thus, we have initiated studies of freshwater fungi as potential sources of new bioactive secondary metabolites.[1,5−7] During the course of this work, we investigated an isolate of *Massarina tunicata* Shearer & Fallah (A-25-1; Lophiostomataceae) collected from a twig submerged in a Wisconsin river. Chemical studies of the EtOAc extract from cultures of this organism have led to the isolation and structure determination of three novel bioactive sesquiterpenoids, which we named massarinolins A−C (**1**−**3**). Details of these studies are presented here.



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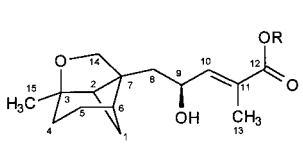

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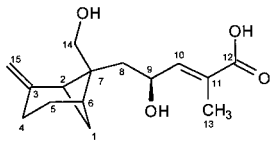

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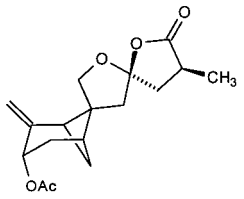

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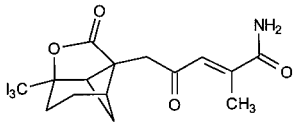

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Massarinolin A (**1**) has the molecular formula $C_{15}H_{18}O_4$, as deduced from ^{13}C NMR and HRFABMS data [$(M + H)^+$ at *m*/*z* 263.1289; Δ −0.6 mmu]. This formula indicated seven degrees of unsaturation. DEPT and ^1H NMR data ($CDCl_3$) revealed the presence of one methyl group, five methylene units, and four methine units. Comparison of DEPT results and the molecular formula indicated the presence of one free OH group. The ^{13}C NMR and DEPT spectra contained signals consistent with an ester carbonyl, two double bonds, a ketal functionality (quaternary carbon signal at δ 114.3), and one additional quaternary carbon. COSY and HMQC (Table 1) experiments defined −CH=CCH_3, $-CH_2O-$, and −CHO− subunits, as well as a $-CHCH_2CHCH_2CH_2C{=}CH_2$ unit, and allowed assignment of all of the protonated carbon signals.

Connectivities between the subunits in **1** were deduced from HMBC correlations (Table 1). Correlations of H_2-8 and H-9 with vinylic carbon C-10, and additional reciprocal correlations between positions 9 and 15, linked C-9 with C-10 to form a methylene cyclohexane ring. HMBC correlations of methine H-7 to oxygenated methine C-5, and from methine H-9 to C-5 and to quaternary carbon C-6, along with an unusually strong four-bond C−H correlation from H-8b to oxymethylene C-14, suggested the presence of a bicyclic substructure with bridged cyclobutane and six-membered rings as shown in **1**. The $^4J_{CH}$ coupling between H-8b and C-14 could be attributed to the fact that there are two possible W-type four-bond coupling pathways between these nuclei and to the possibility that there may be some orbital overlap between carbons C-6 and C-8.[8] Strong four-bond homonuclear coupling has been observed for protons that have analogous dispositions across four-membered rings.[8−10] In fact, a 5.7 Hz coupling was indeed observed between H-7 and H-9 in the ^1H NMR spectrum of **1** and confirmed by a strong COSY cross-peak.

Ketal carbon C-4 was connected to C-3 and to the isolated oxygenated methylene carbon C-14 via an ether linkage, on the basis of HMBC correlations from H-3 and H_2-14 to C-4. Although no direct evidence for the connection between C-4 and C-5 was provided by the HMBC data, the chemical shifts of C-4 and C-5 (Table 1) suggested two possible substructures, as shown in **1a** and **1b**. Both of these substructures are consistent with the NMR and MS data for massarinolin A. In an effort to determine the correct substructure, ^1H NMR data for **1** were recorded in other NMR solvents. A spectrum recorded in acetone-d_6 showed a coupling ($^3J_{H-H}$ = 6.4 Hz) between H-5 (δ 4.27) and a

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Table 1. NMR Data for Massarinolin A (**1**)[a]

position	^{1}H δ (mult; J_{H-H} in Hz)[b]	^{13}C δ[c]	HMBC correlations (^{13}C-no.)[d]	selected NOESY correlations (^{1}H-no.)[b]
1		171.5		
2		134.6		
3	6.95 (q; 1.3)	143.1	1, 2, 4, 13	5
4		114.3		
5	4.22 (br s)	81.2	9, 14	3, 7, 8a, 9
6		56.1		
7	2.63 (m)	35.5	5, 6, 9, 11	
8a	2.27 (ddd; 10, 5.7, 5.7)		7, 9, 10, 12	5
8b	1.57 (d; 10)	28.3	6, 7, 9, 10, 12, 14[e]	12a
9	3.14 (dd; 5.7, 5.7)	50.2	5, 6, 7, 8, 10, 11, 15	5, 15a
10		148.2		
11a	2.58 (m)	23.7	10, 12, 15	15b
11b	2.22 (dddd; 17, 9.4, 1.2, 1.2)		7, 9, 10, 12, 15	15b
12a	1.98 (dddd; 12, 12, 4.2, 2.4)			
12b	1.64 (m)	24.3	6, 7, 8, 11	14b
13	1.94 (d; 1.5)	11.0	1, 2, 3	
14a	3.99 (d; 9.6)	72.8	4, 5, 6, 7, 9	9, 11, 12, 15a,b
14b	3.93 (d; 9.6)		4, 6, 7, 9	7, 12b
15a	4.87 (m)		9, 11	9, 14a
15b	4.84 (m)	110.6	9, 11	11b, 14a

[a] All NMR data were recorded using $CDCl_3$ solutions. [b] Recorded at 600 MHz. [c] Recorded at 75.5 MHz. [d] Recorded at 600 MHz (^{1}H dimension). [e] Denotes a 4-bond correlation.

hydroxy proton signal (δ 4.87) that was not observed when the spectrum was recorded in $CDCl_3$. This result, along with the disappearance of the hydroxy proton signal and collapse of the H-5 signal to a singlet upon addition of CD_3-OD to this solution, revealed that the free hydroxy group must be located at C-5 and indicated that the correct substructure must be **1a**.

The relative stereochemistry shown for structure **1** was deduced by analysis of NOESY data and by comparison to the stereochemistry reported for its closest known relative, expansolide A (**4**).[10] As in **4**, H-7 and H-9 of **1** were assigned a cis disposition in the opposite corners of the four-membered ring based on the unusually large $^4J_{H-H}$ coupling constant between these protons.[10] The relative configurations at C-4 and C-5 were defined as depicted in structure **1** on the basis of strong NOESY correlations of H-5 with H-3, H-8a, and H-9. Although expansolide A (**4**) was reported to undergo partial conversion to its C-4 epimer (expansolide B) upon standing in solution at room temperature, massarinolin A (**1**) was not observed to undergo this process under similar conditions.

Massarinolin B (**2**) has the molecular formula $C_{15}H_{22}O_4$, as deduced from ^{13}C NMR and HRFABMS data [$(M + H)^+$ at m/z 267.1609; Δ −1.3 mmu], requiring five unsaturation equivalents. ^{1}H, ^{13}C, and DEPT NMR data revealed the presence of two methyl groups, five methylene carbons (including one bound to oxygen), an olefinic CH, three sp^3 methine carbons (one bound to oxygen), one carboxyl carbonyl, and three other nonprotonated carbons (Table 2). These results, together with the molecular formula of **2**, indicated the presence of two exchangeable protons and required that **2** be tricyclic. HMQC data were used to identify carbons bound to hydrogens. Although the COSY data for **2** defined two isolated spin systems, **A** ($^{13}CH_3{}^{11}C$═ $^{10}CH^9CH(OH)^8CH_2$−) and **B** (−$^{14}CH_2O$−), a third spin system **C** (−$^4CH_2{}^5CH_2{}^6CH^2CH^1CH_2$− or −$^4CH_2{}^5CH_2{}^6CH$-$^1CH_2{}^2CH$−) could not be unambiguously assigned because of ^{1}H NMR signal overlap of a methine proton resonance (H-2) and one of the methylene proton resonances (H-1a) at δ 2.14. This ambiguity was removed by analysis of HMBC correlations (Table 3).

HMBC correlations of H_3-13 and the vinylic proton H-10 to the carboxyl carbon (C-12) indicated that C-12 was connected to the olefin at C-11. HMBC correlations of H-9 and H_2-8 with nonoxygenated quaternary carbon C-7, of H_2-8 with the isolated oxymethylene carbon (C-14), and of H_2-14 with C-8 revealed connections of spin systems **A** and **B** to quaternary carbon C-7. Connection of the resulting substructure with the third spin system **C** was then evaluated. HMBC correlations of H_2-8 with C-2 and C-6, but not with C-1, required connection of both C-2 and C-6 to C-7 and indicated that spin system **C** must be a −$^4CH_2$-$^5CH_2{}^6CH^1CH_2{}^2CH$− unit. Correlations of H-14a with C-2 and C-6 supported this assignment. The alternative −$^4CH_2$-$^5CH_2{}^6CH^2CH^1CH_2$− spin system was ruled out because integration of such a unit into the structure would generate a cyclopropane ring, and the chemical shifts observed are not consistent with such a unit.

HMBC correlations of H_3-15 to the oxygenated quaternary carbon C-3, as well as to C-2 and C-4, indicated the connection of C-3 to C-15, C-2, and C-4. The oxygen atom bound to C-3 must be connected to C-14 to form an ether linkage on the basis of an HMBC cross-peak from H_2-14

Table 2. NMR Data for Massarinolins B (**2**) and C (**3**)[a]

	^{1}H δ (mult.; J_{H-H} in Hz)		^{13}C δ	
	2[b]	**3**[c]	**2**[d]	**3**[e]
1a	2.14 (m)	2.34 (ov. m)	23.5	24.8[f]
1b	1.50 (m)	1.50 (m)		
2	2.13 (m)	2.76 (dd; 5.3, 5.3)	53.6	51.2
3			87.6	151.5
4a	1.87 (m)	2.63 (m)	33.4	26.9
4b	1.61 (m)	2.34 (ov. m)		
5a	1.88 (m)	2.03 (m)	23.8	24.0[f]
5b	1.78 (m)	1.92 (m)		
6	2.31 (m)	2.34 (ov. m)	40.3	38.4
7			55.5	47.6
8a	1.99 (dd; 14, 3.9)	1.99 (dd; 15, 3.7)	41.8	41.5
8b	1.83 (m)	1.95 (dd; 15, 8.8)		
9	4.45 (ddd; 8.7, 8.6, 4.5)	4.70 (ddd; 8.8, 8.8, 3.7)	67.1	66.8
10	6.67 (dq; 8.6, 1.2)	6.75 (m)	145.3	146.0
11			128.5	128.2
12			171.5	171.5
13	1.84 (d; 1.2)	1.87 (d; 1.4)	12.7	12.8
14a	3.90 (d; 9.6)	3.55 (d; 12)	72.7	64.9
14b	3.60 (d; 9.6)	3.29 (d; 12)		
15a	1.23 (s)	4.64 (br s)	25.3	107.5
15b		4.60 (br s)		

[a] All spectra were recorded using CD_3OD solutions. [b] Recorded at 600 MHz. [c] Recorded at 360 MHz. [d] Recorded at 75.5 MHz. [e] Recorded at 90.7 MHz. [f] These assignments are interchangeable.

Table 3. Long-Range Heteronuclear and NOESY Correlations for Massarinolins B (**2**) and C (**3**)[a]

^{1}H position	HMBC correlations (^{13}C-no.) **2**[b]	selective INEPT correlations (^{13}C-no.) 3[c]	selected NOESY correlations (^{1}H-no.) **2**[b]	selected NOESY correlations (^{1}H-no.) **3**[b]
1a	2, 3, 5, 6			
1b	2, 3, 5, 6, 7		4a	4a
2	3, 4, 6, 7, 8, 15	1, 3, 6, 7, 15	8b, 15	8b, 15b
4a	5, 6	3, 5, 15		1b
4b	2, 3, 5, 6			
5a	6, 7		14a	14a
5b	4			
6	1, 2, 4, 7, 8		9	
8a	2, 6, 7, 9, 14			
8b	2, 6, 7, 9, 14		2, 10, 14b	2, 10, 14b
9	7, 8, 10, 11		6, 13, 14b	13, 14b
10	12, 13	11, 12, 13	8b	8b
13	10, 11, 12		9	9
14a	2, 3, 6, 7	2, 7, 8	5a	5a
14b	3, 6, 7, 8		8b, 9	8b, 9
15a	2, 3, 4		2	
15b		3		2

[a] All spectra were recorded using CD_3OD solutions. [b] Recorded at 600 MHz (^{1}H dimension). [c] Recorded at 360 MHz (^{1}H dimension).

to C-3, thus forming a tricyclic system as shown in **2**. The remaining two exchangeable protons required by the molecular formula must be attributed to a free carboxylic acid group and a hydroxy group. The presence of a carboxylic acid group was confirmed by conversion of **2** to the corresponding methyl ester **5** by treatment with trimethylsilyldiazomethane[11] in MeOH. The OH group was positioned at the only available site (C-9), and this assignment was later confirmed by acylation of **2** in the course of applying Helmchen's method to determine the absolute stereochemistry.

In the interpretation of the HMBC data for this compound, the possibility of the presence of four-bond heteronuclear correlations was carefully considered, since this substructure contains a four-membered ring, which had already proven to lead to an unusually strong four-bond ^{1}H−^{1}H correlation in **1**. Dreiding models suggest that four-bond heteronuclear correlations would generally be expected only between certain protons attached to the four-membered ring and any attached carbon substituents that have W-type geometric relationships with one of these protons. No such correlations were observed in this case.

The relative stereochemistry shown for the tricyclic system of **2** was proposed by analogy to that of the closest known analogue, pinthunamide (**6**).[12] The oxatricyclic ring system found in **2** is almost identical with that found in **6** except for the replacement of the ester group with an ether moiety. Massarinolin B (**2**) also differs from **6** in that it contains secondary alcohol and carboxylic acid groups on the side chain in place of the ketone and primary amide groups found in **6**. Dreiding molecular models of **2** and the previously reported X-ray model of **6** indicate that massarinolin B (**2**) must adopt the same relative stereochemistry as in **6** to satisfy the geometric requirements of the ring system. The C-10/C-11 double bond in **2** was assigned the *E* configuration on the basis of a strong NOESY correlation between H-9 and H_3-13, together with the upfield ^{13}C NMR chemical shift of C-13 (δ 12.7) in **2**, which matches up well with the corresponding signal for pinthunamide (**6**; δ 14.0).

To determine the absolute configuration at C-9 of **2**, samples of methyl ester **5** were converted to (*R*/*S*)- and (*R*)-2-phenylbutyrate esters. Formation of the (*R*)-phenylbutyrate ester of **5** was confirmed by a significant downfield shift of H-9 from δ 4.45 to 5.45, appearance of NMR signals for the acyl group, and HRFABMS analysis. Formation of the mixture of *R*/*S* products was confirmed in a similar manner. It was possible to make chemical shift assignments for most of the proton signals of the (*R*)- and (*S*)-phenylbutyrate esters of **5**, although several signals overlapped in the region from δ 1.7 to 2.5 in the spectrum of the mixture. Significant upfield shifts of the H-10, H_3-13, and OCH_3 signals, as well as downfield shifts of the H_2-1, H-6, H_2-14, and H_3-15 signals, for the (*S*)-phenylbutyrate ester relative to those of the (*R*)-phenylbutyrate ester were clearly observed. On the basis of Helmchen's rules,[13] the configuration at C-9 was deduced to be *S* (Figure 1). Unfortunately, it was not possible to correlate the stereochemistry of the bicyclo[3.1.1]heptane portion of **2** with that of the side chain, so the absolute stereochemistry of the ring system in **2** could not be determined by correlation with the absolute configuration at C-9. However, regardless of the absolute configuration of the tricyclic portion, the protons in this unit would experience the same type of shift effects due to the aromatic ring introduced by the formation of the 2-phenylbutyrate esters of **5**. The absolute stereochemistry for the bicyclo[3.1.1]heptane subunit common to **1**−**3** has not been reported for any member of this group, although the prior publications arbitrarily showed opposite configurations for **4** and **6**.[10,11,13] The corresponding subunits of compounds **1**−**6** are all depicted here in the same (arbitrarily chosen) absolute configuration.

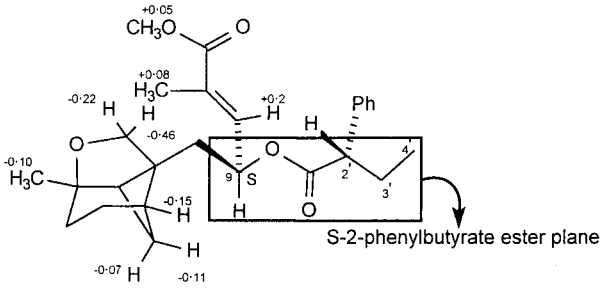

Figure 1. Bisecting plane of the (*S*)-2-phenylbutyrate ester of massarinolin B methyl ester and the observed chemical shift differences ($\Delta\delta = \delta_R - \delta_S$) for selected protons of the (*S*)- and (*R*)-2-phenylbutyrate esters.

The molecular formula of massarinolin C (**3**) was established as $C_{15}H_{22}O_4$ on the basis of HRFABMS and ^{13}C NMR data [$(M + Na)^+$ at *m*/*z* 289.1424; Δ −0.8 mmu]. The ^{1}H NMR spectrum of compound **3** was similar to that of **2** except for the presence of a pair of vinylic methylene proton resonances at δ 4.64 and 4.60 and the absence of the H_3-15 methyl proton resonance (Table 2). ^{13}C NMR and DEPT data revealed the presence of two new olefinic carbons in place of the C-15 methyl and the oxygenated quaternary carbon of **2**. In addition, the MS and DEPT data indicated that compound **3** must have three exchangeable protons. These data suggested that compound **3** differs from **2** by opening of the furan ring in **2** and replacement of the C-15 methyl group with an exocyclic olefin unit.

^{1}H NMR assignments for compound **3** were proposed by analysis of COSY data and by comparison to the assignments for **2**. A series of selective INEPT experiments (Table 3) were carried out, leading to confirmation of the gross structure of massarinolin C as **3**. For example, polarization transfer to carbon signals corresponding to C-3 (δ 151.5) and C-15 (δ 107.5) observed upon irradiation of the H-2 signal (δ 2.76) confirmed the placement of the exocyclic olefin group at C-3. The relatively large ^{13}C NMR chemical shift difference between the C-14 carbon resonances in **2**

and in **3**, changes in shifts and *J* values for the C-14 protons, and the absence of any long-range correlation between H_2-14 and C-3 in **3** all supported the proposed structural difference between **2** and **3**.

The relative stereochemistry and olefin geometry shown for massarinolin C (**3**) are proposed on the basis of NOESY data and ^{13}C NMR chemical shifts for **3**. A strong NOESY correlation between the H-14a signal at δ 3.55 and the H-5a signal (δ 2.03) indicated that they are spatially close, placing H-14a in an axial position on the cyclohexane ring. The double bond between C-10 and C-11 in **3** was again assigned the *E* geometry on the basis of the strong NOESY correlation between H-9 and H_3-13, together with the ^{13}C NMR chemical shift comparison of the relevant values in **3** with those of **2** and **6**.

Massarinolins A (**1**) and B (**2**) possess unusual tetracyclic and tricyclic ring systems. The ring system found in compound **1** has been previously encountered only in expansolides A and B (e.g., **4**), reported as metabolites of *Penicillium expansum*.[10] Compounds related to massarinolins B and C (e.g., **6**) have been isolated from *Ampulliferina* sp.[12,14] Massarinolins A−C appear to be sesquiterpenoids biosynthesized from a farnesyl-type precursor,[12,15] and massarinolins A and B are presumably cyclization products of massarinolin C.

To our knowledge, these new compounds are the first secondary metabolites to be reported from any member of the genus *Massarina*, although there is at least one prior report of antagonistic activity for a different *Massarina* species (*M. aquatica*).[3] In standard disk assays at 200 μg/disk, **1** and **2** were active against *Bacillus subtilis* (ATCC 6051), affording zones of inhibition of 17 and 8 mm, respectively. Compound **1** was also active against *Staphylococcus aureus* (ATCC 29213), causing a 10-mm zone of inhibition. Massarinolin C (**3**) did not exhibit any activity against Gram-positive bacteria. None of these compounds were active in assays against a strain of *Candida albicans* (ATCC 14053) at 200 μg/disk. Further biological evaluation of these compounds is in progress.

Experimental Section

Fungal Material. *M. tunicata* Shearer & Fallah was isolated from a submerged twig collected from the Lemonweir River in Adams County, WI, on July 31, 1992. This isolate was assigned accession number A-25-1 in the C. A. Shearer culture collection at the University of Illinois, and a subculture has been deposited at the American Type Culture Collection in Rockville, MD. This fungus is classified in the Lophiostomataceae, Pleosporales, Loculoascomycetes. *M. tunicata* is a new species of *Massarina*. Details of its taxonomic characteristics will be published elsewhere.

General Experimental Procedures. THF was freshly distilled from Na under inert atmosphere before use. NMR spectra were recorded using $CDCl_3$, CD_3OD, or acetone-d_6 solutions, and chemical shifts were referenced relative to the corresponding residual solvent signals (δ 7.24/77.0, 3.30/49.0, and 2.04/29.8, respectively). Carbon multiplicities were established by DEPT experiments. HMQC experiments were optimized for $^nJ_{CH} = 135$ Hz. Descriptions of other NMR parameters, as well as NMR, MS, UV, and IR instrumentation, have been provided elsewhere.[16]

Production, Isolation, and Characterization of Massarinolins A−C (1−3). Six flasks, each containing 400 mL of potato dextrose broth (Difco) that had been sterilized at 120 °C for 15 min and then cooled to room temperature, were individually inoculated with 1-cm^2 agar plugs taken from stock cultures of *M. tunicata*. Flask cultures were inoculated at 25−28 °C and aerated by agitation on an orbital shaker at 150 rpm for a period of 30 days. Extraction of the filtered fermentation broth with EtOAc (5 × 1 L) provided an organic phase that was dried with $MgSO_4$ and then concentrated using a rotary evaporator to yield 1.75 g of crude extract. The crude extract was subjected to silica gel VLC, eluting with a stepwise gradient system ($CHCl_3$−CH_3OH). The fraction containing **1** (fraction A) eluted at 1% CH_3OH in $CHCl_3$, and the fraction containing **2** and **3** (Fraction B) eluted at 7% CH_3OH in $CHCl_3$. Fraction A (92 mg) was subjected to chromatography on Sephadex LH-20 (1:1 CH_2Cl_2/MeOH), and the largest subfraction (83 mg) was further separated by preparative reversed-phase HPLC using a gradient from 40 to 100% CH_3CN in H_2O over 60 min (Rainin Dynamax-60 Å C_{18} column; 2.14 × 25 cm; 8-μm particle size; 10 mL/min; UV detection at 215 nm) to yield **1** (3.2 mg). Fraction B (168 mg) was subjected to preparative reversed-phase HPLC using a gradient from 30 to 45% CH_3CN in H_2O over 20 min and then 45% to 100% CH_3CN in H_2O over 5 min. Subfractions containing **2** and **3** (2−4; total mass 92 mg) were further purified individually by semipreparative reversed-phase HPLC using a gradient from 10 to 100% CH_3CN in 0.1% HCOOH over 45 min (Beckman Ultrasphere C_8 column; 1.0 × 25 cm; 5-μm particle size; 2 mL/min; UV detection at 215 nm) to yield **2** (16 mg) and **3** (11.5 mg).

Massarinolin A (1): pale yellow oil; $[\alpha]_D$ +19° (*c* 0.18 g/dL; 23 °C; $CHCl_3$); HPLC t_R 20.9 min (under the conditions above); UV (MeOH) 210 (ϵ 6500), 270 (1500); IR 3603, 2932, 1769, 1611, 1314, 1279 cm^{-1}; EIMS (70 eV) *m/z* 262 (M^+, rel int 1.0), 244 (7), 194 (19), 149 (18), 119 (33), 105 (30), 96 (66), 93 (100), 79 (70), 68 (50), 55 (36); ^1H, ^{13}C, HMBC, and NOESY NMR data, Table 1; HRFABMS (thioglycerol matrix) obsd *m/z* 263.1289 $(M + H)^+$, calcd for $C_{15}H_{19}O_4$ 263.1283.

Massarinolin B (2): colorless oil; $[\alpha]_D$ +54° (*c* 0.65 g/dL; 27 °C; MeOH); HPLC t_R 24.0 min (under the conditions above); UV (MeOH) 224 (ϵ 4600); IR 3595, 2929, 1693, 1289, 1019 cm^{-1}; EIMS (70 eV) *m/z* 251 ($M - CH_3$, rel int. 0.5), 193 (31), 151 (28), 147 (17), 133 (21), 105 (19), 95 (53), 79 (31), 67 (25), 55 (31); ^1H and ^{13}C NMR data, Table 2; HMBC and NOESY data, Table 3; HRFABMS (3-NBA matrix) obsd *m/z* 267.1609 $(M + H)^+$, calcd for $C_{15}H_{23}O_4$ 267.1596.

Massarinolin C (3): colorless oil; $[\alpha]_D$ +6.3° (*c* 0.8 g/dL; 27 °C; MeOH); HPLC t_R 26.5 min (under the conditions above); UV (CH_3OH) 226 (ϵ 4500); IR 3296 (br), 2933, 1694, 1287, 1247, 1030 cm^{-1}; EIMS (70 eV) *m/z* 248 ($M-H_2O$, rel int. 3), 193 (18), 180 (14), 136 (27), 119 (27), 105 (49), 91 (100), 79 (82), 67 (40), 55 (47); ^1H and ^{13}C NMR data, Table 2; HMBC and NOESY data, Table 3; HRFABMS (glycerol matrix) obsd *m/z* 289.1424 $(M + Na)^+$, calcd for $C_{15}H_{22}O_4Na$ 289.1416.

Massarinolin B Methyl Ester (5). To a solution of 4 mg (15 μmol) of **2** in 1.5 mL of MeOH was added portions of a 2 M solution of trimethylsilyldiazomethane in hexane ($TMSCHN_2$; Aldrich; ca. 140 μL) until a yellow color persisted. The solution was stirred for 12 h and then concentrated under N_2 flow. The residue was subjected to reversed-phase semipreparative HPLC using a gradient from 50 to 100% CH_3CN in H_2O over 30 min (Rainin Microsorb C_{18} column; 1.0 × 25 cm; 5-μm particle size; 2 mL/min; UV detection at 215 nm) to give methyl ester **5** (2.7 mg, 64% yield, HPLC t_R 13.2 min): ^1H NMR data (CD_3OD, 300 MHz) δ 2.13 (m; H-1a), 1.50 (m; H-1b), 2.12 (m; H-2), 1.81−1.91 (ov. m; H-4a, H-5a, H-8b), 1.60 (m; H-4b), 1.76 (m; H-5b), 2.30 (m; H-6), 1.98 (dd; J_{H-H} = 14.4, 4.1 Hz, H-8a), 4.45 (ddd; J_{H-H} = 8.8, 8.8, 4.1 Hz, H-9), 6.66 (dq; J_{H-H} = 8.5, 1.5 Hz, H-10), 1.85 (d; J_{H-H} = 1.5 Hz, H_3-13), 3.89 (d; J_{H-H} = 9.5 Hz, H-14a), 3.59 (d; J_{H-H} = 9.5 Hz, H-14b), 1.23 (s; H_3-15), 3.73 (s; OCH_3).

(*R*)-2-Phenylbutyrate Ester of 5. To a solution of 1,3-dicyclohexylcarbodiimide (5.4 mg, 26 μmol) in 10 mL of anhydrous THF were added solutions of (*R*)-2-phenylbutyric acid (4 μL, 26 μmol), massarinolin B methyl ester (**5**; 1.6 mg, 5.7 μmol), and a catalytic amount of 4-(*N*,*N*-dimethylamino)-pyridine (0.5 mg) in THF. The mixture was allowed to stir for 48 h, and the solvent was then evaporated under N_2 flow. The residue was redissolved in 1.5 mL of Et_2O and extracted sequentially with 2% CH_3COOH (2 mL), 3% $NaHCO_3$ (2 mL), and H_2O (2 × 2 mL). The organic phase was evaporated and

subjected to semipreparative reversed-phase HPLC using a gradient from 80 to 100% CH_3OH in H_2O over 40 min (Beckman Ultrasphere C_{18} column; 1.0 × 25 cm; 5-μm particle size; 1.5 mL/min; UV detection at 215 nm) to afford 2.2 mg (90% yield, HPLC t_R 30.7) of the (*R*)-2-phenylbutyrate ester of **5**: ^{1}H NMR (CD_3OD, 600 MHz) δ 1.98 (ov. m; H-1a, H-2), 1.37 (m; H-1b), 1.95−2.06 (ov. m; H-4a, H-5a, H-8a), 1.56 (m; H-4b), 1.74 (m; H-5b), 2.11 (m; H-6), 1.88 (br. m; H-8b), 5.45 (ddd; J_{H-H} = 8.6, 8.6, 4.9 Hz, H-9), 6.52 (dq; J_{H-H} = 8.7, 1.5 Hz, H-10), 1.92 (d; J_{H-H} = 1.5 Hz, H_3-13), 1.10 (s; H_3-15), 3.23 (s; H_2-14), 3.73 (s; OCH_3), 3.44 (dd; 7.7, 7.7, H-2′), 2.04/1.75 (m; H_2-3′), 0.83 (dd; J_{H-H} = 7.7, 7.7 Hz, H_3-4′), 7.20−7.35 (ov. m; H_5−Ar); HRESIMS obsd *m*/*z* 449.2324 $(M + Na)^+$, calcd for $C_{26}H_{34}O_5Na$ 449.2304.

^{1}H NMR data for the (*S*)-2-phenylbutyrate ester of 5 prepared using the procedure above: (CD_3OD, 600 MHz) δ 2.05 (ov. m; H-1a, H-2), 1.48 (m; H-1b), 1.95−2.06 (ov. m; H-4a, H-5a, H-8a), 1.58 (m; H-4b), 1.76 (m; H-5b), 2.26 (m; H-6), 1.84 (ov. m, H-8b), 5.54 (ddd; J_{H-H} = 8.5, 8.5, 4.0 Hz, H-9), 6.32 (dq; J_{H-H} = 8.5, 1.5 Hz; H-10), 1.84 (d; J_{H-H} = 1.5 Hz; H_3-13), 3.69 (d; J_{H-H} = 9.1 Hz, H-14a), 3.45 (d; J_{H-H} = 9.1 Hz, H-14b), 1.20 (s; H_3-15), 3.68 (s; OCH_3), 3.46 (ov. m; H-2′), 2.04/1.75 (m; H_2-3′), 0.88 (dd; J_{H-H} = 7.7, 7.7 Hz, H_3-4′), 7.20−7.35 (ov. m; H_5-Ar).

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