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Massarilactones A and B: novel secondary metabolites from the freshwater aquatic fungus *Massarina tunicata*

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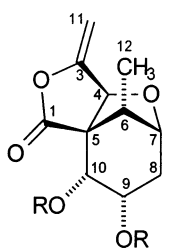
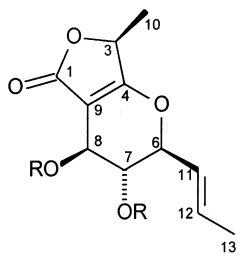
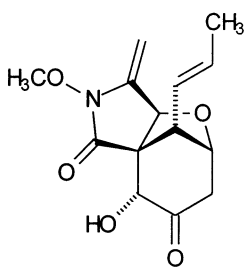
Abstract—Massarilactones A and B (**1** and **2**) have been isolated from cultures of the freshwater aquatic fungus *Massarina tunicata*. The structures, including absolute stereochemistry, were determined by X-ray diffraction analysis of their bis(4-bromobenzoate) derivatives.

Our preliminary studies of freshwater aquatic fungi have led to the isolation of several new bioactive compounds,[1–3] including three sesquiterpenoid metabolites described recently from the aquatic fungus *Massarina tunicata* Shearer & Fallah (A-25-1; =ATCC 201760).[1] Investigations of scale-up cultures of *M. tunicata* have led to the isolation of two new polyketide-derived antibacterial lactones that we have named massarilactones A and B (**1**–**2**), both of which contain unusual ring systems. Details of the isolation and structure elucidation of **1** and **2** are presented here.

Fractionation of the ethyl acetate extract of *M. tunicata* liquid cultures by chromatography on silica gel, followed by Sephadex LH-20, and/or reversed-phase HPLC, afforded compounds **1** and **2**.[4] The molecular formula of massarilactone A (**1**) was determined to be $C_{11}H_{14}O_5$ (five unsaturations) on the basis of NMR and HRFABMS data. The ^1H and ^{13}C NMR data (Table 1) and DEPT results for massarilactone A suggested the presence of an ester group, a -$CHCH_3$ moiety, an sp^3 methylene unit, four oxymethine protons, and an oxygenated 1,1-disubstituted double bond. These data ac-







1 R = H
3 R = Ac
4 R = COC_6H_4Br

2 R = H
6 R = Ac
7 R = COC_6H_4Br

5

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Table 1. NMR data for **1** in $CDCl_3$

C#	^1H[a] δ (mult; J_{H-H} in Hz)	^{13}C[b] δ
1	–	171.7
3	–	156.6
4	5.69 (br t; 2.1)	76.9
5	–	61.5
6	1.97 (br q; 7.2)	46.2
7	4.22 (m)	83.6
8	2.26 (dd; 16, 3.3)	38.6
	1.82 (ddd; 16, 5.7, 1.8)	
9	4.26 (br dd; 5.4, 5.4)	66.4[c]
10	4.22 (m)	69.6[c]
11	4.79 (br t; 2.7)	89.7
	4.58 (br dd; 2.7, 2.1)	
12	1.15 (d; 7.2)	14.3

[a] Recorded at 300 MHz.
[b] Recorded at 75 MHz.
[c] Assignments may be interchanged.

counted for all but two exchangeable protons, and indicted that the structure of massarilactone A (**1**) is tricyclic. ^1H–^1H decoupling experiments identified a -CH_2CHO- subunit, and revealed that the *exo*-methylene signals (H_2-11) were allylically coupled to the oxymethine proton signal at δ 5.69 (H-4). Treatment of massarilactone A (**1**) with acetic anhydride resulted in the formation of a diacetate (**3**), allowing assignment of both exchangeable protons as secondary alcohol groups. The structure of massarilactone A was assigned as **1** on the basis of detailed NMR analysis and was ultimately confirmed by X-ray diffraction analysis.

The bis(4-bromobenzoate) ester (**4**) of massarilactone A was prepared by treatment of **1** with 4-bromobenzoyl chloride. Crystals of **4** suitable for analysis by X-ray crystallography were obtained by slow evaporation of an acetone solution. The final X-ray crystallographic model of **4** (Fig. 1)[5] revealed the structure and absolute stereochemistry of massarilactone A, as shown in **1**.

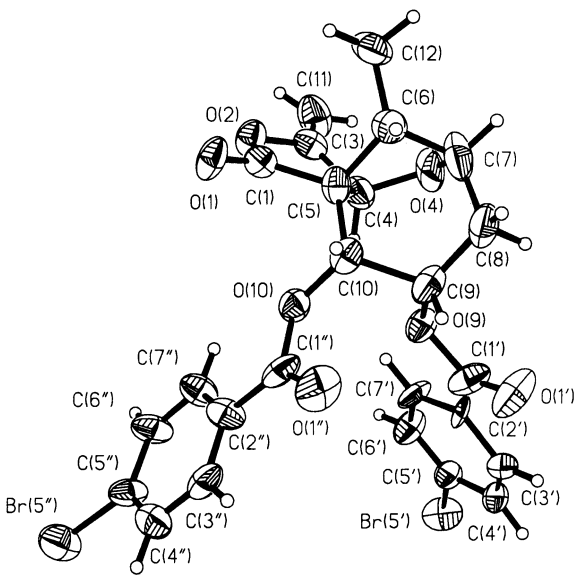

Figure 1. Final X-ray model of **4**.

Table 2. NMR data for **2** in $CDCl_3$

C#	^1H[a] δ (mult, J_{H-H} in Hz)	^{13}C[b] δ	Selective INEPT[a] correlations
1		172.4	
3	4.81 (q, 6.9)	74.2	1, 4, 9, 10
4		177.8	
6	4.67 (br dd, 8.2, 6.6)	84.3	4, 7, 8, 11, 12
7	3.80 (dd, 5.1, 6.6)	71.5	8, 9, 11
8	4.49 (br d, 5.1)	64.2	1, 4, 6, 7, 9
9		100.3	
10	1.43 (d, 6.9)	17.1	3, 4
11	5.66 (ddq, 15, 8.1, 1.5)	124.9	
12	5.90 (ddq, 15, 6.6, 0.7)	133.5	6, 11, 13
13	1.73 (dd, 6.6, 1.5)	17.8	

[a] Recorded at 300 MHz.
[b] Recorded at 75 MHz.

NMR assignments for **1** were made on the basis of chemical shifts, DEPT data, and comparison of its spectral data with those from the known compound spirostaphylotrichin F (**5**).[6]

Massarilactone B (**2**)[4] was determined to be an isomer of **1** on the basis of HRFABMS and ^{13}C NMR data, but these data also suggested the presence of significant structural differences. The ^1H, ^{13}C, and DEPT NMR data (Table 2) for **2** indicated the presence of two methyl groups, four oxymethine units, a *trans*-disubstituted olefin, and two exchangeable protons. Decoupling experiments permitted the assignment of two isolated spin systems corresponding to an isolated -$OCHCH_3$ moiety (C3–C10) and a trioxygenated *trans*-2-hexene unit. The chemical shifts of the three remaining, non-protonated carbons (δ 177.8, 172.4, 100.3) suggested that they comprise a β-alkoxy-α,β-unsaturated lactone unit.[7] The two exchangeable protons were assigned to hydroxy groups at C-7 and C-8 after analysis of ^1H

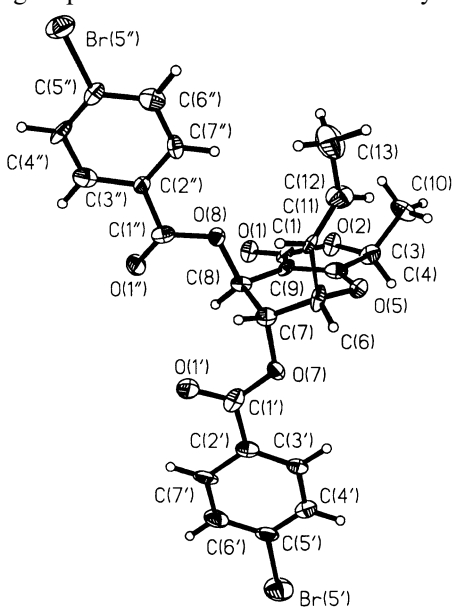

Figure 2. Final X-ray model of **7**.

NMR data for the diacetate **6** formed by treatment of **2** with acetic anhydride. The remainder of the structure and NMR assignments for **2** were proposed on the basis of selective INEPT data (Table 2). As was the case for **1**, X-ray crystallographic analysis[5] of the bis-(4-bromobenzoate) ester of **2** (**7**; Fig. 2) confirmed the structure of massarilactone B and permitted assignment of its absolute stereochemistry as shown.

To our knowledge, the methanofuro[3,4-*b*]oxepin ring system found in **1** has not been previously described, although metabolites that contain the similar methanooxepino[2,3-*c*]pyrrole ring system (e.g. spriostaphylotrichin F; **5**) have been reported from *Staphylotrichum coccosporum*.[6] Similarly, it appears that no natural products having the furo[3,4-*b*]pyran ring system found in massarilactone B (**2**) have been previously reported. However, larger ring systems incorporating such a system are known, and a synthetic intermediate possessing this ring system has been prepared.[8]

Massarilactones A and B both appear to be derived from the same type of polyketide precursor, with addition of a three-carbon unit accounting for carbons 3, 4, and 11. These compounds bear close biogenetic resemblance to several other fungal metabolites, including rosigenin, the curvupallides, and the spirostaphylotrichins.[9–11] Biosynthetic studies of members of this class (e.g. **5**) have suggested that they are formed by condensation of a polyketide chain with an unidentified C_4 unit, most likely either an amino acid (e.g. aspartic acid) or a citric acid cycle intermediate.[10,11]

Massarilactones A (**1**) and B (**2**) exhibited antibacterial activity against *Bacillus subtilis* (ATCC 6051) in standard disk assays, affording zones of inhibition of 19 and 16 mm, respectively, at 200 μg/disk. Massarilactone B was also active against *Staphylococcus aureus* (ATCC 29213) at the same level, causing a zone of inhibition of 12 mm. Neither compound showed activity in assays against *Aspergillus flavus* (NRRL 6541), *Fusarium verticillioides* (ATCC 24378), or *Candida albicans* (ATCC 14053) at 200 μg/disk.

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4. Massarilactone A (**1**): 34 mg obtained from 8 L of fermentation broth; $[\alpha]_D$ +8.7°C (*c* 0.3 g/dL; 24°C; CH_2Cl_2); UV (CH_3OH) 210 (ε 2200); ^1H and ^{13}C NMR data, Table 1; HRFABMS (NaI/3-NBA) obsd *m*/*z* 249.0720, calcd for $C_{11}H_{14}O_5$+Na, 249.0739. Massarilactone B (**2**): 70 mg from 8 L of broth; $[\alpha]_D$ −109° (*c* 2.2 g/dL; 28°C; CH_3OH); UV (CH_3OH) 236 (ε 9000), 270 (ε 6300); ^1H, ^{13}C, and selective INEPT NMR data, Table 2; HRFABMS (LiI/glycerol) obsd *m*/*z* 227.0912, calcd for $C_{11}H_{14}O_5$+H, 227.0919.
5. X-Ray data for **4** were collected on an Enraf–Nonius CAD4 diffractometer (Mo Kα radiation) using θ–2θ scans. The structure was solved using a MULTAN direct methods program, and refined using full-matrix least-squares. Crystals of **4** (0.42×0.20×0.08 mm) were monoclinic (space group $P2_1$) with cell dimensions $a=9.443(3)$, $b=17.089(5)$, $c=7.552(3)$ Å. The 7822 measurements yielded 4147 independent reflections (309 parameters) after equivalent data were averaged and Lorenz and polarization corrections were applied. The final refinement gave $R_1=0.0561$, $wR_2=0.0878$. X-Ray analysis of compound **7** was performed at the University of Minnesota using a Siemens SMART system at 173(2) K. Crystals of **7** (0.45×0.19×0.045 mm) were also monoclinic (space group $P2_1$) with cell dimensions $a=7.69990(10)$, $b=28.0555(3)$, $c=11.6064(2)$ Å. The specimen was determined to be a rotational twin with the twin law (transposed, by rows) [1, 0, −1/7; 0, −1; 0, 0, −1]. The 8489 measurements yielded 8489 independent reflections (619 parameters). The final refinement gave $R_1=0.0914$ and $wR_2=0.2088$. Atomic coordinates for both compounds have been deposited at the Cambridge Crystallographic Data Centre.
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