Gloiosiphones A and B, Novel Metabolites from the Red Marine Alga Gloiosiphonia verticillaris

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GLOIOSIPHONES A AND B, NOVEL METABOLITES FROM THE RED MARINE ALGA *GLOIOSIPHONIA VERTICILLARIS*

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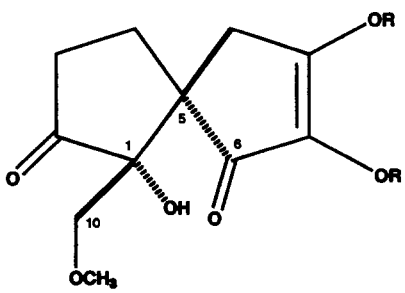
ABSTRACT.—Gloiosiphones A [**1**] and B [**3**] have been isolated from the temperate red marine alga, *Gloiosiphonia verticillaris*. Gloiosiphone A possesses a new C_{10} carbon skeleton composed of a 1-methylspiro[4.4]nonane ring, trivially termed the gloiosiphane ring system. It was isolated as the dimethyl derivative **2** obtained upon treatment of the crude antimicrobial fractions with CH_2N_2. Metabolite **3** is a structurally related C_5 natural product. The structures of **2** and **3** were assigned on the basis of spectral analysis.

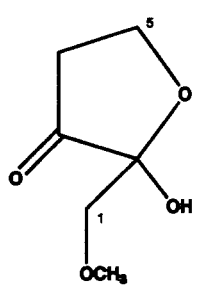
Red marine algae have been the subject of extensive investigation for new biomedicinal agents, and a large number of novel natural products have been isolated from these life forms during the last two decades. For example, the genus *Laurencia* has been found to produce a wide variety of secondary metabolites including halogenated terpenoids and acetogenins in tropical species (1) and unusual oxylipins (2,3) and polyheterocyclic compounds in temperate species (4–8). *Gloiosiphonia verticillaris* (Farlow) Smith (Cryptonemiales, Gloiosiphoniaceae) is an annual red marine alga abundant in Oregon in the early summer and is typically found in low intertidal areas scoured by sand. As a result of our survey of Oregon marine algae for their biomedicinal potential, we found the crude lipid extract of this alga exhibited antimicrobial activity against *Staphyloccus aureus, Bacillus subtilis, Escherichia coli,* and *Salmonella typhimurium*. However, during efforts to isolate the antimicrobial natural products from a portion of the active material, most of the activity was lost, presumably due to the instability of these compounds to activated Si gel chromatography. The remainder of the original bioactive fractions was treated with CH_2N_2 to improve the chromatographic characteristics of these metabolites. The resulting methylated fraction was subjected to further chromatography, leading to the isolation of a colorless oily derivative, dimethylgloiosiphone A [**2**]. Slightly more polar fractions from the original chromatography, which were inactive in antimicrobial assays, yielded natural product **3** without treatment of CH_2N_2. We report here the isolation and structural elucidation of compounds **2** and **3**.

RESULTS AND DISCUSSION

G. verticillaris was collected from exposed tide pools at Seal Rock and Strawberry Hill, Oregon in May 1991. The CH_2Cl_2-MeOH (2:1) extract, which showed antimicro-



1 R=H
2 R=Me



3

bial activity as described above, was subjected to Si gel vacuum chromatography with a gradient of EtOAc in hexanes. A portion of the bioactive fraction was further subjected to normal cc and hplc; however, the bioactivity was lost during this process. Analysis of the ^{1}H-nmr spectrum of the bioactive fraction indicated that it contained a monomethoxy (δ 3.35, s) compound, which we have named gloiosiphone A [**1**]. In order to improve the chromatographic behavior and chemical stability of metabolites in these fractions, they were methylated using excess ethereal CH_2N_2. Repetitive normal phase hplc gave pure derivative **2**. A more polar fraction (60% EtOAc/hexanes) from vacuum chromatography showed some similarity to compound **2** in its ^{1}H-nmr features. Additional cc of this fraction on Si gel yielded compound **3**.

DIMETHYLGLOIOSIPHONE A [**2**].—Derivative **2** was isolated as a colorless oil that analyzed for $C_{13}H_{18}O_6$ by hrms. The ^{1}H-nmr spectrum of **2** showed three sharp methyl singlets at δ 4.07, 3.82, and 3.36. ^{1}H-^{13}C HMQC (9) data revealed that these protons were attached to carbons at δ 58.26 (s), 59.42 (s), and 59.69 (s), respectively, indicating the presence of three methoxyl groups in the molecule.

Detailed spectral analysis of **2** revealed six partial structures **a–f** (Figure 1). Strong absorptions at λ max 262 nm (ε 11300) in the uv and ν max 1620 cm^{-1} in the ir spectra and three signals for nonprotonated carbons at δ 199.79, 170.94, and 134.62 indicated a fully substituted enone in derivative **2**. In the HMBC (10) spectrum methoxy protons at δ 4.07 and 3.82 showed long-range correlations to the olefinic carbons of the enone (δ 170.94 and 134.62), yielding partial structure **a**. ^{1}H- and ^{13}C-nmr spectra clearly showed the presence of two isolated methylenes in **2**. One set of these appeared at δ 3.77 (1H, d, 10.5) and 3.47 (1H, d, 10.5) and was located on a carbon at δ 71.7 by HMQC. In the ^{1}H-^{1}H LRCOSY (11) spectrum, these protons showed correlations to an exchangeable hydroxyl proton at δ 3.29 (1H, s) and a methoxyl resonance at δ 3.36 (3H, s). Based on close correlations in ^{1}H-nmr chemical shifts with the methoxyl resonance in the underivatized and bioactive fraction, this methyl ether was identified as a naturally occurring constituent in gloiosiphone A [**1**]. In a deuterium-exchanged sample of **2**, a quaternary carbon at δ 80.30 showed a −0.14 ppm shift, indicating that it bore the hydroxyl group. Taken together, these data defined partial structure **b**. As shown by ^{1}H-^{13}C HMQC, the second pair of methylene protons, appearing at δ 2.97 (1H, d, 17.1) and 2.14 (1H, d 17.1), was attached to a carbon at δ 32.00 (partial structure **c**).

Further analysis of the nmr data revealed partial structures **d–f**. A ^{13}C-nmr signal at δ 213.51 (s) and a strong ir absorption band at 1750 cm^{-1} indicated the presence of an unconjugated ketone (partial structure **d**). ^{1}H-^{1}H COSY revealed a spin system of four protons at δ 2.70 (1H, m), 2.41 (1H, m), 2.36 (1H, m), and 1.99 (1H, m). These protons

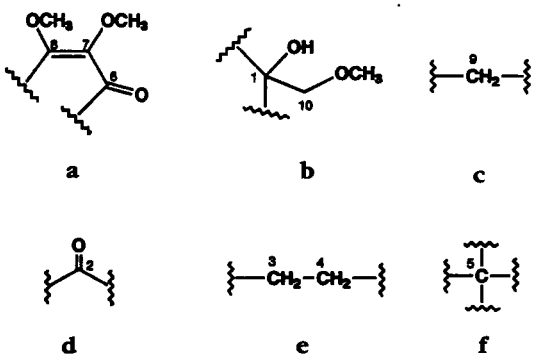


FIGURE 1. Partial structures **a–f** for dimethylgloiosiphone A [**2**].

were shown by ^{1}H-^{13}C HMQC data to be attached to two carbons at δ 32.30 (t) and 28.48 (t), yielding substructure **e**. The remaining quaternary carbon signal at δ 52.84 was assigned to substructure **f**. Partial structures **a–f** accounted for all of the atoms in derivative **2**.

Consideration of the molecular formula indicated that these partial structures **a–f** should be linked to form two rings. Building this bicyclic structure from these partial structures was accomplished by ^{1}H-^{13}C HMBC (Figure 2). The H_2-9 protons showed long-range ^{1}H-^{13}C couplings to C-5, -6, -7, and -8. Further, H_a-9 (δ 2.14) also coupled to C-4 while H_b-4 (δ 2.41) showed coupling to C-6. These couplings allowed connections of partial structures: **a**(C-6)–**f**–**c**–**a**(C-8), **e**(C-4)–**f**–**c**, and **a**(C-6)–**f**–**e**(C-4). Long-range ^{1}H-^{13}C couplings between H_b-9 and C-1, 1-OH and C-5, and H_b-10 (δ 3.77) and C-5 indicated that partial structures **b** and **f** were also connected. Finally, H_2-3, H_2-10, and 1-OH showed long-range correlations to C-2, allowing connections of partial structures **b**–**d**–**e**(C-3). These connections complete the planar structure of dimethyl gloiosiphone A [**2**], 1-hydroxy-7,8-dimethoxy-1-methoxymethylspiro[4.4]non-7-ene-2,6-dione.

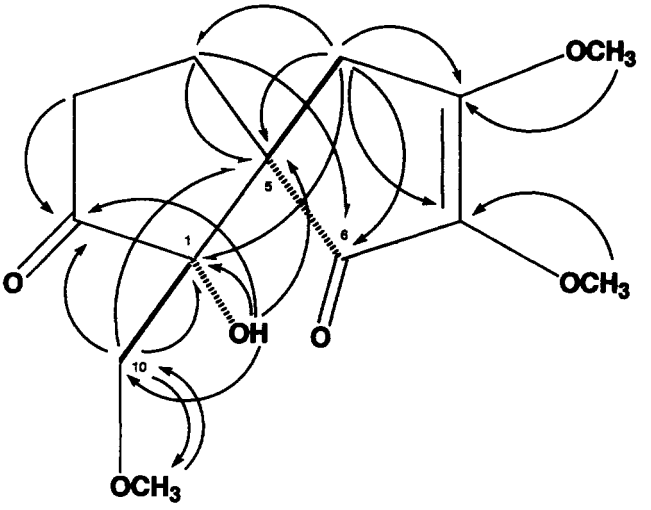


FIGURE 2. Results of the HMBC experiment for dimethylgloiosiphone A [**2**]. ^{1}H-^{13}C long-range couplings are indicated by arrows (double arrow indicates that all protons at designated carbon show long-range coupling; single arrow indicates that only one of the methylene protons shows long-range coupling).

The relative stereochemistry at C-1 and C-5 of **2** was determined by NOESY. NOe correlations between the H_a-10 (δ 3.47) and H_b-9 (δ 2.97) indicated a cis relationship of C-9 and C-10. Thus, the relative stereochemistry of **2** was assigned as 1*R**,5*R**. Dimethylgloiosiphone A [**2**] is apparently a racemate, as it was optically inactive.

GLOIOSIPHONE B [**3**].—Six carbon signals (2 quaternary, 3 methylene, and 1 methyl) were shown by ^{13}C-nmr and DEPT experiments. The eims and cims of **3** showed $[M-OH]^+$ at *m/z* 129; in combination with a detailed analysis of nmr data, a molecular formula of $C_6H_{10}O_4$ could be deduced. The close similarity of the hydroxyl and unconjugated ketone stretches of **2** and **3** was shown in their ir spectra. However, **3** was found to be uv-inactive and possessed no enone absorption bands in its ir spectrum. The

TABLE 1. ^{1}H- and ^{13}C-nmr Data ($CDCl_3$) and Assignments for **2** and **3**.

Position	Compound **2**		Position	Compound **3**	
	δ_H	δ_C		δ_H	δ_C
1		80.30 C	2		96.04 C
2		213.51 C	3		208.77 C
3a	2.36 (1H, m)	32.30 CH_2	4	2.58 (2H, m)	34.21 CH_2
3b	2.70 (1H, m)				
4a	1.99 (1H, m)	28.48 CH_2	5a	4.32 (1H, d, 8.8)	62.52 CH_2
4b	2.41 (1H, m)		b	4.33 (1H, dd, 8.8, 1.5)	
5		52.84 C			
6		199.79 C			
7		134.62 C			
8		170.94 C			
9a	2.14 (1H, d, 17.1)	32.00 CH_2			
9b	2.97 (1H, d, 17.1)				
10a	3.47 (1H, d, 10.5)	71.72 CH_2	1a	3.50 (1H, d, 10.2)	72.76 CH_2
10b	3.77 (1H, d, 10.5)		b	3.62 (1H, d, 10.2)	
1-OH	3.29 (1H, s)		2-OH	3.40 (1H, s)	
7-OMe	3.82 (3H, s)	59.42 Me			
8-OMe	4.07 (3H, s)	58.26 Me			
10-OMe	3.36 (1H, s)	59.69 Me	1-Me	3.45 (3H, s)	59.87 Me

nmr spectra (Table 1) indicated **3** also had structural features similar to those of **2** but was of overall smaller size. One pair of methylene protons [δ 3.62 (1H, d, 10.2) and 3.50 (1H, d, 10.2)] was located on a carbon at δ 72.76 by HMQC. These two protons showed long-range correlations to an exchangeable proton at δ 3.40 (1H, s) and a methoxyl resonance at δ 3.45 (3H, s), indicating that **3** possessed the same partial structure **b** as in derivative **2**. A strong ir absorption at 1770 cm^{-1} and carbon signal at δ 208.77 (s) indicated a ketone in **3** (subunit **d** of **2**). A spin system consisting of four protons at δ 4.33 (1H, dd), 4.32 (1H, d), and 2.58 (2H, m) was shown by COSY, and the protons were attached to carbons at δ 62.52 and 34.21 by HMQC (similar to substructure **e** of **2**). Therefore, three substructures, similar to subunits **b**, **d**, and **e** of **2**, accounted for all ^{1}H and ^{13}C signals of **3**. The gross structure of **3** could be easily constructed by HMBC (Figure 3). The chemical shifts for C-2 (δ 96.04) and C-5 (δ 62.52) indicated that they were connected by an ether linkage.

Gloiosiphone B [**3**] is a structural analogue of laurencione (4), which was isolated from *Laurencia spectabilis*. Like laurencione, gloiosiphone B is also a naturally occurring racemate. However, in contrast to laurencione, gloiosiphone B does not exist in solution as a mixture of cyclic and acyclic forms (4).

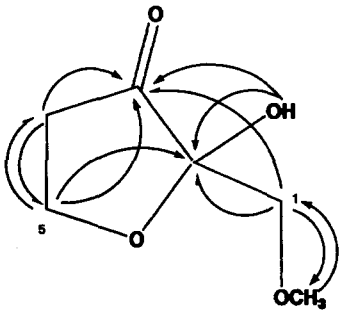


FIGURE 3. Results of the HMBC experiment for gloiosiphone B [**3**]. ^{1}H-^{13}C long-range couplings are indicated by arrows (double arrow indicates that all protons at designated carbon show long-range coupling; single arrow indicates that only one of the methylene protons shows long-range coupling).

Gloiosiphone B [**3**] and dimethylgloiosiphone A [**2**] were found to be inactive in antimicrobial bioassays. Gloiosiphone A [**1**] possesses a novel polyoxygenated 1-methylspiro[4.4]nonane ring. This is the first example of this unique ring system in nature, and we have trivially named the parent alkane as the gloiosiphane ring system (Figure 4). While the biogenetic origin of gloiosiphone A [**1**] is unknown at this point, it conceivably could derive from gloiosiphone B [**3**] and reductic acid (12). [Reductic acid 2-methyl ether was tentatively identified as a very minor component in vacuum chromatography fraction 5: uv ($CHCl_3$) λ max 262 nm (ε 18400), 278 nm (ε 14400); ir (neat) ν max 3340, 2930, 1740, 1620, 1460, 1350, 1300, 1250, 1200, 1170, 1120, 1060 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.81 (1H, s), 4.14 (3H, s), 2.49 (2H, m), 2.41 (2H, m).]

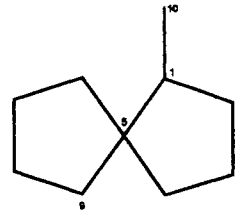


FIGURE 4. Gloiosiphane ring system.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on Bruker AM 400 and AC 300 and AM 500 spectrometers. All shifts are reported relative to an internal TMS standard. Ms was recorded on Kratos MS 50 TC and Finnigan 4023 mass spectrometers. Uv spectra were recorded on a Hewlett-Packard 8452A UV-VIS spectrophotometer and ir spectra on a Nicolet 5 DXB FT 15 spectrophotometer. Hplc was performed with Waters M-6000 and M-45 pumps, U6K injectors, and either an R401 differential refractometer or a Waters Lambda-Max 480 lc spectrophotometer. Merck aluminum-backed tlc sheets were used for tlc, and all solvents were distilled prior to use.

COLLECTION AND EXTRACTION.—Deep-rose-colored thalli of *G. verticillaris* were collected from the exposed tide pools at Seal Rock and Strawberry Hill on the Oregon coast on May 12 and May 25, 1991. A voucher specimen has been deposited at the Department of Botany and Plant Pathology Herbarium at Oregon State University. The fresh algae were immediately frozen with dry ice until workup. The defrosted tufts (610 g dry wt) were extracted by repetitive steeping in CH_2Cl_2-MeOH (2:1) (about 10 liters total), affording 5.0 g of a dark green syrup. The crude extract was subjected to standard antimicrobial sensitivity assays at 2 mg/disc, and activity was observed to two Gram-positive bacteria, *B. subtilis* (ATCC 6081, 12 mm) and *St. aureus* (ATCC 12600, 12 mm), as well as two Gram-negative bacteria, *E. coli* (ATCC 11775, 7.5 mm) and *Sa. typhimurium* (ATCC 14028, 8.0 mm).

FRACTIONATION AND METHYLATION.—The bioactive crude material was applied to a Si gel column in the vacuum mode and chromatographed in a gradient of EtOAc/hexanes. Of the resulting six fractions, the third and fourth (50% EtOAc/hexanes) showed antimicrobial activity and nearly the same mixture of compounds by tlc, and possessed ^1H-nmr ($CDCl_3$, partial) bands at δ 3.75 (1H, d), 3.68 (1H, d), 3.35 (3H, s), 2.56 (1H, d), 2.48 (1H, d), 1.7–2.8 (4H, m). Fraction 4 (ca. 1.1 g) was subjected to further separations using Si gel cc and hplc (17% iPrOH/hexanes, Phenomenex, 10 μm, 500×10 mm). Unfortunately, none of the resulting fractions showed antimicrobial activity.

In order to improve the chromatographic behavior and chemical stability of the constituents in this active material, the remainder of fractions 3 and 4 (152 mg), which contained a mixture of compounds similar to fraction 4 was treated with excess ethereal CH_2N_2 for 7 min. The resulting mixture was then subjected to further chromatography after evaporation of the solvent.

ISOLATION OF DIMETHYL GLOIOSIPHONE A [**2**].—Repetitive hplc on a Phenomenex Si column (10 μm, 500×10 mm) of the above methylated mixture with 2–7% iPrOH/hexanes resulted in a colorless oily compound, **2** (3.8 mg). Tlc analysis of **2** showed that it was a uv-active and acid-charring (50% H_2SO_4) compound. Dimethylgloiosiphone A [**2**] had the following spectral characteristics: uv (CH_3CN) λ max 262 nm (ε 11300), 194 nm (ε 11900); ir (neat) ν max 3440, 2950, 1750, 1700, 1620, 1460, 1350, 1300, 1200,

1100, 1040 cm^{-1}; hreims *m/z* $[M]^+$ 270.1113 ($C_{13}H_{18}O_6$, 1.0 mmu error); eims *m/z* (rel. int. %) $[M]^+$ 270 (25), $[M-H_2O]^+$ 252 (10), $[M-MeOH]^+$ 238 (20), $[C_{10}H_{14}O_5]^+$ 214 (43), $[C_9H_{11}O_5]^+$ 199 (23), $[C_9H_9O_4]^+$ 181 (10), $[M-C_4H_5O_3]^+$ 169 (76), $[C_8H_{11}O_3]^+$ 155 (100), 139 (16), 111 (10), 69 (12), 55 (36); ^1H and ^{13}C nmr ($CDCl_3$) see Table 1.

ISOLATION OF GLOIOSIPHONE B [**3**].—Repetitive chromatography on Si gel of vacuum chromatography fraction 5 with 65% EtOAc/hexanes resulted in a colorless oily compound, **3** (13.5 mg). Tlc analysis showed that **3** was uv-inactive but acid-charring (50% H_2SO_4). Gloiosiphone B had the following spectral characteristics: ir (neat) ν max 3380, 2990, 2920, 2900, 2830, 1770, 1470, 1460, 1400, 1330, 1310, 1260, 1240, 1200, 1150, 1110, 1020 cm^{-1}; eims *m/z* (rel. int., %) $[M-OH]^+$ 129 (100), 128 (10), $[M-CO]^+$ 118 (16), $[C_3H_6O_3]^+$ 101 (13), 98 (25), 87 (14), 73 (18), 60 (21), 55 (24); positive cims *m/z* (rel. int. %) $[M-OH]^+$ 129 (100); ^1H and ^{13}C nmr ($CDCl_3$) see Table 1.

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