Chlorinated C12 Fatty Acid Metabolites from the Red Alga *Gracilaria verrucosa*

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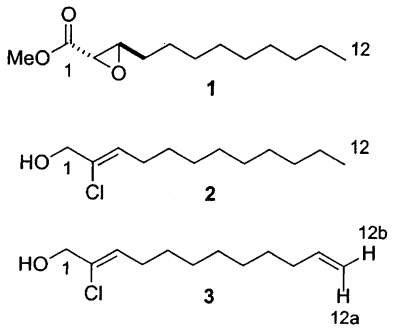
Three compounds containing moieties rarely encountered in nature, viz., 3-nonyloxirane-2 carboxylic acid methyl ester (**1**), 2-chlorododec-2-en-1-ol (**2**), and 2-chlorododec-2,11-dien-1-ol (**3**), were isolated from the red alga *Gracilaria verrucosa,* and their structures were determined by spectroscopic methods.

The red alga *Gracilaria verrucosa* has been the subject of previous investigations, and these have uncovered mainly fatty acid metabolites. The most biologically active were prostaglandins A_2 and E_2 and arachidonic acid, the biosynthetic precursor of the prostaglandins.[1] It was suggested that these prostaglandins were responsible for a gastrointestinal disorder, known as Ogonori poisoning, in Japan in 1990.[2] In addition, analyses of the fatty acid content,[3] lipids,[4] and glycolipids[5] of this species have been conducted. Few other compounds have been isolated from this species apart from floridoside, a glycerol glycoside.[6]

The collection of *G. verrucosa* was sourced in Port Elizabeth, South Africa, and the methanol/dichloromethane extract was made available to us via the Natural Products Branch of the U.S. National Cancer Institute as part of a collaborative screening program. The extract was subjected to a standard solvent partitioning procedure.[7] Silica chromatography of the hexane partition fraction followed by normal-phase HPLC led to the isolation of three fatty-acid-derived compounds, **1** (7 mg), **2** (14 mg), and **3** (46 mg).



The ^{13}C and DEPT-135 NMR spectra of **1** (Table 1) revealed the presence of one ester carbonyl, two oxygenated methines, eight methylenes, and one methoxy and one C-methyl group. This led to a CH formula of $C_{13}H_{24}$, which in combination with the low-resolution FAB mass spectrum *m*/*z* 229 $[M + H^+]$ suggested a molecular formula of $C_{13}H_{24}O_3$. The formula was confirmed by an accurate FAB mass measurement of 251.1625 $[M + Na]^+$ Δ +0.7 ppm from the calculated value. An unsaturation number of two was calculated, which in conjunction with one ester carbonyl group suggested the presence of one ring. All protonated carbons were assigned by a HSQC experiment.

An HMBC correlation between the ester carbonyl C-1 and O*CH*$_3$ suggested the presence of a methyl ester. The ^1H and ^{13}C chemical shifts for the oxygenated methines C-2 and C-3 suggested that they formed part of an oxirane system directly connected to the ester carbonyl. This was confirmed by HMBC correlations from C-1 to H-2 and C-3 to H-2. The substructure comprising of C-1 to C-3 was further extended with the aliphatic chain C-4 to C-12 by an HMBC correlation from C-3 to H-4. The ~2 Hz coupling constant from H-2 to H-3 suggested a *trans* configuration for the oxirane, which was confirmed by the absence of an NOE from H-2 to H-3. As the compound was not biologically active, the absolute stereochemistry was not determined, and the enantiomer presented in the diagram is arbitrary. Epoxides at the C-2 position of fatty-acid-derived compounds are rare in nature. A recent report describes the discovery of such a compound with the same relative stereochemistry from the marine cyanobacterium *Lyngbya semiplena*.[8]

The ^{13}C and DEPT-135 NMR spectra of compound **2** (Table 1) indicated the presence of one sp^2 quaternary carbon, one sp^2 methine, eight methylenes, one oxygenated methylene, and one methyl group. The low-resolution FAB mass spectrum displayed an isotopic cluster (241/243) consistent with the presence of one chlorine atom in the molecule. An accurate mass measurement gave an *m*/*z* of 241.1334 $[M + Na^+]$, Δ −0.4 ppm, from the calculated value for the formula $C_{12}H_{22}O^{35}Cl$. An unsaturation number of one was calculated, which corresponded to a single double bond in the molecule. All protonated carbons were assigned by an HSQC experiment.

The ^{13}C NMR chemical shifts of C-2 (133.15 s) and C-3 (127.85 d) indicated a trisubstituted double bond. The oxygenated methyne (C-1) was attached using HMBC correlations from C-1 to H-3, C-3 to H-1, and C-2 to H-1. The aliphatic chain C-4 to C-12 was attached to the C-1−C-3 substructure by HMBC correlations from C-2 and C-3 to H-4 and C-3 to H-5. The Cl was attached at the remaining position, C-2, by chemical shift additivity.[9] The double bond was assigned the *Z* geometry by the presence of an NOE from H-1 to H-3. Chlorohydrin compounds of this type are rare in nature, with only a few having been reported such as 2-amino-5-chloro-6-hydroxy-4-hexenoic acid from the fungus *Amanita abrupta*.[10]

The ^{13}C and DEPT-135 NMR spectra of **3** revealed one sp^2 quaternary carbon, two sp^2 methines, one sp^2 methylene, seven sp^3 methylenes, and one oxygenated sp^3 methylene. The low-resolution FAB mass spectrum displayed an isotopic cluster at *m*/*z* 239/241 $[M + Na]^+$ consistent with the presence of one chlorine atom in the molecule. An accurate FAB mass measurement gave an *m*/*z* of 239.1168 $[M + Na]^+$ Δ −0.4 ppm from the calculated value for the

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Table 1. ^{13}C NMR (δ/ppm, multiplicity) at 100 MHz and ^{1}H NMR (δ/ppm, proton count, multiplicity, J/Hz) at 400 MHz and $^{1}H-^{1}H$ COSY and HMBC Data in $CDCl_3$ for Compounds **1**−**3**

	compound **1**				compound **2**				compound **3**			
	δ_C	δ_H	COSY H−H	HMBC C→H	δ_C	δ_H	COSY H−H	HMBC C→H	δ_C	δ_H	COSY H−H	HMBC C→H
1	170.0 s			MeO H2	67.2 t	4.13 2H s	H3 H4 OH	H3	66.9 t	4.12 1H s	H3 OH	H3
2	53.2 d	3.18 1H d 2.1		H4	133.2 s			H1 H3 H4	133.0 s			H1 H3 H4
3	58.8 d	3.12 1H ddd 6.5 4.8 1.8	H4	H2 H4	127.9 d	5.75 1H t 7.0	H1 H4	H1 H4 H5	127.6 d	5.75 1H m	H1 H4	H1 H4 H5
4	31.6 t	1.57 2H m		H5	28.6 t	2.18 2H q 7.2	H1 H3 H5		28.1 t	2.16 2H q 7.2	H5	H3
5	29.6 t	1.24 2H m	H4	H4	28.3 t	1.36 2H quint 7.1	H4	H4	28.4 t	1.36 2H m	H6	H4
6	29.6 t	1.24 2H m			29.7 t	1.23 2H dd		H4 H5	29.2 t	1.27 2H m	H5	H4 H5
7	29.4 t	1.24 2H m		H9	29.6 t	1.23 2H m			29.1 t	1.27 2H m		
8	22.8 t	1.24 2H m	H9		29.5 t	1.23 2H m			29.0 t	1.27 2H m	H9	
9	25.9 t	1.42 2H m	H8 H10	H11	29.4 t	1.23 2H m			28.7 t	1.33 2H m	H10	
10	32.0 t	1.23 2H m	H9 H11	H12	32.1 t	1.21 2H m		H9 H12	33.7 t	2.00 2H q 7.1	H11 H12a H12b	H11 H12a H12b
11	29.4 t	1.23 2H m	H10 H12	H12	22.9 t	1.24 2H m	H12		139.2 d	5.76 1H m	H12a H12b	H9 H10
12	14.3 q	0.84 3H t 6.8	H11	H10	14.3 q	0.84 3H t 6.9			114.1 t	4.94 dq 17.1 1.8 4.88 dquint 10.3 1.0	H11	H10 H11
MeO	52.6 q	3.74 3H s										

formula $C_{12}H_{20}O^{35}Cl$. An unsaturation number of two was determined, which corresponds to two double bonds in the molecule. All protonated carbons were assigned by a HSQC experiment.

The ^{1}H NMR spectrum showed the presence of one trisubstituted double bond (δ 5.75, t) and one terminal double bond (δ 5.76, m; 4.94, dq; 4.88, dquint) in the molecule. The presence of an isolated oxygenated methylene was confirmed by a 2H singlet at 4.12 ppm in the ^{1}H NMR spectrum. The structure was assembled as for **2** using HMBC correlations C-1−H-3, C-2−H-1/H-3/H-4, C-3−H-1/H-4/H-5. The Cl was placed at the remaining position on C-2 as for **2**. The alkyl chain was terminated with a double bond, as was evident from the HMBC correlations C-10−H-11/H-12a/H-12b, C-11−H-9/H-10, and C-12−H-10/H-11. Irradiation at δ_H 4.12 ppm using a selective 1D-NOE experiment resulted in enhancement of a triplet at 5.75 ppm, indicating that the geometry of the C-2−C-3 double bond was *Z*.

In conclusion, three compounds containing moieties rarely encountered in nature were isolated from *G. verrucosa*. Compounds **1**−**3** appear to originate from the same biosynthetic root, dodec-2-enoic acid. No biological activity was found for any of the compounds presented.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Bellingham & Stanley P20 polarimeter. IR spectra were recorded on an Ati Mattson Genesis Series FTIR spectrometer. ^{1}H, ^{13}C, and 2D NMR experiments were recorded on a Varian Unity Inova 400 MHz spectrometer, in $CDCl_3$ solution. Chemical shifts are reported in parts per million (δ) downfield relative to residual $CHCl_3$ at 7.27 pm. All mass spectra were obtained on a JEOL The Mstation JMS-700 high-resolution mass spectrometer. HPLC separations were carried out on an Alltech Econosphere SiO_2 column (4.6 × 250 mm, 10 mm particle/100 Å pore size) using a Spectraseries P100 isocratic pump and monitored with a Waters refractive index detector.

Extraction and Isolation. The red alga *Gracilaria verrucosa* (order Gigartinales, family Gracilariaceae) was collected at 0.5 m offshore from Port Elizabeth, South Africa, in October 1998 (collection number OCDN6226), and a voucher sample is kept at the Natural Products Branch, National Cancer Institute. The alga was extracted with 1:1 MeOH/CH_2Cl_2, and the solvent was removed. The extract (2.15 g) was partitioned between water and CH_2Cl_2. The solvent was removed from the CH_2Cl_2 layer, and the resulting oil was partitioned between *n*-hexane and 10% aqueous MeOH. The hexane fraction was dried under reduced pressure to give 500 mg of a residue, which was subjected to silica chromatography. The column was packed in hexane, and the eluent was gradually increased in polarity from 0 to 100% CH_2Cl_2 in hexane and finally to 100% methanol. Further purification was achieved by normal-phase HPLC using a solvent mixture of hexane and ethyl acetate (85:15) to afford **1** (7 mg), **2** (14 mg), and **3** (46 mg).

3-Nonyloxirane-2-carboxylic acid methyl ester (1): colorless oil; $[\alpha]^{23}_D$ +36.4° (*c* 0.11, CH_2Cl_2); IR (neat) 2924, 1756, 1458, 1207, and 1025 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; LRFABMS *m/z* 229 $[M + H]^+$; HRFABMS *m/z* 251.1625 $[M + Na]^+$ (calcd for $C_{13}H_{24}O_3Na$, 251.1623).

2-Chlorododec-2-en-1-ol (2): colorless oil; IR (neat) 3329, 2923, 2852, 1458, and 1001 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; LRFABMS *m/z* 241 and 243 $[M + Na]^+$; HRFABMS *m/z* 241.1334 $[M + Na]^+$ (calcd for $C_{12}H_{23}O^{35}ClNa$, 241.1335).

2-Chlorododec-2,11-dien-1-ol (3): colorless oil; IR (neat) 3321, 2930, 2854, 1463, 1012, and 908 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; LRFABMS *m/z* 239 and 241 $[M + Na]^+$; HRFABMS *m/z* 239.1168 (calcd for $C_{12}H_{21}O^{35}ClNa$, 239.1179).

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