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Antineoplastic unsaturated fatty acids from Fijian macroalgae

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ABSTRACT

Phytochemical analysis of Fijian populations of the green alga *Tydemania expeditionis* led to the isolation of two unsaturated fatty acids, 3(ζ)-hydroxy-octadeca-4(*E*),6(*Z*),15(*Z*)-trienoic acid (**1**) and 3(ζ)-hydroxy-hexadeca-4(*E*),6(*Z*)-dienoic acid (**2**), along with the known 3(ζ)-hydroxy-octadeca-4(*E*),6(*Z*)-dienoic acid (**4**). Investigations of the red alga *Hydrolithon reinboldii* led to identification of a glycolipid, lithonoside (**3**), and five known compounds, 15-tricosenoic acid, hexacosa-5,9-dienoic methyl ester, β-sitosterol, 10(*S*)-hydroxypheophytin A, and 10(*R*)-hydroxypheophytin A. The structures of **1–3** were elucidated by spectroscopic methods (1D and 2D NMR spectroscopy and ESI-MS). Compounds **1**, **2**, and **4**, containing conjugated double bonds, demonstrated moderate inhibitory activity against a panel of tumor cell lines (including breast, colon, lung, prostate and ovarian cells) with IC_{50} values ranging from 1.3 to 14.4 μM. The similar cell selectivity patterns of these three compounds suggest that they might act by a common, but unknown, mechanism of action.

1. Introduction

Unsaturated fatty acids are widely distributed in human foods including vegetable oils, meat, milk, and soy products (Jacobsen, 2004), fulfilling important physiological functions. Docosahexaenoic acid and arachidonic acid are important constituents in mammalian cell membranes (Sala-Vila et al., 2006) and are crucial to brain and eye development in human infants (Birch et al., 1997). Consumption of omega-3 and omega-6 fatty acids has been associated with reduced mortality from cardiovascular disease, suppressed arthritis-associated inflammation, and decreased risk of cancer (Simopoulos 2002). Specifically, arachidonic acid was shown to inhibit proliferation of chronic myeloid leukemia cells by inducing apoptotic cell death (Rizzo et al., 1999). However, the proliferation of prostate cancer cell lines (PC3 and LNCap) was stimulated by arachidonic acid due to the increased formation of 5-HETE, a 5-lipoxygenase product important to prostate cancer cells (Ghosh and Myers, 1998). In contrast, conjugated linoleic acids were reported to inhibit human prostate cancer and to reduce metastases of cancers to lung tissue (Cesano et al., 1998). These fatty acids were also reported to block growth and systemic spread of human breast cancer via mechanisms independent of the host immune system (Visonneau et al., 1997), perhaps by peroxidation of intracellular lipids (Devery et al., 2001).

Glycolipids, which incorporate both fatty acid and carbohydrate moieties, have also been shown to be active against tumor cells. For example, galactolipids with octadecatrienoyl residues were found to possess potent activity against murine leukemia and human colon adenocarcinoma cells (Jung et al., 1996), and nigricanosides A and B, ether-linked glycoglycerolipids, showed antimitotic activity against human breast and colon cancer cells (Williams et al., 2007). Glycolipids also have ecological functions such as herbivore deterrence in the brown seaweed *Fucus vesiculosus* (Deal et al., 2003) and inhibition of bacterial pathogens in the terrestrial plant *Arabidopsis thaliana* (Andersson et al., 2006).

Marine algae are a rich source of unsaturated fatty acids (Khotimchenko et al., 2002). During our ongoing effort to search for novel natural products as pharmaceutical leads, extracts of the green alga *Tydemania expeditionis* and of the red alga *Hydrolithon reinboldii* were found to be potently cytotoxic in an invertebrate toxicity assay. Herein, we report the isolation and structural elucidation of two novel unsaturated fatty acids (**1**–**2**) and a novel glycolipid (**3**) from these two species, and their inhibitory effects on 11 cancer cell lines.

2. Results and discussion

Guided by a rotifer (invertebrate) toxicity assay, investigation of the ethyl acetate-soluble fraction of *T. expeditionis* led to the

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isolation of two new fatty acids, 3(ζ)-hydroxy-octadeca-4(*E*),6(*Z*),15(*Z*)-trienoic acid (**1**) and 3(ζ)-hydroxy-hexadeca-4(*E*),6(*Z*)-dienoic acid (**2**), along with the known 3(ζ)-hydroxy-octadeca-4(*E*),6(*Z*)-dienoic acid (**4**). Similarly, investigation of the cytotoxic hexane-soluble fraction of *H. reinboldii* led to a new glycolipid, lithonoside (**3**), along with five known compounds, 15-tricosenoic acid, hexacosa-5,9-dienoic acid methyl ester, β-sitosterol, 10 (*S*)-hydroxypheophytin A, and 10 (*R*)-hydroxypheophytin A.

Compound **1** was isolated as a colorless oil. HRESIMS analysis indicated a quasimolecular ion $[M–H]^-$ at *m*/*z* 293.2069, corresponding to a molecular formula of $C_{18}H_{30}O_3$, with four units of unsaturation.

The ^{1}H NMR spectrum of **1** (Table 1) indicated four conjugated olefinic protons at δ 5.62 (1 H, *dd*, *J* = 6.5, 15.5 Hz, H-4), 6.49 (1 H, *dd*, *J* = 11, 15.5 Hz, H-5), 5.95 (1 H, *t*, *J* = 11 Hz, H-6) and 5.41 (1H, *dt*, *J* = 8.0, 11 Hz, H-7), two non-conjugated olefinic protons at δ 5.35 (1H, *m*, H-15) and 5.46 (1 H, *m*, H-16), an oxymethine proton at δ 4.10 (1H, *dd*, *J* = 6.5, 13 Hz, H-3), a methylene envelope at 1.32, and a terminal methyl group at δ 0.95 (3H, *t*, *J* = 7.5 Hz, H-18), which suggested hydroxylated and unsaturated fatty acid skeleton. The optical rotation for **1** was zero, indicating that **1** is likely present as a racemic mixture, consistent with fatty acids previously reported (Stavri et al., 2006). The large coupling constant between H-4 and H-5 (15.5 Hz) indicated that these two protons were *trans*-oriented. In contrast, the 11 Hz coupling for both $\Delta^{6,7}$ and $\Delta^{15,16}$ indicated they were *cis*-oriented. Analysis of the ^{13}C NMR and DEPT spectra of **1** indicated presence of six olefinic carbon atoms, a methyl group, an oxymethine, and nine methylenes. The carboxyl group was not seen in the ^{13}C NMR spectrum, but a clear correlation of H-2 at δ 2.25 to a carbonyl at 179.0 ppm was observed in the HMBC spectrum. Therefore, the carbon spectral data further confirmed the skeleton revealed by ^{1}H NMR spectroscopy.

The full NMR assignments and connectivities of **1** were determined by ^{1}H ^{1}H COSY, HSQC, and HMBC spectroscopic data analysis. Some key HMBC correlations are shown in Fig. 1. The proton at δ 4.10 (H-3), attached to a carbon at δ 72.3, showed HMBC correlations to C-2, C-4, and C-5, indicating the location of the oxymethine at C-3. In the ^{1}H ^{1}H COSY spectrum, H-5 showed cross-peaks with H-6 and H-7, and H-8 was correlated to H-7 as well as to the methylene envelope at H-9–H-13. The location of the third double bond at C-15 and C-16 was established by the following HMBC correlations: H-18 → C-17 (δ 20.7) and C-16 (δ 133.6), and H-17 → C-16 and C-15 (δ 124.5). Accordingly, the structure of compound **1** was established to be 3(ζ)-hydroxy-octadeca-4(*E*),6(*Z*),15(*Z*)-trienoic acid.

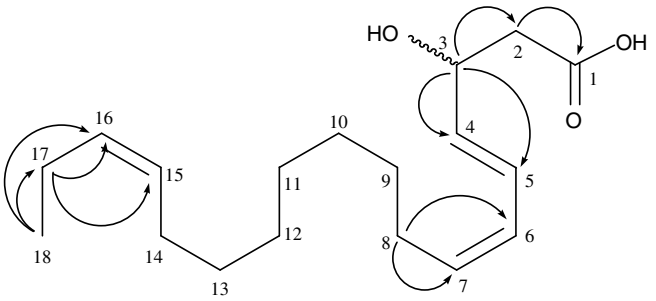

Fig. 1. Key HMBC correlations for **1**.

Compound **2** was also isolated as a colorless oil. The HRESIMS analysis indicated a quasimolecular ion $[M–H]^-$ at *m*/*z* 267.1966, corresponding to a molecular formula of $C_{16}H_{28}O_3$, possessing three units of unsaturation. Compared to the one separated and two conjugated double bonds of **1**, the ^{1}H NMR spectroscopic data of **2** only suggested two conjugated double bonds, with olefinic proton signals at δ 5.60 (1H, *dd*, *J* = 6.5, 15 Hz, H-4), 6.49 (1H, *dd*, *J* = 11, 15 Hz H-5), 5.96 (1H, *t*, *J* = 11 Hz, H-6) and 5.41 (1H, *m*, H-7), which were confirmed by the four olefinic carbon resonances at δ 136.3 (C-4), 125.5 (C-5), 128.4 (C-6) and 131.9 (C-7). The other signals were similar to those of **1**. As with **1**, the carboxyl group was not visible in the ^{13}C NMR spectrum, but was observed in the HMBC spectrum by a clear correlation of H-2 at δ 2.34 to a carbon at 177.0 ppm. Accordingly, the structure of compound **2** was established to be 3(ζ)-hydroxy-hexadeca-4(*E*),6(*Z*)-dienoic acid.

Compound **3** was isolated as a white powder from the cytotoxic hexane-soluble extract of the coralline red alga *H. reinboldii*. HRESIMS analysis indicated a quasimolecular ion $[M+Na]^+$ at *m*/*z* 801.5503, corresponding to a molecular formula of $C_{45}H_{78}O_{10}$. The ^{1}H NMR spectrum (Table 2) showed four geminal protons attached to carbon atoms bearing an oxygen functionality at δ 4.38 (*dd*, *J* = 3.5, 12 Hz, H-1a), 4.19 (*dd*, *J* = 6.5, 12 Hz, H-1b), 3.73 (*dd*, *J* = 6.5, 11 Hz, H-3a), and 3.88 (*dd*, *J* = 5.5, 11 Hz, H-3b) and a methine at 5.30 (1H, *m*, H-2), indicating the presence of a glycerol moiety, which was also confirmed by ^{13}C and DEPT signals at δ 63.4 (*t*, C-1), 70.5 (*d*, C-2) and 68.8 (*t*, C-3).

The appearance of a triplet at δ 0.87 (*J* = 7.0 Hz, H-20″) in the ^{1}H NMR spectrum of **3** was assigned to a terminal methyl of a fatty acid chain, and the multiplets at δ 2.31 (H-2″), 1.69 (H-3″) and 2.09 (H-4″) were due to the α, β, γ-methylenes, respectively, linked to the ester carbonyl functionality. These signals, together with the broad multiplet at δ 2.80 arising from allylic methylene protons and olefinic methine proton resonances overlapping at δ 5.35, were assigned to an unsaturated acyl fatty chain. The eight carbon signals in the low-field region (δ 129.4, 128.2, 129.2, 129.0, 128.7, 128.5, 127.9 and 130.9) indicated four separated double bonds in this chain. The small coupling constants (<11 Hz) revealed by the homonuclear *J*-spectrum (Derome, 1987; Sánchez-Sampedro et al., 2007) suggested a *Z*-configuration for all double bonds. Similarly, a triplet at δ 0.85 (*J* = 7.0 Hz, H-16′) belonging to the terminal methyl group, broad methylene resonances at δ 1.20–1.30 due to $(CH_2)_n$ of the aliphatic chain, and a multiplet at δ 2.05 (H-2′) associated with the α-methylene linked to the ester carbonyl functionality, were attributed to a saturated fatty acyl chain. The presence of two acyl fatty acids was reinforced by the MS/MS sodium adduct fragments *m*/*z* 497, which represented loss of eicosatetraenoic acid, and *m*/*z* 545, which represented loss of palmitic acid. Thus, the two chains were concluded to consist of eicosatetraenoyl and palmitoyl

Table 1
^{1}H and ^{13}C NMR spectroscopic data of **1–2** (in CD_3OD)

Position	**1**		**2**	
	δc DEPT	δ_H (mult., J in Hz)	δc DEPT	δ_H (mult., J in Hz)
1	179.0, *s*		177.0, *s*	
2	35.3, *t*	2.25 (2H, *m*)	37.4, *t*	2.34 (1H, *m*)
				1.52 (1H, *m*)
3	72.3, *t*	4.10 (1H, *dd*, 6.5, 13)	72.4, *d*	4.06 (1H, *dd*, 6.5, 13)
4	135.7, *d*	5.62 (1H, *dd*, 6.5, 15.5)	136.3, *d*	5.60 (1H, *dd*, 6.5, 15)
5	125.7, *d*	6.49 (1H, *dd*, 11, 15.5)	125.5, *d*	6.49 (1H, *dd*, 11, 15)
6	128.3, *d*	5.95 (1H, *t*, 11)	128.4, *d*	5.96 (1H, *t*, 11)
7	132.1, *d*	5.41 (1H, *dt*, 8.0, 11)	131.9, *d*	5.41 (1H, *m*)
8	27.6, *t*	2.18 (2H, *m*)	27.6, *t*	2.20 (2H, *m*)
9	25.7, *t*	1.59 (2H, *m*)	25.3, *t*	1.60 (1H, *m*)
				1.41 (1H, *m*)
10	29.7, *t*	1.32 (2H, *m*)	29.8, *t*	1.33 (2H, *m*)
11	29.4, *t*	1.32 (2H, *m*)	29.6, *t*	1.33 (2H, *m*)
12	29.3, *t*	1.32 21H, *m*)	29.5, *t*	1.33 21H, *m*)
13	29.2, *t*	1.32 (2H, *m*)	29.1, *t*	1.33 (2H, *m*)
14	20.7, *t*	2.05 (2H, *m*)	32.0, *t*	1.33 (2H, *m*)
15	124.5, *d*	5.35 (1H, *m*)	22.7, *t*	1.33 (2H, *m*)
16	133.6, *d*	5.46 (1H, *m*)	13.4, *t*	0.91 (3H, *t*, 7.5)
17	20.7, *t*	2.05 (2H, *m*)		
18	13.5, *q*	0.95 (3H, *t*, 7.5)		