A New monogalactosyldiacylglycerol (MGDG), a known monogalactosylmonoacylglycerol (MGMG) and a known polyunsaturated fatty acid methyl ester (PUFAME) were isolated from the marine dinoflagellate Karenia mikimotoi. The planar structure of the glycolipids was elucidated using MS and NMR spectroscopic analyses and comparisons to the known glycolipid to confirm its structure. The isolation of PUFAME strongly supports the polyunsaturated fatty acid fragment of these glycolipids. The relative configuration of the sugar was deduced by comparisons of 3JHH values and proton chemical shifts with those of known glycolipids. All isolated compounds MGDG, MGMG and PUFAME (1-3) were evaluated for their antimicrobial and anti-inflammatory activity. All compounds modulated macrophage responses, with compound 3 exhibiting the greatest anti-inflammatory activity.

Keywords: dinoflagellate; Karenia mikimotoi; glycolipids; monogalactosyldiacylglycerol; monogalactosylmonoacylglycerol; digalactosyldiacylglycerols; polyunsaturated fatty acid methyl ester; Staphylococcus aureus; Escherichia coli; Candida albicans; anti-inflammatory activity

1. Introduction

Microorganisms isolated from marine environments have been considered as a good source for exploration of novel natural products [1a, b]. Karenia mikimotoi (Miyake and Kominami ex Oda) Hansen et Moestrup (former name Gymnodinium aureolum, G. nagasakiense, G. mikimotoi) is a harmful dinoflagellate species with a worldwide distribution that forms massive blooms in coastal waters in the temperate regions of Europe [2–5], East Asia [6,7] and the east coast of United States of America [8]. K. mikimotoi has been associated with fish kills around the globe. The toxic components produced by K. mikimotoi that have been associated with fish kills are hemolysins. Hemolytic compounds, or hemolysins, are typically uncharacterized substances that can lyse red blood cells (RBCs) by altering membrane permeability [9]. Other components of K. mikimotoi blooms that have a negative effect on co-occurring species include: lipids, sterols, and/or polyunsaturated fatty acids [8,10]. Additionally, digalactosylmonoacylglycerols and a polyunsaturated fatty acids isolated from K. mikimotoi have been shown to be hemolytic [11] and ichthyotoxic [11]. Monogalactosyldiacylglycerols (MGDGs), monogalactosylmonoacylglycerols (MGMGs), and digalactosyldiacylglycerols (DGDGs) have attracted much attention in recent years because of their biological activities, such as anti-tumor-promoting [12,13], oxygen scavenging [14], anti-viral [15,16], anti-inflammatory [17] and anti-hyperlipidemic [18] properties. The activity of these compounds seems to be strictly related to the acyl chain length, considering of the anti-tumor-promoting activity...
of MGDGs; Akito Nagatsu et al. demonstrated that most MGDGs were less potent than galactosyl glycerol also MGDGs with a myristoyl group at sn-1 position were more potent than those at sn-2 position. Although MGDGs with highly unsaturated acyl group, which have myristoyl group at sn-2 position, showed relatively strong effect [19]. The length of the acyl chain is important for the activity, six carbon atoms resulting in the maximum effect, rather than the position of the ester function and the nature of the sugar (galactose or glucose) [20]. Glycoglycerolipids are usually available from natural sources in only limited quantities and difficult to separate mixtures [21]. In particular, it is difficult to isolate MGDGs and MGMGs with different acyl groups from K. mikimoi, because they have almost the same polarity and size.

During the investigation of the K. mikimoi strain CC21, for secondary metabolites, a new galactolipid, named (2S)-3-O-β-D-galactopyranosyl-1-O-3,6,9,12,15-octadecapentaenoyl-2-O-tetradecanoylglycerol (1), a known galactolipid (2S)-3-O-β-D-galactopyranosyl-1-O-3,6,9,12,15-octadecapentaenoylglycerol (2) [22] and a known PUFAME, Methyl (3Z,6Z,9Z,12Z,15Z)-octadeca-3,6,9,12,15-pentaenoate (3) [22] were isolated and identified.

2. Results and Discussion

Monogalactosyldiacylglycerol (1) was obtained as an optically active amorphous white solid, ([α]β20 – 16.7, c 0.30, MeOH). The molecular formula of 1 was established as C41H68O14 on the basis of HRMS ESI TOF mass spectrum (m/z 743.4727 [M + Na]+ (calcd for C41H66O14Na, 743.4710)) (Supporting Information, Figure S7). The IR spectrum of 1 revealed absorptions indicative of hydroxyl (3361 cm−1), ester (1736 cm−1), and glycosidic moieties (1085 cm−1). 1 was submitted to ESI-MS. The positive ion mode gave the ions at m/z 721.9 [M+H]+, 703.9 [M+H+Na]+ (Supporting Information, Figure S9).

Analyses of the COSY together with HSQC and heteronuclear multiple-bond coherence (HMBC) spectra led to the identification of a terminal β-galactopyranose unit with 13C NMR signals at δc 105.4 (C-1″), 72.5 (C-2″), 75.0 (C-3″), 70.3 (C-4″), 76.9 (C-5″), and 62.5 (C-6″) and a glycerol moiety with carbon signals at δc 64.5 (C-1), 71.8 (C-2), and 68.8 (C-3). In its 1H NMR spectrum, signals were observed corresponding to two methyls at δh 0.90 (3H, t, J = 7.3 Hz) and 0.98 (3H, t, J = 7.6 Hz), numerous oxymethylenes and oxymethines between δh 3.36 and 4.44, one oxymethine at δh 5.27 (m, H-2), methylene hydrogens between δh 1.20 and 3.15, and olefinic hydrogens between δh 5.35 and 5.56. The 23C and 1H NMR (Table 1) signals of 1 were similar to those of (2S)-3-O-β-D-galactopyranosyl-1-O-3,6,9,12,15-octadecapentaenoylglycerol [22] with the exception of signals at δc 174.6, 35.2, 26.1, 30.6, 30.2-30.8, 33.1, 23.8, 14.5 and δh 2.33 (t, J = 7.4 Hz, H-2″), 1.61 (m, H-3″), 1.33 (m, H-4″-H13″), and 0.90 (t, J = 7.3 Hz, H-14″) suggesting the presence of 2-O-tetradecanoyl moiety. The anomeric H-1″ proton of 1 at δh 4.22 (d, J = 7.5 Hz) showed HMBC cross-peaks with C-3 (δc 68.8) of the glycerol moiety and C-2″ (δc 72.5). The protons H-1a,b at δh [4.25 (dd, J = 12.1, 6.3 Hz) and 4.43 (dd, J = 12.1, 3.0 Hz)] of the glycerol was correlated to the carbonyl C-1′ (δc 173.1) of the fatty acid side chain. The 2-O-tetradecanoyl moiety was attached at C-2 by the proton chemical shift of C-2, δh 5.27 which appears in the down field shift. These overall analyses determined the structure of 1 as shown in (Figure 3). The anomeric β-configuration of the glycosidic bond was determined on the
basis of the anomic signal at δc 105.4 (C-1") [23]. Bis-allylic carbon signals of Z and E-isomers are observed at δc ca. 27 and ca. 32, respectively [22,24,25], the 26.6 ppm shift suggests that all double bonds have a cis geometry (Z). Because of the small amount of monogalactosyldiacylglycerol (1) produced by K. mikimotoi, we were able to confirm the exact nature of the polyunsaturated fatty acids, by isolation of methyl (3Z,6Z,9Z,12Z,15Z)-octadeca-3,6,9,12,15-pentaenoate in this strain. 1 was subjected to fragmentation mass spectroscopy, the observations of the fragment ion peak at m/z 229 corresponded to a tetradecanoic fatty acid. The fragment ion peak at m/z 559 corresponded to the loss of a sugar moiety. The observation of the fragment ion peak at m/z 331 corresponded to the loss of tetradecanoic acid and the sugar moiety, which clearly supports the polyunsaturated fatty acids description 3,6,9,12,15-octadecapentaenoic acid, this polyunsaturated fatty acid moiety was also support by observation of an ion peak at m/z 257. The tetradecanoic acid was further support by fragment ion peak at m/z 285 which corresponded to the loss of 3,6,9,12,15-octadecapentaenoic acid and a sugar moiety (Figure 2 and Supporting Information, Figure S9).

![Image of monogalactosyldiacylglycerol (1) from Karenia mikimotoi.](image)

**Figure 2.** Positive ESI-MS of the monogalactosyldiacylglycerol (1) from Karenia mikimotoi.

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<th>No.</th>
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<td>3.45, dd (9.7, 3.2)</td>
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**Table 1.** NMR Spectroscopic Data for Monogalactosyldiacylglycerol (1) in CD₃OD, δ in ppm.
Monogalactosylmonoacylglycerol (2) was obtained as an optically active amorphous white solid, ([α]_25^D -62.9, c 0.06, MeOH). The IR spectrum of 2 revealed absorptions indicative of hydroxyl (3366 cm\(^{-1}\)), ester (1726 cm\(^{-1}\)), and glycosidic moieties (1084 cm\(^{-1}\)). The molecular formula of 2 was established as C_{27}H_{42}O_9 on the basis of HRMS ESITOF mass spectrum (m/z 533.2727 [M + Na]^+ (calcd for C_{27}H_{42}O_9Na, 533.2727)) (Supporting Information, Figure S12).

Comparison of NMR spectroscopic data of 2 to those of previously reported spectra showed that the planar structure of 2 was identified as (2S)-3-β-D-galactopyranosyl-1-O-3,6,9,12,15-octadecapentaenoyl-2-O-tetradecanoylglycerol [22].

Methyl (3Z,6Z,9Z,12Z,15Z)-octadeca-3,6,9,12,15-pentaenoate (3) was obtained as a white solid. Its molecular formula C_{19}H_{28}O_2 was determined based on ^1H and ^13C spectroscopic data (NMR) and HRESITOF mass spectrum data (observed [M+H]^+ ion at m/z 289.2169, calculated [M+H]^+ ion at 289.2168) (Supporting Information, Figure S15). Comparison of NMR spectroscopic data of polyunsaturated fatty acid methyl ester 3 to those of the reported one permitted that the planar structure of 3 was identified as methyl (3Z,6Z,9Z,12Z,15Z)-octadeca-3,6,9,12,15-pentaenoate [22,26,27,28]. The isolation of compound 3 is a good support of the structure of a new Monogalactosyldiacylglycerol (1).

All three compounds were assessed for antimicrobial activity on the following potential human pathogens: gram positive bacteria Staphylococcus aureus, gram negative bacteria Escherichia coli, and fungi Candida albicans. None of the compounds exhibited the ability to kill or inhibit growth of either bacterial or fungal species.

All three compounds were assessed for immunomodulatory potential on the RAW 264.7 macrophage cell line exposed to the inflammatory stimulus lipopolysaccharide (LPS). Cells were exposed to LPS for 72 hours prior to the assay, with treatment with the compounds 24 hour prior to testing (48 hours after LPS). As can be seen in Figure 4, the compounds had varying effects on cell parameters. Compounds 2 and 3 both alter cell size (FSC) and complexity (SSC), causing smaller but
more complex cells. Cell surface markers of macrophage activation indicate that compound 1 increased TLR 4 expression, but decreased CD80 expression (as did compound 3). CD80 is a marker of M1, or classical, activation in macrophages. Classically activated M1 macrophages, as seen with an initial response to infection, produce pro-inflammatory cytokines to coordinate other immune cells in a battle against an infection or injury [29-31]. While none of the compounds had an effect on CD206, all three significantly reduced expression of CD124. Both CD206 and CD124 are cell surface markers of the M2 activation state in macrophages. M2 macrophages are considered the alternatively activated and are associated with wound and tissue healing [29-31]. As CD124 is a receptor for the signaling cytokine IL-4, this result indicates that these compounds are reducing macrophage ability to respond to this molecule. In particular, compound 3 appears to have the most aggregate effects, decreasing M1 activation (CD 80) and M2 activation (CD124), in addition to decreasing size and increasing complexity of RAW 264.7 cells.

Figure 4. Flow cytometry results of forward scatter (FSC – panel A), side scatter (SSC – panel B), and marker/receptor expression of TLR4 (panel C), CD80 (panel D), CD206 (panel E), and IL4Rα (panel F). RAW 264.7 macrophages were treated with LPS, then exposed to vehicle control (VC) or one of the three compounds. Results are presented as average relative fluorescent units +/- standard deviation, and * indicates statistical significance from control of p < 0.05 (n = 3).

To assess the functional response to decreases in activation states seen by these compounds on macrophages, media was collected from RAW 264.7 cells treated with LPS for 72 total hours and the test compounds for the 24 hours after the LPS treatment LPS treatment. As seen in Figure 5,
expression of the pro-inflammatory cytokine TNFα was significantly decreased in response to
treatment with compound #3 alone. This functional response is in agreement with the flow
cytometry data (Fig 4), where compound 3 had the most effects on macrophage expression of
activation markers and size complexity. Interestingly, none of the compounds altered expression of
the anti-inflammatory IL-10. IL-10 is typically associated with an M2 phenotype, as it suppresses
immune responses and prevents tissue damage due to inflammation [32]. This data indicates that
compound 3 may have potential as an anti-inflammatory therapy if it can reduce activation and
inflammatory cytokine secretion while maintaining IL-10 secretion and thus its protective effects,
though further studies would need to be conducted to confirm this hypothesis.

Figure 5. ELISA results of cytokine secretion from LPS-stimulated RAW 264.7 macrophages after
treatment with the three compounds or vehicle control (VC). Panel A shows TNFα secretion and
panel B shows IL-10 secretion. Results are presented as average expression in pg/mL +/- standard
deviation, and * indicates statistical significance from control of p < 0.05 (n = 3).

One may expect, with increased expression of TLR4 (Fig 4C), that compound 1 would allow cells
to be more responsive to LPS treatment. However, the increase in TNFα secretion (Fig 5A) was not
statistically significant, and it could be that the concomitant decrease in CD80 expression (Fig 4D)
results in a net neutral activation state for these macrophages.

3. Experimental Section

3.1. General Experimental Procedures

The optical rotation was measured using an Autopol III (Rudolph Research) polarimeter with a
1 cm cell. IR spectra were recorded on a Bruker Fourier transform (FT)-IR model IFS-88 spectrometer.
NMR spectra were obtained with Bruker 500 MHz spectrometer in d4-methanol, using the signals of
the residual solvent protons and the solvent carbons as internal references (δH 3.30 and δC 49.0 ppm
for CD3OD). High Resolution ESITOF-mass spectra were measured on a Waters Xevo G2-XS QTOF
mass spectrometer. The instrument was scanned from 200 to 1500 m/z with the spectra shown
consisting of the sum of 20 scans. Samples for MS analysis were dissolved in either 50:50
water/acetonitrile or 49:49:2 water/acetonitrile/acetic acid before being directly injected at a flow rate
of 10 µL/min. Low-resolution ESI data were measured using a Sciex QTRAP 4000 LC/MS system.
Samples were dissolved in 98% acetonitrile 2% water and 0.1% formic acid and directly infused into
the mass spectrometer using a Harvard syringe pump with a flow rate of 10 µL/min. The mass
spectrometer parameters were: declustering potential 80V, Ion spray voltage 5000V, Temperature 350
C, Gas Source 1 35psi, gas source 2 35psi, interface heater on). Fragments of the target compounds
were produced by ramping the collision energy from 5-130V.

HPLC was conducted on a SHIMADZU LC-10AD Pump coupled with a SHIMADZU SPD-10A
UV/vis detector. The UV wavelength used for separation was 215 nm. The flow rate of the mobile
3.2. Strain and Cultivation

*Karenia mikimotoi*, strain ARC 163, was isolated from the marine waters off Corpus Christi, TX (27.84567,-97.429848). A voucher specimen was deposited by L. Campbell, (2006) and is maintained at the Algal Resources Collection (ARC), University North Carolina Wilmington. The *Karenia* species was identified on the basis of morphology by ARC. The strain was grown in two 10L batch static cultures with LH media, (NaNO$_3$: 75 g/L of pyrogen free DIW, NaH$_2$PO$_4$:H$_2$O 5 g/L, Na:SeO$_3$: 0.0045 g/L, PII Metal Mix, F/2 Vitamin Solution) in 33SU at 22 ºC, and photoperiod of 8:16 for 25-30 days. The finial density was approximately 20x10$^6$ cells/L.

3.3. Extraction and Isolation

After approximately 30 days under static growth, the whole culture was extracted with ethyl acetate (EtOAc), with the ratio EtOAc-Water (1:1 v:v) and evaporated under reduced pressure to yield the crude organic extract (0.4 g).

The ethyl acetate extract was then partitioned between 90% methanol in water and petroleum ether (1:1 v:v) to separate non-polar lipids from polar lipids. The methanol-soluble layer was removed and concentrated under vacuum to give 0.2 g dry weight. The methanol-water layer was then subjected to reversed-phase HPLC (Agilent, Zorbax C$_18$); HRMSESITOF detection at 215 nm) using an isocratic solvent system of H$_2$O/Methanol (2 : 98) fraction contained a mixture of metabolites, which was further fractionated by reversed-phase HPLC (Phenomenex Luna Phenyl-Hexyl, 250 x 4.6 mm, 1.4 mL/min, 5 µm, UV detection at 215 nm) using an isocratic solvent system of 89% MeOH 11% H$_2$O to afford monogalactosyldiacylglycerol (1, $t_r$ = 20 min, 0.7 mg), monogalactosylmonoacylglycerol (2, $t_r$ = 17 min, 0.3 mg) and polysaturated fatty acid methyl ester (3, $t_r$ = 6 min, 0.4 mg).

3.3.1. Monogalactosyldiacylglycerol (1)

Optically active amorphous white solid; ([α]$^2$D –16.7, $c$ 0.30, MeOH); IR (KBr) $\nu_{max}$ 3361, 2923, 2853, 1736, 1277, 1163, 1085 cm$^{-1}$; $^1$H and $^{13}$C NMR data (Table 1); HRMSESITOF $m/z$ 743.4727 [M + Na]$^+$ (calcd for C$_{32}$H$_{60}$O$_{10}$Na, 743.4710).

3.3.2. Monogalactosylmonoacylglycerol (2)

Optically active amorphous white solid: ([α]$^2$D –62.9, $c$ 0.06, MeOH); IR (KBr) $\nu_{max}$ 3366, 2920, 2847, 1726, 1476, 1084 cm$^{-1}$; HRMSESITOF $m/z$ 533.2727 [M + Na]$^+$ (calcd for C$_{22}$H$_{38}$O$_{10}$Na, 533.2727); $^1$H NMR (500 MHz, CD$_2$OD): $\delta$ 5.55 (2H, q, $J$ = 4.9 Hz, H-3', H-4'), 5.39 (1H, m, H-16'), 5.36 (7H, m, H-6', H-7', H-9', H-10', H-12', H-13', H-15'), 5.28 (1H, m, H-2'), 4.43 (1H, dd, $J$ = 12.1, 3.0 Hz, H-1b), 4.27 (1H, dd, $J$ = 12.1, 6.3 Hz, H-1a), 4.23 (1H, d, $J$ = 7.5, H-1'), 3.99 (1H, dd, $J$ = 10.9, 5.4, H-3b), 3.82 (1H, brd, $J$ = 3.2, H-4'”), 3.77 (1H, m, H-3a), 3.75 (2H, m, H-6'”, 3.51 (1H, m, H-2”), 3.51 (1H, m, H-5'”), 3.44 (1H, dd, $J$ = 9.7, 3.2, H-3’”), 3.15 (2H, dd, $J$ = 7.9, 5.9 Hz, H-2’), 2.84 (8H, m, H-5’, H-8’, H-11’, H-14’), 2.07 (3H, m, H-7’, H-9’), $^{13}$C NMR (125 MHz, CD$_2$OD): $\delta$ 172.0 (C-1’), 132.9 (C-16’), 132.6 (C-14’), 127.5 (C-10’), 122.5 (C-3’), 114.4 (C-5’), 74.9 (C-3’), 72.1 (C-2’), 70.4 (C-4’), 70.4 (C-2’), 67.0 (C-3’), 62.6 (C-6’), 62.6 (C-1’), 32.3 (C-2’), 26.7, 26.6 (X2), 26.5 (C-5’, C-8’, C-11’, C-14’), 21.6 (C-17’), 14.7 (C-18’), (Supporting Information, Figure S10 and Figure S11), in agreement with data reported in the literature [22].

3.3.3. Methyl (3Z,6Z,9Z,12Z,15Z)-octadeca-3,6,9,12,15-pentaenanoate (3)

White solid; $^1$H NMR (500 MHz, CD$_2$OD): 5.50-5.59 (2H overlapping, m, alkene-H); 5.24-5.43 (8H overlapping, m, alkene-H), 3.13 (2H, d, $J$ = 5.3 Hz, H-2); 3.67 (3H, s, -OCH$_3$); 2.78-2.84 (four overlapping t, 8H, m, H-5, H-8, H-11, H-14), 2.08 (2H, quintet, $J$ = 7.6, H-17); 0.97 (3H, t, $J$ = 7.6, H-18); $^{13}$C NMR (125 MHz, CD$_2$OD): $\delta$ 172.0 (C-1’), 132.8 (C-16’), 132.5 (C-4’), 129.6, 129.5, 129.3, 129.0,
Compounds were tested for antimicrobial activity using a Minimum Inhibitory Concentration (MIC) assay with the gram-positive bacteria *Staphylococcus aureus*, the gram-negative bacteria *Escherichia coli*, and the fungi *Candida albicans* (Carolina Biological, Greensboro, NC, USA). Bacteria were inoculated in nutrient broth for 24 hours at 37 °C, and fungi were inoculated in Sabouraud broth for 24 hours at 27 °C.

Test compounds and positive controls (10-50 μg/mL doxycycline for bacteria and 50 μg/mL amphotericin B for fungi) were dissolved in methanol, and 100 μL were added to duplicate wells of a 96-well assay plate. Compounds and controls were serially diluted 1:2 in broth. Vehicle control (methanol only) and negative control (no compound) was added to control wells. Following compound/control dilutions, 10 μL of bacteria or fungi were added to each well for a final concentration of 5 x 10^5 CFUs per well. Plates were incubated for 24 hours at the corresponding temperature (37 °C for bacteria, 27 °C for fungi). Plates were read for OD at 600nm to assay for bacteriostatic or fungistatic activity. To assess for bactericidal or fungicidal activity, wells were assayed using an XTT Cell Proliferation Assay Kit (ATCC, Manassas, VA, USA), according to the manufacturer’s instructions.

**3.5. Flow Cytometry and ELISA Experiments**

RAW 264.7 macrophages were seeded in 12-well plates and incubated at 37 °C until cells adhered and grew to confluence before being treated with LPS (50 ng/mL). Cells were incubated for 48 hours prior to treatment with vehicle control (EtOH) or one of the three compounds for another 24 hours (72 total hours LPS). Cells were then harvested, centrifuged, and media removed for ELISA experiments. Cells were blocked with anti-goat serum to block Fc receptors for 15 minutes on ice, then stained for 1 hour with TLR4, CD80, CD206, and CD124 antibody solution. Unbound antibody was removed and cells resuspended in cold PBS prior to analysis on a BD FACS Celesta flow cytometer (BD Biosciences; San Jose, CA, USA).

Expression of Toll-like receptor 4 (TLR4) or CD284/MD-2) was measured using BV650-conjugated rat anti-mouse antibodies directed against CD284/MD-2 (BD Biosciences; San Jose, CA, USA). Expression of mannose receptors (CD206) was measured using Alexa Fluor 488-conjugated rabbit anti-mouse antibodies directed against CD206 (Abcam; Cambridge, MA, USA). Expression of CD80 receptors was measured using phycoerythrin (PE)/CF594-conjugated hamster anti-mouse antibodies directed against CD80 (BD Biosciences; San Jose, CA, USA). Expression of IL4Ra/CD124 receptors was measured using Alexa Fluor 647-conjugated rat anti-mouse antibodies directed against CD124 (BD Pharmingen; San Diego, CA, USA).

Cell media was assayed for TNFα or IL-10 expression using commercially available ELISA kits (R&D systems; Minneapolis, MN, USA) according to the manufacturer’s directions.

**4. Conclusions**

In our aim for isolation of new natural product, the glyceroglycolipids composition of marine dinoflagellate *K. mikimotoi* was investigated. *K. mikimotoi* is a promising candidate strain for sustainable production of high value co-products including long chain polyunsaturated fatty acids (PUFAs), omega-3 fatty acids. A new monogalactosyldiacylglycerol (MGDG, 1) was isolated and characterized. In the best of our knowledge this is the first time monogalactosylmonoacylglycerol (MGMG, 2) and a polyunsaturated fatty acid methyl ester (PUFAME, 3) were isolated from *K. mikimotoi*. Compound 3 may be artifact of the isolation procedure, and may be formed by methanolysis of biological ester. Because of limited amount of compound 1 we were not able to done
an acid hydrolysis, fortunately the isolation of compound 3 further support the fatty acid side chain
of compounds 1 and 2. The biological activities of 1, 2 and 3 were investigated on three human
pathogenic microorganisms. None of the compounds exhibited the ability to kill or inhibit growth of
bacterial species or fungal species, but they did exhibit ability to modulate macrophage activation
and functionality, indicating a potential anti-inflammatory role for this family.5. Conclusions

This section is not mandatory, but can be added to the manuscript if the discussion is unusually
long or complex.

**Supplementary Materials:** The 1H, 13C NMR, DEPT-135, COSY, HSQC, HMBC, HRMSESITOF LRESI-MS, IR,
data of Monogalactosyldiacylglycerol (1); 1H, 13C NMR, HRMSESITOF data of
Monogalactosylmonoacylglycerol (2); 1H, 13C NMR, HRMSESITOF data of Polyunsaturated fatty acid
methyl ester (3) are available online at www.mdpi.com/.

**Author Contributions:** A.S.L. performed the isolation, structure elucidation of monogalactosyldiacylglycerol
(1), monogalactosylmonoacylglycerol (2) and polyunsaturated fatty acid methyl ester (3) and wrote the
manuscript; J.R.M. conducted bioassay testing and writeup, funded the work, and reviewed the paper; B.Y.
performed large cultures; R.R.G. was involved in mass spectrometry and reviewed the manuscript; A.J.B. was
the project leader for guiding the experiments of chemical analysis and writing the manuscript.

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