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Algicidal Activity of Glycerolipids from Brown Alga *Ishige sinicola* toward Red Tide Microalgae

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Bioassay-guided fractionation of a methanol extract of the brown alga, *Ishige sinicola*, led to the isolation of five algicidal compounds. Their structures were determined to be α-monoglycerides of eicosa-5Z,8Z,11Z,14Z-tetraenoic (arachidonic) acid, octadeca-6Z,9Z,12Z,15Z-tetraenoic acid, linoleic acid and oleic acid, and 1-*O*-palmitoyl-3-*O*-(6-sulfo-α-D-quinovopyranosyl)-*sn*-glycerol on the basis of spectroscopic data and a comparison with the data in the literature. These glycerolipids showed moderate-to-high cell lysis activity against the red tide microalgal species, *Heterosigma akashiwo*, *Karenia mikimotoi* and *Alexandrium catenella*, at a concentration of 20 μg/mL.

Key words: algicidal compound; *Ishige sinicola*; α-monoglyceride; 1-*O*-palmitoyl-3-*O*-(6-sulfo-α-D-quinovopyranosyl)-*sn*-glycerol

Red tides caused by harmful algal blooms (HABs) have been reported to occur world-wide and have become a serious problem for public health and fisheries industries in recent years. HAB effects are quite noxious to marine living organisms, including some aquacultured fish, causing significant damage to the ecosystem and having a severe economic impact on aquaculture. Practical countermeasures against red tides are therefore urgently needed.

Like terrestrial plants, seaweeds (macroalgae) are known to produce allelopathic (algicidal) substances which inhibit the growth and/or reproduction of other competing algae. Some of the macroalgal alleochemicals have also been shown to be toxic to microalgae and microorganisms. Consequently, several studies on the anti-microalgal substances in seaweeds have been reported, aiming at the development of a novel strategy for controlling HABs.[1)] Pioneering work was done by Kakisawa *et al.* in 1988.[2)] They isolated the polyunsaturated fatty acid (PUFA), octadeca-6Z,9Z,12Z,15Z-tetraenoic acid (ODTA), as an alleopathic substance from the brown alga, *Cladosiphon okamuranus*, and demonstrated that ODTA and its related PUFAs, as arachidonic acid, had high algicidal activity toward a variety of microalgae involving the red tide microalgal species, *Heterosigma akashiwo*, *Chattonella antiqua*, *C. marina*, *Gymnodinium nagasakiense* (currently *Karenia mikimotoi*) and *G. sanguineum* (currently *Akashiwo sanguenea*). Chiang *et al.* subsequently determined the allelopathic substances in the green alga, *Botryococcus braunii*, to be α-linolenic, oleic, linolenic, and palmitic acids.[3)] We have also isolated three PUFAs, hexadeca-4Z,7Z,10Z,13Z-tetraenoic acid (HDTA), ODTA and α-linolenic acid, from the green alga, *Ulva fasciata*,[4)] and four diterpenes from the brown alga, *Dictyota dichotoma*,[5)] as their algicidal principles. Hong *et al.* have recently isolated heptadeca-5Z,8Z,11Z-trienoic acid from the crustose coralline seaweed, *Lithophyllum yessoense*, and found that this odd-numbered carbon fatty acid had lysis activity against the spores of seaweeds and the cells of several red tide phytoplankton species.[6)]

We screened eleven seaweeds collected from the coastal region of Nagasaki prefecture in Japan, involving seven Phaeophyta and four Rhodophyta, for their cell lysis activity toward *H. akashiwo* and found that a methanol extract of the brown algae, *Ishige sinicola* and *Dictyopteris undulata*, had higher activity (Table 1). We report here the isolation and microalgal cell lysis activity of the algicidal principles of *I. sinicola*.

Chromatographic separation[7)] of the methanol extract of the whole body of *I. sinicola* by monitoring the cell lysis activity against *H. akashiwo* resulted in the isolation of five algicidal compounds **1**–**5** (Fig. 1). The structures of the isolates were determined to be α-monoglycerides of eicosa-5Z,8Z,11Z,14Z-tetraenoic (arachidonic) acid (**1**),[8)] octadeca-6Z,9Z,12Z,15Z-tetraenoic acid (ODTA) (**2**),[9)] linoleic acid (**3**)[10)] and oleic acid (**4**),[11)] and 1-*O*-palmitoyl-3-*O*-(6′-sulfo-α-D-quinovopyranosyl)-*sn*-glycerol (**5**),[12)] by comparing their spectroscopic data (IR, ^{1}H- and ^{13}C-NMR, and MS) and optical rotation values with those reported in the literature.[13–19)] α-Monoglyceride **2** was first isolated from the brown alga, *Sargassum sagamianum*, as a cyclooxygenase-2 (COX-2) inhibitor,[14)] and, to our knowledge, it is the only example of a naturally occurring α-monoglyceride of ODTA. Sulfolipid **5** was first isolated from the green alga, *Ulva pertusa*, as a hemolysin by Fusetani and Hashimoto in 1975.[17)] The same compound was isolated from the green alga, *Bryopsis* sp., as a factor inducing ecdysis (thecal loss) in the toxic dinoflagellate, *Gambierdiscus toxicus*, by Sakamoto *et al.* in 2000.[18)] Sahara *et al.* have recently reported that chemically synthesized SQMGs involving

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Abbreviations: HAB, harmful algal bloom; PUFA, polyunsaturated fatty acid; ODTA, octadeca-6Z,9Z,12Z,15Z-tetraenoic acid; SQDG, sulfoquinovosyl diacylglycerol; SQMG, sulfoquinovosyl monoacylglycerol

Table 1. Algicidal Activity[a] (mortality, %) of the Methanol Extract of Seaweeds from Nagasaki Beach (collected May 2007) against *Heterosigma akashiwo* at Concentrations of 100 and 20 μg/mL after 4 h

	Seaweed species	Algicidal activity (mortality, %)	
		100 μg/mL	20 μg/mL
Brown alga	*Colpomenia sinuosa*	5	nt[b]
	Dictyopteris undulata	100	100
	Eckloniopsis radicosa	1	nt[b]
	Ishige okamurae	10	nt[b]
	Ishige sinicola	100	100
	Sargassum horneri	1	nt[b]
	Sargassum tenuifolium	23	nt[b]
Red alga	*Gelidium elegans*	5	nt[b]
	Gloiopeltis furcata	5	nt[b]
	Hypnea flexicaulis	3	nt[b]
	Laurencia undulata	14	nt[b]

[a]The phytoplankton species was cultured aseptically in a sterilized Guillard's f/2 medium at 20 °C under illumination by a fluorescent lamp (40 μmol photons/m^2s) with a cycle of 12 h light and 12 h dark. Cells in the exponential growth phase were used throughout the experiments. To the phytoplankton cell suspensions (cell density *ca.* 3×10^4 cells/mL) in a 24-well micro plate, the sample solution in MeOH (<2 μL) was added to make a final concentrations of 100 and 20 μg/mL. After 4 h cultivation, the survivability and mortality of the cells were calculated under microscopic observation (×20).
[b]not tested.

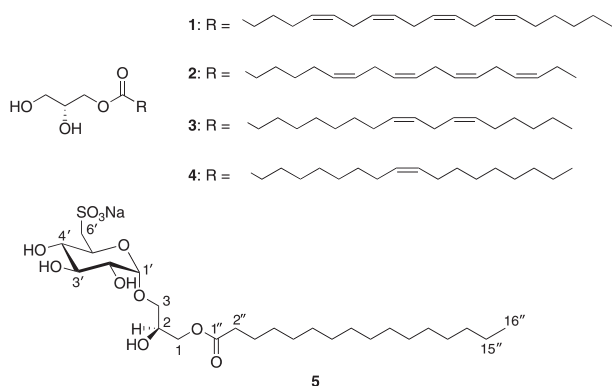

Fig. 1. Structures of Algicidal Compounds **1**–**5**.

5 inspired by cytotoxic SQDGs and SQMGs of the sea urchin, *Strongylocentrotus intermedius*,[20] had weak ($LC_{50} \sim 10^{-5}$ M) *in vivo* anti-tumorial effects on nude mice bearing solid tumors of human lung adenocarcinoma cell line A-549.[21]

The algicidal activity[2,22] of isolated glycerolipids **1**–**5** was assessed against four representative red tide microalgal species, *H. akashiwo* (raphidophyte), *Karenia mikimotoi* (dinoflagellate), *Chattonella marina* (raphidophyte) and *Alexandrium catenella* (dinoflagellate) (Table 2). Free fatty acids corresponding to α-monoglycerides **1**, **3** and **4** were also tested for their activity in comparison. All the compounds except for **4** showed moderate-to-high activity toward *H. akashiwo* and *A. catenella*, but moderate-to-low activity toward *C. marina* at a concentration of 20 μg/mL. Sulfolipid **5** had the highest activity among the isolated compounds. The higher activity of the sulfolipid may have partially been due to its highly hydrophilic property. Monoglycerides **1**, **3** and **4** showed the same level of activity as their corresponding fatty acids in all the

Table 2. Algicidal Activity[a] of Isolated Compounds **1**–**5** and Fatty Acids toward the Lysis of *Heterosigma akashiwo*, *Karenia mikimotoi*, *Chattonella marina* and *Alexandrium catenella* after 4 h at 20 μg/mL (mortality, %)

	H. akashiwo	*K. mikimotoi*	*C. marina*	*A. catenella*
Compound **1**	85.0 ± 7.1	63.3 ± 8.5	15.0 ± 4.1	47.0 ± 11.2
Compound **2**	47.5 ± 4.3	31.0 ± 8.6	32.5 ± 7.5	48.8 ± 4.1
Compound **3**	73.3 ± 8.5	20.0 ± 5.5	28.3 ± 6.2	51.3 ± 4.1
Compound **4**	33.3 ± 8.4	51.3 ± 8.9	20.0 ± 3.5	33.0 ± 8.1
Compound **5**	84.0 ± 7.8	93.0 ± 7.5	76.7 ± 10.2	93.8 ± 4.1
Arachidonic acid	78.3 ± 10.3	73.0 ± 12.1	17.5 ± 4.3	60.8 ± 9.3
Linoleic acid	73.3 ± 30.9	nt[b]	32.5 ± 7.5	50.0 ± 8.4
Oleic acid	20.0 ± 8.2	43.8 ± 10.8	21.3 ± 2.2	20.8 ± 6.7

[a]The algicidal activity was evaluated in the same procedure as described in Table 1.
[b]not tested.

microalgal species (*cf.* **1** and arachidonic acid, **3** and linoleic acid, and **4** and oleic acid). It has been reported that the lysis activity of the free fatty acids decreased as the degree of unsaturation decreased, and that such monounsaturated fatty acids as oleic acid (C18:1) and eicosenoic acid (C20:1) had no or quite low activity for the lysis of *H. akashiwo*[22] and monospores of *Porphyra suborbiculata*;[6] however, such a trend was not obvious in the α-glycerides of *I. sinicola*.

The microalgal cells treated with the glycerolipids of *I. sinicola* soon became swollen within 30 min and finally caused cell lysis. Although the detailed mechanism of action is unknown, these amphiphatic molecules may interact with the cell membrane of microalgae and disrupt osmolarity regulation.

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7) A MeOH (2 L) extract (47.60 g) of air-dried and powdered *I. sinicola* (1042 g) was partitioned between water (400 mL) and CH_2Cl_2 (700 mL). Part of the CH_2Cl_2 extract (12.99 g of 23.80 g) was chromatographed over Diaion HP-20, using gradient elution from 40% to 100% MeOH, to yield five fractions (Fr 1 to Fr 5), in which Fr 3 and Fr 4 were active. The Fr 3 was then chromatographed on Chromatorex ODS DM1020T using gradient elution from 85% to 100% MeOH, to yield an active fraction (Fr 3-3, 62.5 mg) which was separated by HPLC (Cosmosil 5C18 MS-II, 20 × 250 mm, 80% CH_3CN), and subsequent reversed-phase TLC (Merck, RP-18, 90% MeOH) to yield compound **5** (4.5 mg). Part of Fr 4 (1.33 g of 3.71 g) was separated by silica gel chromatography, using a hexane-EtOAc gradient (1:1 to 0:1), and subsequent HPLC (Cosmosil 5C18 MS-II, 90% CH_3CN) to yield compounds **1** (11.6 mg), **2** (5.2 mg), **3** (23.0 mg), and **4** (6.5 mg).
8) Colorless oil, $[\alpha]_D^{25}$ −10.3° (*c* 0.58, MeOH). NMR δ_H (400 MHz, $CDCl_3$): 0.89 (3H, t, $J = 6.8$ Hz), 1.30 (8H, m), 1.70 (2H, m), 2.09 (4H, m), 2.37 (2H, t, $J = 7.8$ Hz), 2.81 (6H, m), 3.60 (1H, dd, $J = 11.7$, 5.8 Hz), 3.70 (1H, dd, $J = 11.7$,

3.9 Hz), 3.93 (1H, m), 4.15 (1H, dd, $J = 11.7, 6.8$ Hz), 4.21 (1H, dd, $J = 11.7, 4.4$ Hz), 5.38 (8H, m).

9) Colorless oil, $[\alpha]_D^{25}$ -5.4° (*c* 0.52, MeOH). NMR δ_H (400 MHz, $CDCl_3$): 0.98 (3H, t, $J = 7.6$ Hz), 1.41 (2H, m), 1.66 (2H, m), 2.08 (4H, m), 2.37 (2H, t, $J = 7.3$ Hz), 2.83 (6H, m), 3.60 (1H, dd, $J = 11.7, 5.8$ Hz), 3.70 (1H, dd, $J = 11.7, 4.3$ Hz), 3.94 (1H, m), 4.15 (1H, dd, $J = 11.7, 5.8$ Hz), 4.21 (1H, dd, $J = 11.7, 5.3$ Hz), 5.38 (8H, m).

10) Colorless oil, $[\alpha]_D^{25}$ -3.9° (*c* 0.77, MeOH). NMR δ_H (400 MHz, $CDCl_3$) 0.89 (3H, t, $J = 6.8$ Hz), 1.31 (14H, m), 1.61 (2H, m), 2.05 (4H, m), 2.35 (2H, t, $J = 7.7$ Hz), 2.77 (2H, t, $J = 6.4$ Hz), 3.59 (1H, dd, $J = 11.7, 5.8$ Hz), 3.69 (1H, dd, $J = 11.7, 3.9$ Hz), 3.93 (1H, m), 4.14 (1H, dd, $J = 11.7, 5.8$ Hz), 4.20 (1H, dd, $J = 11.7, 4.3$ Hz), 5.36 (4H, m).

11) Colorless oil, $[\alpha]_D^{25}$ -2.5° (*c* 0.65, MeOH). NMR δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, $J = 6.8$ Hz), 1.29 (22H, m), 1.63 (2H, m), 2.02 (4H, m), 2.35 (2H, t, $J = 7.6$ Hz), 3.60 (1H, dd, $J = 11.7, 5.8$ Hz), 3.70 (1H, dd, $J = 11.7, 3.9$ Hz), 3.93 (1H, m), 4.15 (1H, dd, $J = 11.7, 5.8$ Hz), 4.21 (1H, dd, $J = 11.7, 4.3$ Hz), 5.35 (2H, m).

12) Colorless oil, $[\alpha]_D^{22}$ $+48.1^\circ$ (*c* 0.54, MeOH). NMR δ_H (600 MHz, CD_3OD) 0.89 (3H, t, $J = 7.0$ Hz), 1.30 (24H, m), 1.61 (2H, quint, $J = 7.5$ Hz), 2.36 (2H, t, $J = 7.5$ Hz), 2.90 (1H, dd, $J = 14.3, 9.3$ Hz), 3.07 (1H, dd, $J = 9.8, 9.0$ Hz), 3.35 (1H, dd, $J = 14.3, 2.0$ Hz), 3.38 (1H, m), 3.40 (1H, dd, $J = 9.1, 3.8$ Hz), 3.64 (1H, t, $J = 9.1$ Hz), 4.05 (1H, dd, $J = 10.2, 3.5$ Hz), 4.09 (3H, m), 4.19 (1H, dd, $J = 13.8, 6.3$ Hz), 4.77 (1H, d, $J = 3.8$ Hz).

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