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New Chemical Constituents from *Oryza sativa* Straw and Their Algicidal Activities against Blue-Green Algae

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ABSTRACT: Five new constituents, 5,4′-dihydroxy-7,3′-dimethoxyflavone-4′-*O*-*β*-D-xylopyranosyl-(2a→1b)-2a-*O*-*β*-D-xylopyranosyl-(2b→1c)-2b-*O*-*β*-D-xylopyranosyl-2c-octadecanoate (**1**), 5,4′-dihydroxy-7,3′-dimethoxyflavone-4′-*O*-*α*-D-xylopyranosyl-(2a→1b)-2a-*O*-*α*-D-xylopyranosyl-(2b→1c)-2b-*O*-*α*-D-xylopyranosyl-(2c→1d)-2c-*O*-*α*-D-xylopyranosyl-2d-octadecanoate (**2**), kaempferol-3-*O*-*α*-D-xylopyranosyl-(2a→1b)-2a-*O*-*α*-D-xylopyranosyl-(2b→1c)-2b-*O*-*α*-D-xylopyranosyl-(2c→1d)-2c-*O*-*α*-D-xylopyranosyl-2d-hexadecanoate (**3**), methyl salicylate-2-*O*-*α*-D-xylopyranosyl-(2a→1b)-2a-*O*-*α*-D-xylopyranosyl-(2b→1c)-2b-*O*-*α*-D-xylopyranosyl-(2c→1d)-2c-*O*-*α*-D-xylopyranosyl-(2d→1e)-2d-*O*-*α*-D-xylopyranosyl-(2e→1f)-2e-*O*-*α*-D-xylopyranosyl-(2f→1g)-2f-*O*-*α*-D-xylopyranosyl-(2g→1h)-2g-*O*-*α*-D-xylopyranosyl-2h-geranilan-8′,10′-dioic acid-1′-oate (**4**), and oleioyl-*β*-D-arabinoside (**5**), along with eight known compounds, were isolated from a methanol extract of *Oryza sativa* straw. The structures of the new compounds were elucidated using one- and two-dimensional NMR spectroscopies in combination with IR, ESI/MS, and HR-ESI/FTMS. In bioassays with blue-green algae, the efficacies of the algicidal activities of the five new compounds (**1**−**5**) were evaluated at concentrations of 1, 10, and 100 mg/L. Compound **5** had the highest growth inhibition (92.6 ± 0.3%) for *Microcystis aeruginosa* UTEX 2388 at a concentration of 100 ppm (mg/L). Compound **5** has high potential for the ecofriendly control of weeds and algae harmful to water-logged rice.

KEYWORDS: *Oryza sativa L., Gramineae, rice straw, new chemical constituents, algicidal activity*

INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food in Asia and generally exists as two types, white hulled and colored hulled. The most common type (85%) is white-hulled rice. The germination of rice is of great agricultural importance and has long been known to be influenced by compounds present in the seed coat (hull).[1,2] The compounds momilactones A and B from rice hulls cause germination and growth inhibition in the rice roots.[3−5] They are also found in rice leaves and rice straw as phytoalexins.[6,7]

Rice straw is reapplied in large amounts to paddy and upland fields, especially greenhouse croppings, as an organic material for soil improvement. The degradation products of rice straw in the soil may influence the growth of crops in both nutritional and physiological terms. The elucidation of the mechanism of humus formation from rice straw is also important for understanding its influence on plant growth.[8] Phenolic substances are widely distributed in various plants, including the different parts of rice plants. Some of these substances, which enter the soil from plants, are plant growth inhibitors and cause dieback disease or other abnormal growth. It has been reported that *p*-coumaric acid and other phenolic acids, for instance, inhibited the growth of upland rice plants. Kuwatsuka and Oshima[9] isolated and/or identified *p*-hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acids from rice leaves. Inamatsu[10] also found *p*-coumaric acid in methanol extracts of rice straw and recognized that the amount of the acid decreased during the heaping of rice straw.

Phenolic compounds were reported from rice straw on the basis of HPLC or GC analysis.[8,11] Identification of allelopathic compounds, including momilactones A and B, from rice straw and their biological activities have been reported.[11−17] Sakuranetin, a flavonone phytoalexin, from ultraviolet-irradiated rice leaves has been reported.[7]

Previous observations, in a series of unreplicated field trials and laboratory culture, have shown that decomposing barley straw can prevent the growth of *Microcystis aeruginosa* and other blue-green unicellular and green filamentous algal species.[18] Previously reported compounds from rice straw were mostly studied using either HPLC or GC analysis. There are no reports of rice straw compounds obtained by extraction and separation techniques and their identification by spectroscopic analysis. The objective of this study is the identification of new constituents with algicidal growth inhibition properties in rice straw.

This is the first report of the isolation of five new constituents, along with eight known compounds, from the

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Table 1. ^{1}H NMR Spectroscopic Data of 1, 2, and 3 (in Methanol-d_4)[a]

position	1	2	3
2			
3	6.60 br s	6.61 br s	
4			
5			
6	7.46 d (2.5)	7.40 d (2.5)	7.31 d (3.0)
7			
8	6.80 d (2.5)	6.79 d (2.5)	6.80 d (3.9)
9			
10			
1′			
2′	6.71 br s	6.69 br s	7.03 d (7.8)
3′			7.44 d (7.8)
4′			
5′	7.48 d (8.4)	7.41 d (7.8)	7.10 d (8.4)
6′	6.91 d (8.4)	6.90 d (7.8)	6.92 d (8.4)
1a	4.40 d (7.3)	5.28 d (4.5)	4.32 d (4.2)
2a	4.06 dd (7.3, 6.5)	4.21 dd (4.5, 6.3)	4.16 dd (4.2, 6.3)
3a	3.81 m	3.57 m	3.52 m
4a	3.52 m	3.46 m	3.30 m
5a	3.86 br s	3.83 d (6.2), 3.81 d (6.3)	3.64 d (6.0), 3.67 d (5.8)
1b	4.39 d (7.5)	5.15 d (5.6)	5.84 d (4.8)
2b	4.33 dd (7.5, 5.8)	4.23 dd (5.6, 6.1)	4.45 dd (4.8, 6.9)
3b	3.79 m	3.52 m	3.35 m
4b	3.44 m	3.44 m	3.42 m
5b	3.65 d (6.2), 3.69 d (6.5)	3.70 d (6.8), 3.68 d (6.8)	3.74 d (6.0), 3.72 d (7.8)
1c	4.33 d (7.8)	5.05 d (5.2)	5.39 d (3.6)
2c	4.23 dd (7.8, 6.2)	4.01 dd (5.2, 5.8)	4.03 dd (3.6, 5.8)
3c	3.56 m	3.50 m	3.99 m
4c	3.38 m	3.41 m	4.36 m
5c	3.62 d (9.0), 3.60 d (9.0)	3.65 d (6.5), 3.63 d (6.7)	3.81 d (7.1), 3.78 d (7.2)
1d		4.73 d (4.1)	5.95 d (6.6)
2d		4.44 dd (4.1, 6.2)	4.73 dd (6.6, 5.4)
3d		3.47 m	4.09 m
4d		3.39 m	4.25 m
5d		3.61 d (6.6), 3.59 d (6.6)	3.88 d (7.2), 3.84 d (6.2)
1″			
2″	2.31 t (7.2)	2.33 t (7.2)	2.23 t (7.2)
3″−17″	1.59−1.14	2.24−1.15 m	
3″−15″			1.61−1.26
18″	0.88 t (6.5)	0.88 t (6.3)	
16″			0.88 t (6.3)
7, 3′ (OMe)	3.91, 3.94	3.91 br s, 3.88 br s	

[a]Coupling constants in parentheses are given in hertz.

straw of *O. sativa*. All of the known compounds were identified by comparison with standards and literature data.[13,19] The bioactivities of the new isolated compounds were evaluated as natural algicides against the growth of a blue-green alga, *M. aeruginosa* UTEX 2388.

MATERIALS AND METHODS

General. Melting points were determined using a model IA9100 melting point apparatus (Electrochemical Engineering, Seoul, South Korea). Optical rotations were measured with a model AA-10 polarimeter (Instrument Ltd., Seoul, South Korea). UV spectra were measured with a TU-1800$_{PC}$ UV−vis spectrophotometer. IR spectra were recorded on a Thermo Scientific FT-IR model Nicolet 6700 (USA) spectrophotometer at the Korea Institute of Science and Technology (KIST) Seoul, South Korea. ^{1}H and ^{13}C NMR spectra were obtained at 600 and 150 MHz, respectively, using a Bruker Avance-600 spectrometer, available at the National Instrumentation Center for Environmental Management (NICEM), College of Agriculture and Life Science, Seoul National University (SNU), Seoul, South Korea. NMR spectra were obtained in deuterated chloroform, pyridine-d_5, and methanol-d_4 using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in parts per million (δ) and coupling constants (J) in hertz. High-resolution ESI/FT mass spectra were recorded on a Thermo-Finnigan LTQ-Orbitrap instrument (Thermo Scientific, USA) equipped with a Dionex U 3000 HPLC system (NICEM, Seoul National University). All chemicals were of analytical grade. *n*-Hexane, ethyl acetate, methanol, ethanol, sulfuric acid, and vanillin were purchased from Daejung Chemicals and Metals (Seoul, South Korea). Thin-layer chromatography was performed on precoated silica gel 60 F_{254} plates (Merck). Visualization of the TLC plates was performed using 5% H_2SO_4 in ethanol spray reagent. Column chromatography was performed using silica gel (70−230 mesh) and LiChroprep RP-18 [40−63 μm; octadecyl silica (ODS) gel] from Merck. Authentic

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standards of β-sitosterol, stearic acid, palmitic acid, oleic acid, and D-xylose were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Materials. The rice (*O. sativa* L.) straw used in the present study was collected after harvesting of rice cereal at the Konkuk University experimental farm, Yeoju, South Korea, in October 2010. The collected samples were dried in the laboratory in the temperature range of 25−30 °C for 3 weeks with some modifications of a previous study.[15] A voucher specimen (reference code ILPUM variety) has been dried and deposited in the herbarium of the Department of Applied Life Science, Konkuk University.

Extraction of Rice Straw. Dried *O. sativa* straw (10 kg) was immersed in methanol (55 L) for 1 week at room temperature, and then the supernatant was concentrated under vacuum to yield 78 g of extract. This material was suspended in water and extracted with hexane, ethyl acetate, and *n*-butanol successively and evaporated to produce hexane (9.4 g), ethyl acetate (11.2 g), and *n*-butanol (14.2 g) extracts.

Isolation of Compounds from Hexane Extract. The hexane extract (9.4 g) was column chromatographed (CC) over silica gel (70−230 mesh, 200 g, 800 mm × 25 mm) with hexane and ethyl acetate solvents and initially yielded three known compounds, *n*-octacos-9-enyl propionate (11 mg), 1-tetratriacontanol (16 mg), and tritriacontane-4,12-diene (7 mg).

Isolation of Compounds from Ethyl Acetate Extract. The EtOAc extract (11.2 g) was subjected to normal-phase CC over silica gel (70−230 mesh, 400 g, 900 mm × 45 mm), yielding 30 fractions (each fraction 200 mL), with the following eluants: fraction 1 in hexane, fractions 2−5 in hexane/EtOAc (9:1), fractions 6−11 in hexane/EtOAc (8:2), fractions 12−15 in hexane/EtOAc (7:3), fractions 16−20 in hexane/EtOAc (1:1), fractions 21 and 22 in EtOAc, fractions 23−26 in EtOAc/MeOH (9.5:0.5), and fractions 27−30 in EtOAc/MeOH (9:1). Fraction 6 was crystallized and, after purification by CC, yielded β-sitosterol (23 mg). This was confirmed by comparison with an authentic sample from Sigma and a previously isolated compound. Fraction 11, which was further purified by CC over silica gel with methylene dichloride and methanol, produced two pure compounds: *n*-tetracontan-15α-ol (6 mg) and *n*-tritetracontan-5α-ol (9 mg). Fractions 23−26, after CC over silica gel and Lichroprep RP-18 (ODS), yielded one white compound in powder form. This was identified as gallic acid (13 mg). Fraction 27, after rechromatography on a silica gel column with chloroform and methanol, yielded one pure compound: β-sitosterol-3-*O*-β-D-glucoside (12 mg).

Isolation of Compounds from *n*-Butanol Extract. The entire *n*-butanol extract (14.2 g) was subjected to normal-phase CC over silica gel (70−230 mesh, 800 g, 1200 mm × 35 mm), yielding 32 fractions (each fraction 250 mL), with the following eluants: fractions 1 and 2 with $CHCl_3$, fractions 3−5 with $CHCl_3$/MeOH (99:1), fractions 6−11 with $CHCl_3$/MeOH (98:2), fractions 12−15 with $CHCl_3$/MeOH (97:3), fractions 16−20 with $CHCl_3$/MeOH (96:4), fractions 21−24 with $CHCl_3$/MeOH (9.5:0.5), fractions 25−28 with $CHCl_3$/MeOH (9:1), fractions 29 and 30 with $CHCl_3$/MeOH (8.8:1.2), and fractions 31 and 32 with $CHCl_3$/MeOH (8.5:1.5). All fractions were examined by TLC. Fractions 21−24 were combined and rechromatographed with CH_2Cl_2/MeOH mixtures (9.9:01, 9.8:0.2, 9.7:0.3, 9.6:0.4, 9.5:0.5, 9.4:0.6, 9.3:0.7, 9.2:0.8, 9.1:0.9, and 9:1) to obtain 10 fractions. Fractions 6−8 (4.2 g) were combined and rechromatographed over Lichroprep RP-18 (40−63 μm; ODS gel, 80 g, 400 mm × 15 mm, each fraction 50 mL), eluting sequentially with methanol containing 80, 60, 40 20, 10, and 0% water to yield three compounds: **1** (34 mg, 0.00034%), **2** (28 mg, 0.00028%), and **3** (22 mg, 0.0022%). Fractions 9 and 10 (3.8 g) were combined and rechromatographed over Lichroprep RP-18 (40−63 μm, ODS gel, 60 g, 300 mm × 15 mm, each fraction 50 mL), eluting sequentially with methanol containing 80, 60, 40, 20, 10, and 0% water to yield two compounds: **4** (29 mg, 0.00029%) and **5** (27 mg, 0.00027%).

*5,4′-Dihydroxy-7,3′-dimethoxyflavone-4′-O-β-D-xylopyranosyl-(2a→1b)-2a-O-β-D-xylopyranosyl-(2b→1c)-2b-O-β-D-xylopyranosyl-2c-octadecanoate (**1**):* yellow solid; R_f 0.45 ($CHCl_3$/MeOH; 9:1); mp 214−216 °C; $[\alpha]_D^{22}$ −53.2 (*c* 0.1, MeOH); UV (MeOH), λ_{max} 265, 308, 342 nm; IR (KBr), ν_{max} 3455, 3363, 3261, 2926, 2841, 1721, 1690, 1604, 1513, 1431, 1346, 1265, 1176, 1070, 1027, 839 cm^{-1}; ESI/MS, *m*/*z* (relative intensity) 977 $[M + H]^+$ ($C_{50}H_{73}O_{19}$) (5.1), 663 (11.3), 577 (4.5), 531 (6.8), 399 (11.5), 313 (9.1), 267 (24.8), 166 (43.2); HR-ESI/FTMS, *m*/*z* 977.4759 (calcd for $C_{50}H_{73}O_{19}$, 977.4746). For ^1H and ^{13}C NMR data, see Tables 1 and 2.

Table 2. ^{13}C NMR Spectroscopic Data of 1, 2, and 3 (in Methanol-d_4)

position	1	2	3
2	159.32	158.95	157.67
3	103.41	104.93	136.25
4	184.12	184.04	177.44
5	163.02	164.88	161.31
6	110.74	110.68	112.40
7	166.31	166.48	162.57
8	105.66	105.40	95.78
9	161.93	162.99	153.63
10	110.39	111.67	107.80
1′	122.01	123.45	121.13
2′	116.28	116.94	120.18
3′	149.63	149.69	119.06
4′	147.52	147.12	142.15
5′	123.50	129.67	123.35
6′	117.88	117.21	117.24
1a	104.99	102.25	102.77
2a	78.24	78.62	88.29
3a	77.93	77.99	75.38
4a	72.24	72.51	71.06
5a	63.45	63.47	63.59
1b	104.65	100.12	101.29
2b	80.57	80.57	82.77
3b	75.28	77.39	75.62
4b	71.75	72.33	71.35
5b	62.81	62.81	62.80
1c	102.34	101.79	93.77
2c	82.73	79.22	78.20
3c	75.06	74.53	75.20
4c	71.45	71.75	69.71
5c	62.63	62.64	64.53
1d		101.36	91.33
2d		82.57	78.01
3d		74.19	72.77
4d		71.43	73.98
5d		62.07	62.63
1″	172.26	166.48	170.31
2″	35.92	35.53	35.50
3″−17″	35.53−21.99	30.89−22.10	
3″−15″			32.39−22.39
18″	14.35	18.06	
16″			14.57
7, 3′ (OMe)	56.84, 56.50	57.20, 56.82	

*5,4′-Dihydroxy-7,3′-dimethoxyflavone-4′-O-α-D-xylopyranosyl-(2a→1b)-2a-O-α-D-xylopyranosyl-(2b→1c)-2b-O-α-D-xylopyranosyl-(2c→1d)-2c-O-α-D-xylopyranosyl-2d-octadecanoate (**2**):* yellow solid; R_f 0.42 ($CHCl_3$/MeOH; 9:1); mp 224−225 °C; $[\alpha]_D^{22}$ −47.8 (*c* 0.1, MeOH); UV (MeOH), λ_{max} 261, 304, 338 nm; IR (KBr), ν_{max} 3447, 3379, 3283, 2993, 2903, 2837, 1722, 1678, 1606, 1513, 1452, 1344, 1260, 1069, 838 cm^{-1}; ESI/MS, *m*/*z* (relative intensity) 1109 $[M + H]^+$ ($C_{55}H_{81}O_{23}$) (1.3), 399 (7.8), 313 (6.1), 267 (7.3), 166 (9.5); HR-ESI/FTMS, *m*/*z* 1109.5175 (calcd for $C_{55}H_{81}O_{23}$, 1109.5169). For ^1H and ^{13}C NMR data, see Tables 1 and 2.

Kaempferol-3-O-α-D-xylopyranosyl-(2a→1b)-2a-O-α-D-xylopyranosyl-(2b→1c)-2b-O-α-D-xylopyranosyl-(2c→1d)-2c-O-α-D-xylopyr-

Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data of 4 (in Methanol-d_4)[a]

position	^{1}H NMR	^{13}C NMR	position	^{1}H NMR	^{13}C NMR
1		142.82	1f	4.53 d (3.8)	98.23
2		166.28	2f	4.02 m	84.21
3	8.01 dd (7.2, 2.4)	137.34	3f	3.72 m	77.71
4	7.80 m	125.01	4f	3.58 m	71.45
5	6.79 m	138.23	5f	3.58 d (6.1)	62.86
6	7.24 dd (7.8, 2.7)	147.15	1g	4.46 d (4.6)	90.65
7		169.81	2g	4.03 m	84.68
1a	5.89 d (4.8)	90.65	3g	3.68 m	78.94
2a	4.09 m	82.56	4g	3.72 m	71.04
3a	3.88 m	74.91	5g	3.30 d (5.8)	65.77
4a	3.66 m	73.93	1h	4.40 d (3.6)	90.30
5a	3.69 br s	64.08	2h	4.17 m	86.54
1b	5.71 d (4.8)	102.92	3h	3.70 m	79.47
2b	4.07 m	83.38	4h	3.75 m	69.34
3b	3.90 m	78.09	5h	3.29 m	65.35
4b	3.65 m	73.24	1′		174.23
5b	3.67 br s	65.14	2′	2.68 m	41.17
1c	5.39 d (3.7)	94.01	3′	2.55 m	39.24
2c	4.03 m	83.25	4′	1.35 m	35.54
3c	3.87 m	74.40	5′	1.31 m	31.12
4c	3.60 m	73.01	6′	1.27 m	26.95
5c	3.65 d (6.5)	64.46	7′	2.46 m	39.40
1d	5.12 d (7.3)	93.75	8′		181.37
2d	4.01 m	83.49	9′	1.13 d (6.4)	25.90
3d	3.85 m	75.83	10′		178.93
4d	3.56	72.38	OMe	3.78 br s	54.87
5d	3.52 br s	64.61			
1e	4.75 d (4.8)	103.23			
2e	3.95 m	83.75			
3e	3.82 m	76.79			
4e	3.54 m	71.76			
5e	3.57 d (6.6)	64.88			

[a]Coupling constants in parentheses are given in hertz.

*anosyl-2d-hexadecanoate (**3**):* yellow gum; R_f 0.37 ($CHCl_3$/MeOH; 9:1); $[\alpha]_D^{22}$ −42.1 (*c* 0.1, MeOH); UV (MeOH), λ_{max} 267, 311, 348, nm (log 4.2, 2.8, 2.1); IR (KBr), ν_{max} 3437, 3329, 3265, 2914, 2849, 1722, 1708, 1647, 1601, 1514, 1451, 1376, 1217, 1037, 841 cm^{-1}; ESI/MS, *m*/*z* 1053 $[M + H]^+$ ($C_{51}H_{73}O_{23}$) (1.3), 371 (26.5), 285 (17.1), 269 (13.4), 239 (33.6), 166 (5.8); HR-ESI/FTMS, *m*/*z* 1053.4551 (calcd for $C_{51}H_{73}O_{23}$, 1053.4542). For ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

*Methyl salicylate-2-O-α-D-xylopyranosyl-(2a→1b)-2a-O-α-D-xylopyranosyl-(2b→1c)-2b-O-α-D-xylopyranosyl-(2c→1d)-2c-O-α-D-xylopyranosyl-(2d→1e)-2d-O-α-D-xylopyranosyl-(2e→1f)-2e-O-α-D-xylopyranosyl-(2f→1g)-2f-O-α-D-xylopyranosyl-(2g→1h)-2g-O-α-D-xylopyranosyl-2h-geranilan-8′,10′-dioic acid-1′-oate (**4**):* light brown solid; mp 234−235 °C; R_f 0.27 ($CHCl_3$/MeOH; 9:1); $[\alpha]_D^{22}$ −89.1 (*c* 0.1, MeOH); IR (KBr), ν_{max} 3516, 3425, 3379, 3281, 1732, 1721, 1698, 1635, 1512, 1433, 1345, 1210, 1025 cm^{-1}; ESI/MS, *m*/*z* 1423 $[M + H]^+$ ($C_{58}H_{87}O_{40}$) (2.3), 743 (2.8), 611 (5.6), 283 (3.7), 232 (100), 215 (16.1), 151 (14.8); HR-ESI/FTMS, *m*/*z* 1423.4779 (calcd for $C_{58}H_{87}O_{40}$, 1423.4772). For ^{1}H and ^{13}C NMR data, see Table 3 (D-xylose; R_f 0.27 (*n*-butanol/water/acetic acid; 4:1:5); $[\alpha]^{20}_D$ +18.8° (*c* 2, water).

*Oleioyl-β-D-arabinoside (**5**):* light yellow semisolid; $[\alpha]_D^{22}$ −19.1 (*c* 0.1, MeOH); IR (KBr), ν_{max} 3415, 3345, 2905, 2843, 1721, 1650, 1451, 1374, 1213, 1038, 838 cm^{-1}; ^{1}H NMR (MeOD; 600 MHz), δ 5.37 (1H, m, H-9), 5.28 (1H, m, H-10), 4.43 (1H, d, *J* = 7.5 Hz, H-1′), 4.12 (1H, m, H-2′), 3.89 (1H, m, H-3′), 3.74 (1H, m, H-4′), 3.63 (2H, br s, H_2-5′), 2.32 (2H, t, *J* = 7.2 Hz, H_2-2), 2.26 (2H, m, H_2-11), 2.08 (2H, m, H_2-8), 1.59 (2H, m, CH_2), 1.31 (2H, m, CH_2),1.27 (18H, br s, 9 × CH_2), 0.86 (3H, t, *J* = 6.0 Hz, Me); ^{13}C NMR (MeOD, 150 MHz), δ 175.26 (C-1), 131.03 (C-9), 129.25 (C-10), 105.48 (C-1′), 71.29 (C-2′), 70.01 (C-3′), 66.37 (C-4′), 64.21 (C-5′), 35.07 (C-2), 33.21 (CH_2), 32.80 (CH_2), 30.91 (3 × CH_2), 30.70 (4 × CH_2), 30.61 (CH_2), 30.35 (CH_2), 29.28 (CH_2), 28.30 (CH_2), 26.67 (CH_2), 26.13 (CH_2), 23.87 (CH_2), 14.58 (Me-18); ESI/MS, *m*/*z* 415 $[M + H]^+$ ($C_{23}H_{43}O_6$); HR-ESI/FTMS, *m*/*z* 415.3067 (calcd for $C_{23}H_{43}O_6$, 415.3060).

Acid Hydrolysis of 1−4. Compounds **1**−**4** (10 mg each) were heated at 70−80 °C with diluted 2 M hydrochloric acid (2.5 mL) in 70% aqueous ethanol (3 mL) for 30 min. The reaction mixture was dried under vacuum, neutralized with aqueous sodium bicarbonate, and extracted with chloroform (3 × 5 mL) to separate the flavonoid moiety. The chloroform extract was washed with water (3 × 5 mL), dried over anhydrous sodium sulfate, and evaporated to produce aglycone moieties. The mother liquor, after separation of the flavonoid, was treated with dilute 2 M hydrochloric acid to liberate the free fatty acids and was re-extracted with chloroform (3 × 5 mL) to obtain the fatty acids. The presence of D-xylose in the aqueous solution was determined by co-TLC.

Acid Hydrolysis of 5. Compound **5** (5 mg) was refluxed with 2 mL of 1 M hydrochloric acid/dioxane (1:1, v/v) in a water bath for 4 h. The reaction mixture was evaporated to dryness and partitioned four times between chloroform and water. The chloroform extract was concentrated and contained the aglycone portion, whereas the water extract contained D-arabinose (co-TLC with authentic sample).

Biological Assays for Algicidal Activity. The bioactivities of the five compounds identified in the present study were assessed using previously reported methods.[16,20] Briefly, the blue-green alga *M. aeruginosa* UTEX 2388 was obtained from the Biological Resource

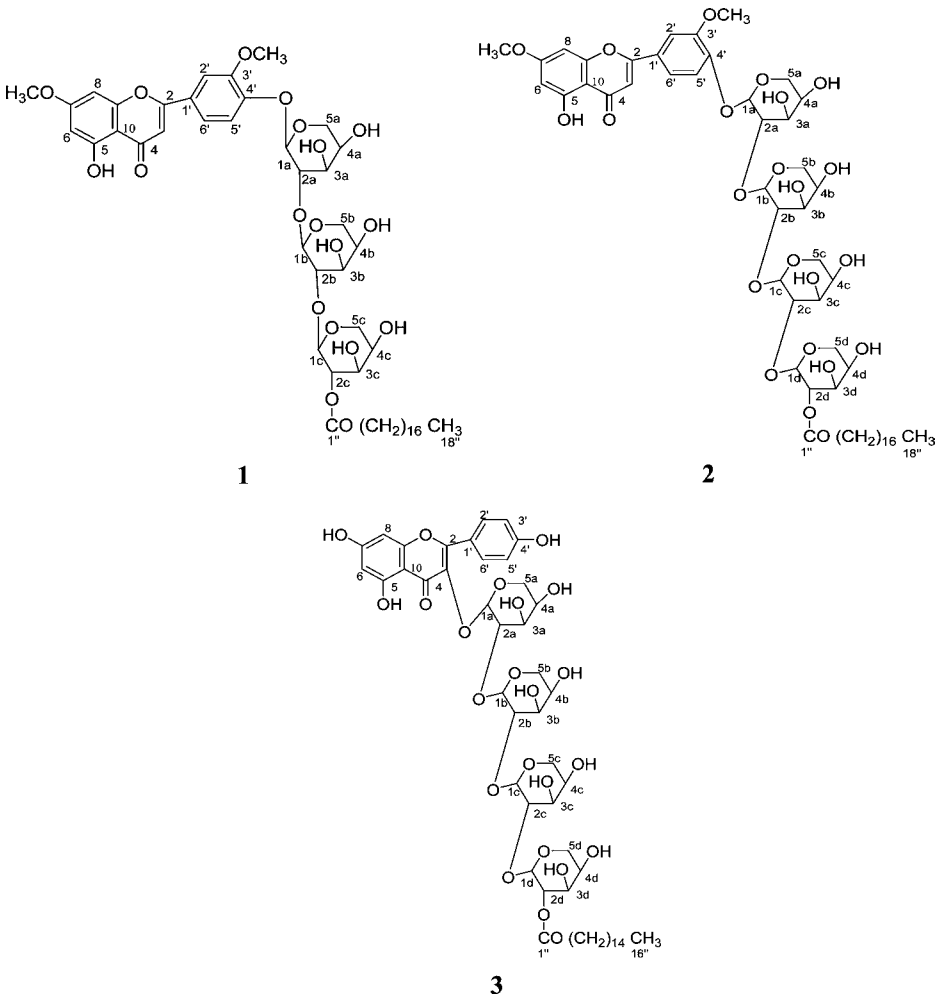

Figure 1. Chemical structures of compounds **1**−**3**.

Center (Korea Research Institute of Biological and Biotechnology, Daejeon, Korea). The cells were transferred weekly to new media and maintained at 25 °C in Allen[21] medium. To investigate the sensitivity of the algae, compounds **1**−**5** were diluted in DMSO, filtered through a 0.2 μm filter (Satorius, Göttingen, Germany), and added to sterilized Allen medium at final concentrations of 1, 10, and 100 mg/L. After inoculation of the test alga, the cultures were propagated at 25 °C for a 10 h dark/14 h light photoperiod (light intensity = 60 μmol/m^2 s). The algicidal activity against *M. aeruginosa* was evaluated for 6 days after addition of the test compounds. At the end of the incubation period, the absorbance at 670 nm of an aliquot of each liquid culture was measured using a LabSystems (Helsinki, Finland) type 352 microplate reader. The values were transformed into dry mass using previously determined equations. The flasks were arranged as a randomized complete block with triplicates per treatment.

Statistical Analysis. Statistical analyses were performed using the general linear model of the statistical analysis program SAS (version 9.2; SAS Institute Inc., Cary, NC, USA).[22] The experimental design was completely randomized with triplicates, and all data were represented as the mean with the standard deviation (±SD). The least significant difference (LSD) test was performed for a 0.05 probability level.

RESULTS AND DISCUSSION

Compound **1** was obtained as a pale yellow amorphous solid from $CHCl_3$/MeOH (9.5:0.5, v/v) eluants (Figure 1). It tested positive with the ferric chloride test for a phenolic moiety. The UV absorption maxima, 272, 313, and 330 nm, were typical for a flavonoid.[23−25] A one-proton singlet resonance at δ 6.60 and the corresponding upfield vinylic carbon signal at δ 103.41 (C-3) suggested a flavone skeleton.[26,27] On addition of sodium acetate, there was no UV absorption band shift, suggesting a bound C-7 hydroxyl group. A bathochromic shift of 40 nm on addition of $AlCl_3$ and $AlCl_3$/HCl indicated the presence of a chelated hydroxyl group at C-5.[26] The IR spectrum displayed absorption bands for hydroxyl groups (3455, 3363, 3261 cm^{-1}), an ester function (1721 cm^{-1}), carbonyl groups (1690 cm^{-1}), and aromatic rings (1604, 1513 cm^{-1}). On the basis of the ^{13}C NMR and mass spectra, the molecular ion peak of **1** was assigned as m/z 977 and was consistent with the molecular formula of a flavonol triglycosidic ester $C_{50}H_{73}O_{19}$. High-resolution ESI/FTMS provided the exact mass of the protonated molecular ion, which was consistent with this molecular formula.

The ^{1}H NMR spectrum of **1** indicated a flavonoid moiety, as it displayed two one-proton meta-coupled doublets at δ 7.46 (J = 2.5 Hz) and 6.80 (J = 2.5 Hz) and three one-proton signals as a singlet at δ 6.71 and ortho-coupled doublets at δ 7.48 (J = 8.4 Hz) and 6.91 (J = 8.4 Hz), which indicated an AX system for ring A and an ABX system for ring B, and were assigned to H-6, H-8, H-2′, H-5′ and H-6′ protons, suggesting a 3′- and 4′-oxygenated substitution pattern in ring B. The sugar units in **1** were identified as β-D-xylopyranose by analysis of the coupling constants of the anomeric signals of sugar protons as one-

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proton doublets at δ 4.40 (J = 7.3 Hz, H-1a), 4.39 (J = 7.5 Hz, H-1b), and 4.33 (J = 7.8 Hz, H-1c). The remaining sugar protons appeared as multiplets at δ 4.33−3.38 for H-3a, -3b, -3c and H-4a, -4b, -4c. The methylene protons in sugars appeared as double doublets 3.84, 3.88 (J = 6.2, 57.2), 3.65, 3.69 (J = 6.2, 6.5), and 3.62, 3.60 (J = 7.1, 7.2). A three-proton triplet at δ 0.88 (J = 6.5 Hz) and two three-proton broad singlets at δ 3.94 and 3.91 correlated with a primary C-18″ methyl and two methoxy protons, respectively. The ^{13}C NMR spectrum of **1** showed signals for a C-4 flavone carbonyl carbon at δ 184.12; other flavone carbon signals were observed between δ 166.31 and 103.41, anomeric carbons at 104.99 (C-1a), 104.65 (C-1b), and 102.34 (C-1c), ester carbon at δ 172.26 (C-1″), fatty acid methyl carbon at δ 14.35 (C-18″), methoxy carbons at δ 56.84 and 56.50, and other sugar carbons between δ 82.73 and 62.63. The ^{1}H and ^{13}C NMR values of ester and flavone moieties were compared with those described for similar compounds in the literature.[19,25,28] Furthermore, the ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) exhibited the presence of a β-xylopyranosyl moiety ($J_{1'',2''}$ = 7.3, 7.5, and 7.8 Hz) for anomeric protons. The appearance of the C-4′ signal at δ 147.52 in the ^{13}C NMR spectrum supported the attachment of the sugar moiety at this carbon.[29] The presence of ^{1}H NMR signals in the deshielded region at δ 4.06 (H-2a), 4.33 (H-2b), and 4.23 (H-2c) and the respective carbon signals at δ 78.24 (C-2a), 80.57 (C-2b), and 82.73 (C-2c) suggested (2→1) linkage of the sugar units.

The ^{1}H−^{1}H COSY spectrum of **1** showed correlations of H-6 with H-8 and OMe (ring A); H-2′ with OMe (ring B); H-5′ with H-6′; H-2a with H-1a, H-3a, and H-1b; H-2b with H-1b and H-1c; and H_3-18″ with H-17″. The HMBC spectrum of **1** exhibited interactions of H-6, H-8, and OMe with C-7; H-3 with C-4; H-5′, H-6′, and H-1a with C-4′; H-1a, H-3b, and H-1b with C-2a; and H-2c and H_2-2″ with C-1″, confirming (2→1) linkages of the sugar units, attachment of the sugar chain at C-4′, and an ester group at C-2c. The HSQC correlations were used to assign all protons and carbon atoms in the molecule; some common correlations are H-1a with C-1a;, H-1b with C-1b, and H-1c with C-1c. The ^{1}H and ^{13}C NMR spectroscopic data of the flavone unit were compared with the reported data for such molecules.[30]

Acid hydrolysis of **1** yielded flavones, D-xylose, and stearic acid; these were identified by co-TLC. On the basis of the analysis of the spectroscopic data and the chemical reactions, the structure of **1** was formulated as 5,4′-dihydroxy-7,3′-dimethoxyflavone-4′-*O*-β-D-xylopyranosyl-(2a→1b)-2a-*O*-β-D-xylopyranosyl-(2b→1c)-2b-*O*-β-D-xylopyranosyl-2c-octadecanoate, which is a new flavone glycoside.

Compound **2** was obtained as a yellow solid mass from $CHCl_3$/MeOH (9.5:0.5, v/v) eluants (Figure 1). It tested positive in the ferric chloride test for a phenolic group. The UV absorption maxima, at 270, 309, and 331 nm, were typical of a flavonoid.[23−25] A one-proton singlet resonance at δ 6.61 and the corresponding upfield vinylic carbon signal at δ 104.93 (C-3) suggested a flavone skeleton.[26,31] Addition of sodium acetate did not show any shift in the UV absorption band shift, suggesting a bound C-7 hydroxyl group. A bathochromic shift of 40 nm on addition of $AlCl_3$ and $AlCl_3$/HCl indicated the presence of a chelated hydroxyl group at C-5.[26] The IR spectrum displayed absorption bands for hydroxyl groups (3447, 3379, 3283 cm^{-1}), an ester function (1722 cm^{-1}), a carbonyl group (1678 cm^{-1}), aromatic rings (1606, 1513 cm^{-1}), and a long aliphatic chain (838 cm^{-1}). On the basis of ^{13}C NMR and mass spectra, the molecular ion peak of **2** was assigned as m/z 1109, consistent with the molecular formula of a flavonol tetraglycosidic ester $C_{55}H_{81}O_{23}$. High-resolution ESI/FTMS provided the exact mass of the protonated molecular ion, which was consistent with this molecular formula.

The ^{1}H NMR spectrum of **2** indicated a flavonoid moiety, as it displayed two meta-coupled doublets at δ 7.40 (J = 2.5) and 6.79 (J = 2.5) and three one-proton signals as a singlet at δ 7.41 (J = 7.8) and as ortho-coupled doublets at δ 7.41 (J = 7.8) and 6.90 (J = 7.8), indicating an AX system for ring A and a ABX system for ring B and which were assigned to H-6, H-8, H-2′, H-5′, and H-6′ protons, suggesting a 3′- and 4′-oxygenated substitution pattern in ring B. The sugar units in **2** were identified as α-D-xylopyranose by analysis of the coupling constants of the anomeric signals of sugar protons as one-proton doublets at δ 5.28 (J = 4.5 Hz, H-1a), 5.15 (J = 4.7 Hz, H-1b), 5.05 (J = 4.3, H-1c), and 4.73 (J = 4.1 Hz, H-1d), respectively. The remaining sugar protons appeared as multiplets at δ 4.44−3.39 for H-3a, -3b, -3c, -3d and H-4a, -4b, -4c, -4d. The methylene protons in sugars appeared as double doublets at δ 3.83, 3.81 (J = 6.2, 3.3), 3.70, 3.68 (J = 6.8, 6.8), 3.65, 3.63 (J = 6.5, 6.7), and 3.61, 3.59 (J = 6.6, 6.6). A three-proton triplet at δ 0.88 (J = 6.3 Hz) and two methoxy proton broad singlets at δ 3.91 and 3.88 were attributed to primary C-18″ methyl, C-7, and C-3′ protons, respectively. The ^{13}C NMR spectrum of **1** showed signals for a C-4 flavone carbonyl carbon at δ 184.04; other flavone carbons resonated between δ 166.48 and 104.93, with anomeric carbons at δ 102.25 (C-1a), 100.12 (C-1b), 101.79 (C-1c), and 101.36 (C-1d), ester carbon at δ 166.48 (C-1″), fatty acid methyl carbon at δ 18.06 (C-18″), methoxy carbons at δ 57.20 and 56.82, and other sugar carbons between δ 82.57 and 62.07. The presence of the ^{1}H NMR signals in the deshielded region at δ 4.21 (H-2a), 4.23 (H-2b), 4.01 (H-2c), and 4.41 (C-1d), and the corresponding carbon signals between δ 82.57 and 77.39, suggested (2→1) linkage of the sugar units, and confirmed attachment of the ester group at C-2d. The ^{1}H and ^{13}C NMR values of the ester and flavone moieties were compared with those reported in the literature for similar compounds.[19,26,32] Furthermore, the ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) exhibited the presence of an α-xylopyranosyl moiety ($J_{1'',2''}$ = 4.5, 4.7, 4.3, and 4.1 Hz, for anomeric protons). The appearance of the C-4′ signal at δ 147.12 in the ^{13}C NMR spectrum supported the attachment of the sugar moiety at this carbon.[29]

The ^{1}H−^{1}H COSY spectrum of **2** showed correlation of H-6 with H-8 and OMe and of H-2′ with H-6′ and OMe functions at C-7 and C-3′. The ^{1}H−^{1}H COSY interactions of H-2a with H-1a, H-3a, and H-1b; of H-2b with H-1a and H-1c; and of H-2c with H-1c and H-4d indicated (2→1) linkages of the sugar units; H_3-18″ was linked with H-17″. The HMBC interactions of H-5′, OMe, H-2′, and H-1a with C-4, H-2a, H-2b, and H-3b with C-1b; H-2c with C-1d and H-2d; and H_2-2″ with C-1″ confirmed the attachment of the sugar unit at C-4′ and an ester function at C-2d; H_3-18″ was coupled with C-17″ and C-16″. The HSQC correlations were used to assign all of the protons and carbons in the molecule, and some important correlations are H-1a with C-1a, H-1b with C-1b, H-1c with C-1c, and H-1d with C-1d. The ^{1}H and ^{13}C NMR spectroscopic data of the flavone unit were compared with the reported values of these compounds.[30]

Acid hydrolysis of **2** yielded flavones, D-xylose, and stearic acid, confirmed by comparison using co-TLC. On the basis of

the analysis of the spectroscopic data and the chemical reactions, the structure of **2** was established as 5,4′-dihydroxy-7,3′-dimethoxyflavone-4′-*O*-α-D-xylopyranosyl-(2a→1b)-2a-*O*-α-D-xylopyranosyl-(2b→1c)-2b-*O*-α-D-xylopyranosyl-(2c→1d)-2c-*O*-α-D-xylopyranosyl-2d-octadecanoate, which is a new flavone glycoside.

Compound **3** was obtained as a yellow gum mass from $CHCl_3$/MeOH (9.5:0.5, v/v) eluants (Figure 1). It tested positive in the ferric chloride test for a phenolic moiety. The UV spectrum of **3** showed absorption maxima typical of a flavonoid, at 267, 311, and 348 nm.[23,24] The characteristic UV absorption maxima were in agreement with those in the literature.[26] The IR spectrum showed characteristic absorption bands for hydroxyl groups (3437, 3329, 3265 cm^{-1}), an ester function (1722 cm^{-1}), a keto group (1708 cm^{-1}), and aromatic rings (1601, 1514, 1037 cm^{-1}). On the basis of ^{13}C NMR and ESI/MS spectra, the molecular ion peak of **3** was assigned as m/z 1053, consistent with the molecular formula of a flavonol tetraglycosidic ester $C_{51}H_{73}O_{23}$. High-resolution ESI/FTMS provided the exact mass of the protonated molecular ion, which was consistent with this molecular formula.

The ^1H NMR spectrum of **3** indicated a flavonoid moiety, as it displayed two meta-coupled doublets at δ 7.31 (J = 3.0) and 6.80 (J = 3.9), ascribed to H-6 and H-8 in a 5,7-oxygenated ring. Four ortho-coupled doublets, at 7.03 (J = 7.8), 7.44 (J = 7.8), 7.10 (J = 8.4), and 6.92 (J = 8.4), were assigned to H-2′, H-3′, H-5′, and H-6′, suggesting a 4′-oxygenated substitution pattern in ring B; a meta-coupled AX system corresponding to the H-6 and H-8 protons in ring A and ortho-coupled protons characteristic of an AA′XX′ spin system of a para-substituted phenyl ring were found for ring B. The sugar units in **3** were identified as α-D-xylopyranose by analysis of the coupling constant of anomeric signals of sugar protons as one-proton doublets at δ 4.32 (J = 4.2 Hz, H-1a), 5.84 (J = 4.8 Hz, H-1b), 5.39 (J = 3.6 Hz, H-1c), and 5.95 (J = 4.6 Hz, H-1d). The remaining sugar protons appeared as multiplets at δ 4.73−3.30 for H-3a, -3b, -3c, -3d and H-4a, -4b, -4c, -4d. The methylene protons in sugars appeared as double doublets δ 3.64, 3.67 (J = 6.0, 5.8), 3.72, 3.74 (J = 6.0, 7.8), 3.78, 3.81 (J = 7.1, 7.2), and 3.84, 3.88 (J = 6.2, 7.2). A three-proton triplet at δ 0.83 (J = 6.3 Hz) was attributed to methylene H_2-2′ protons adjacent to the ester function of the C-16″ primary methyl protons. The ^{13}C NMR spectrum of **3** showed signals for a C-4 flavone carbonyl carbon at δ 177.44; other flavone carbons resonated between δ 162.57 and 95.78, anomeric carbons at δ 102.77 (C-1a), 101.29 (C-1b), 93.77 (C-1c), and 91.33 (C-1d), ester carbon at δ 170.31 (C-1′), and fatty acid methyl carbon at δ 14.57 (C-16″). The ^1H and ^{13}C NMR spectroscopic data of the flavonol unit were compared with reported spectroscopic values for these compounds.[19,25,28,33−35] The appearance of the C-3 signal at δ 136.25 in the ^{13}C NMR spectrum supported the attachment of the sugar moiety at this carbon.[29,30,32] The presence of the sugar ^1H NMR signals of H-2a, H-2b, H-2c, and H-2d between δ 4.73 and 4.03, and the ^{13}C NMR signals at δ 88.29 (C-2a), 82.77 (C-2b), 78.20 (C-2c), and 78.01 (C-2d) in the deshielded region suggested (2→1) linkages of the sugar units. Furthermore, the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) exhibited the presence of an α-xylopyranosyl moiety ($J_{1'',2''}$ = 4.2, 4.8, 3.6, 4.6 Hz).

The ^1H−^1H COSY spectrum of **3** showed correlations of H-6 with H-8; H-2′ with H-3′ and H-6′; H-5′ with H-6′; H-2a with H-1a, H-3a, and H-1b; H-2b with H-1b and H-1c; H-1d with H-2c and H-2d; and H_3-16″ with H-15″ and H-14″. The HMBC spectrum of **3** exhibited interactions of H-1a with C-3 and C-2a; H-1b with C-2a; and H-2d with C-1d and C-1″, confirming (2→1) sugar units and attachment of the innermost sugar at C-3. The esterification of palmitic acid was at C-2d of the terminal sugar, as the proton signal at δ 4.73 (C-2d) interacted with the signal at δ 170.31 for the C-1″ ester carbon of palmitic acid. The HSQC correlations were used to assign all protons and carbon atoms with the corresponding units, and established possible links with other parts of the molecule; some important correlation in the HSQC spectrum are H-1a with C-1a; H-1b with C-1b, H-1c with C-1c; and H-1d with H-1c.

Acid hydrolysis of **3** yielded D-xylose, kaempferol, and palmitic acid, confirmed by co-TLC. The sugar was confirmed to be D-xylose from the sign of its optical rotation ($[\alpha]_D$ = +18.8°) and co-TLC with an authentic sample. On the basis of the analysis of the spectroscopic data and the chemical reactions, the structure of **3** was established as kaempferol-3-*O*-α-D-xylopyranosyl-(2a→1b)-*O*-α-D-xylopyranosyl-(2b→1c)-*O*-α-D-xylopyranosyl-(2c→1d)-*O*-α-D-xylopyranosyl-2d-hexadecanoate, which is a new flavonol glycoside.

Compound **4** (Figure 2), a methoxysalicylic octaxylosidic ester, was obtained as a light brown solid mass from $CHCl_3$/MeOH (9:1, v/v) eluants. It showed positive tests for glycosides and had IR absorption bands for hydroxyl groups (3516, 3425, 3379, 3281 cm^{-1}), ester functions (1732, 1721 cm^{-1}), a carboxylic group (1698 cm^{-1}), and aromatic rings (1635, 1512, 1025 cm^{-1}). On the basis of ^{13}C NMR and mass spectra, the molecular ion peak of **4** was assigned as m/z 1422, corresponding to a phenolic acid glycosidic ester $C_{58}H_{86}O_{40}$. High-resolution ESI/FTMS provided the exact mass of the

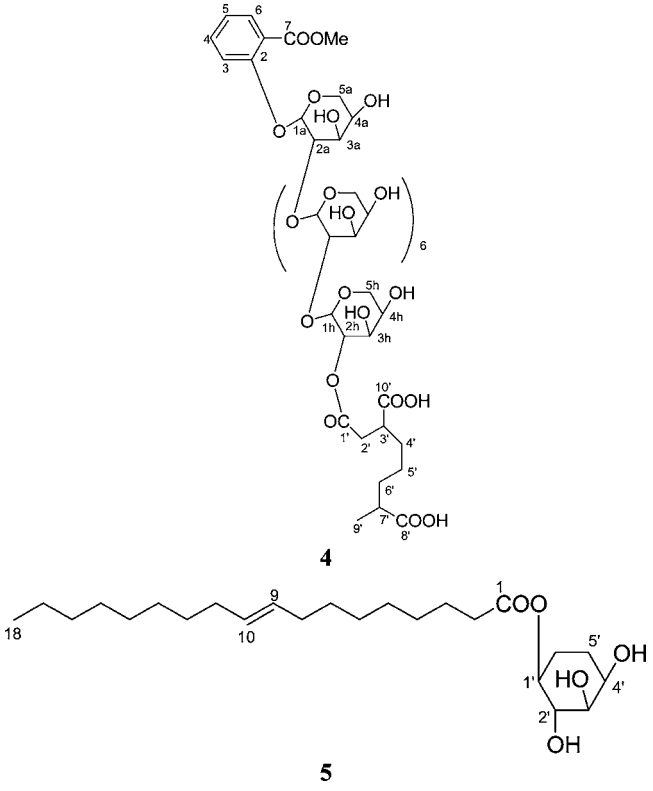

Figure 2. Chemical structures of compounds **4** and **5**.

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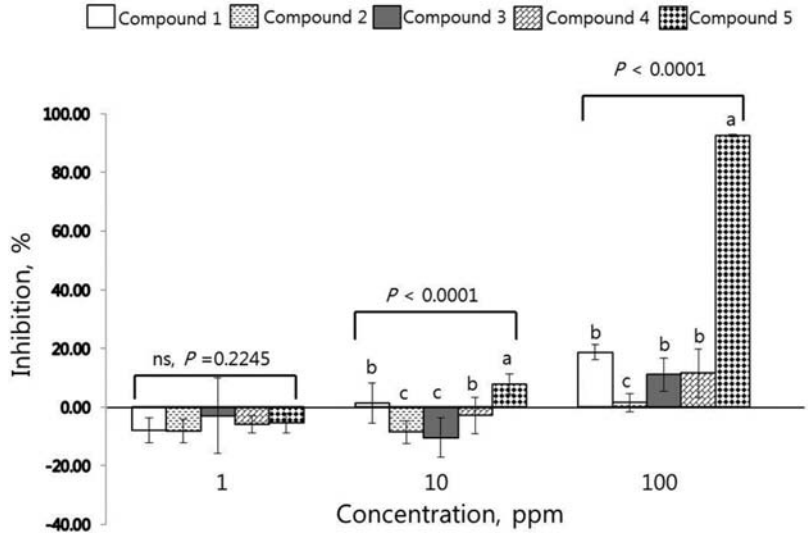

Figure 3. Inhibitory effects of compounds isolated from rice straw extracts on growth of blue-green alga, *Microcystis aeruginosa* UTEX 2386. Data represent the mean ± SD of triplicates. Net growth of algae during the test period was 1.57 mg DW/mL medium in untreated control.

protonated molecular ion, which was consistent with this molecular formula.

The ^{1}HNMR spectrum of **4** displayed two one-proton double doublets at δ 8.01 (J = 7.2, 2.4 Hz) and 7.24 (J = 7.8, 2.7 Hz), and two one-proton multiplets at δ 7.80 and 6.79, assigned to aromatic H-3, H-6, H-4, and H-5 protons, respectively. Eight one-proton doublets between δ 5.89 and 4.40, with coupling interactions from δ 3.6 to 6.5 Hz, were assigned to anomeric H-1a to H-1h protons in the L configuration. The other sugar protons appeared at δ 4.17−3.29. A three-proton broad singlet at δ 3.78 and a doublet at δ 1.13 d (J = 6.4 Hz) were attributed to methoxy and C-9′ secondary methyl protons, respectively. The methylene protons resonated from δ 2.68 to 1.27. The ^{13}C NMR spectrum of **4** exhibited signals for carboxylic carbons at δ 181.37 (C-8′) and 178.93 (C-10′), ester carbons at δ 169.81 (C-7) and 174.23 (C-1′), aromatic carbons between δ 166.28 and 125.01, a methoxy carbon at δ 54.87, anomeric carbons from δ 103.23 to 90.30, other sugar carbons in the range δ 86.54 to 62.86, and methine, methylene, and methyl carbons from δ 41.17 to 25.90. The presence of H-2a to H-2h signals in the deshielded region between δ 4.17 and 3.95 in the ^{1}H NMR spectrum and carbon signals from δ 86.54 to 82.56 in the ^{13}C NMR spectrum suggested (2 →1) linkages of the sugar units.

The ^{1}H−^{1}H COSY spectrum of **4** exhibited correlations of H-3 with H-4, H-5, and H-1a; H-2a with H-1a, H-3a, and H-1b; H-2h with H-1h, H-3h, and H_2-2′; and Me-9′ with H-7′ and H_2-6′. The HMBC spectrum of **4** displayed interactions of C-2 with H-3, H-4, and H-1a; C-2a with H-1a, H-3a, and H-1c; C-2h with H-1h, H-3h, and H-4h; C-1′ with H-2h, H_2-2′, and H-3′; and C-8′ with H-7′, Me-9′, and H_2-6′. HSQC measurements enabled full assignments of the ^{1}H and ^{13}C NMR chemical shifts of **4** to be made. Acid hydrolysis of **4** yielded D-xylose as the sugar component. On the basis of this evidence, the structure of **4** was assigned as methyl salicylate-2-*O*-*α*-D-xylopyranosyl-(2a→1b)-2a-*O*-*α*-D-xylopyranosyl-(2b→1c)-2b-*O*-*α*-D-xylopyranosyl-(2c→1d)-2c-*O*-*α*-D-xylopyranosyl-(2d→1e)-2d-*O*-*α*-D-xylopyranosyl-(2e→1f)-2e-*O*-*α*-D-xylopyranosyl-(2f→1g)-2f-*O*-*α*-D-xylopyranosyl-(2g→1h)-2g-*O*-*α*-D-xylopyranosyl-2h-geranilan-8′,10′-dioic acid-1′-oate.

Compound **5** was obtained as a light yellow semisolid mass from $CHCl_3$/MeOH (9.5:0.5, v/v) fractions. The IR spectrum showed characteristic absorption bands for hydroxyl groups (3415, 3345 cm^{-1}), an ester function (1721 cm^{-1}), and unsaturation (1650 cm^{-1}). On the basis of ^{13}C NMR and positive ESI/MS spectra, the molecular ion peak of **5** was assigned as m/z 415, consistent with the molecular formula of an oleioyl monoglycosidic ester $C_{23}H_{42}O_6$. High-resolution ESI/FTMS provided the exact mass of the protonated molecular ion, which was consistent with this molecular formula.

The ^{1}H NMR spectrum of **5** displayed two one-proton multiplets at δ 5.37 and 5.28, assigned to vinylic H-9 and H-10 protons, respectively, and a one-proton doublet at δ 4.43 (J = 7.5), assigned to H-1′. Three multiplets at δ 4.12, 3.89, and 3.74 were assigned to H-2′, H-3′, and H-4′, and one broad singlet at δ 3.63 was assigned to H-5′. A two-proton multiplet at δ 2.26 was assigned to oxygenated methylene, and other methylene proton signals were observed between δ 1.27 and 2.26. A three-proton triplet at δ 0.86 (J = 6.0 Hz) was assigned to primary C-18 methyl protons. The ^{13}C NMR spectrum of **5** displayed carbon signals for ester carbon at δ 175.26 (C-1), vinylic carbons at δ 131.03 (C-9) and 129.25 (C-10), anomeric carbon at δ 105.48 (C-1′), sugar carbons at δ 71.2−64.21, the remaining methylene carbons in the range δ 33.21−23.87, and oxygenated methylene at δ 35.07.

The ^{1}H−^{1}H COSY spectrum of **5** displayed correlations of H-2 with H-3 and H-4; H-9 with H-10, H-11, and H-8; H-18 with H-17 and H-16; and H-1′ with H-2′ and H-3′. The HMBC spectrum showed correlations of H-2 and H-3 with C-1 and of H-17, H-16, and H-15 with C-18. HSQC measurements led to full assignments of the ^{1}H and ^{13} C NMR chemical shifts of **5**. Acid hydrolysis of **5** with dilute HCl yielded *β*-D-arabinose and oleic acid (TLC comparable). On the basis of spectroscopic data and chemical reactions, the structure of **5** was established as oleoyl *β*-D-arabinoside.

The known compounds *n*-octacos-9-enyl propionate, 1-tetratriacontanol, *β*-sitosterol, *n*-tetracontan-15*α*-ol, tritriacontane-4,12-diene, *n*-tritetracontan-5*α*-ol, gallic acid, and *β*-sitosterol-3-*O*-*β*-D-glucoside were identified by comparison with literature data[13,19] and standards.

Algicidal Activities of Isolated Compounds. Rice cultivated by water logging is susceptible to vigorous algal

growth. Algal growth reduces the water temperature and inhibits photosynthesis by blocking the light. The growth inhibitory activities of compounds **1**−**5** against blue-green algae (*M. aeruginosa* UTEX 2388 was used) are shown in Figure 3. Compound **5** gave the highest inhibition of *M. aeruginosa* growth at a concentration of 100 ppm (mg/L). At this concentration, growth inhibition of the blue-green algae by compound **5** was 92.6 ± 0.3%; it was statistically significant ($P < 0.0001$). Furthermore, at the concentration of 10 ppm, compound **5** had also a relatively higher inhibition effect (7.8 ± 3.5%) of the *M. aeruginosa* growth (mg/L) compared to those of other compounds (Figure 3). None of the compounds in the present study inhibited *M. aeruginosa* growth at a concentration of 1 ppm (mg/L).

Various bioactive compounds, including phenols, long-chain fatty acids, and benzene derivatives, have been reported to be present in rice hulls.[12] Among these, momilactones A and B, isolated from rice hulls, are well-known to strongly suppress the germination of lettuce seeds and the growth of rice roots.[4] The inhibitory strength of compound **5** in the present study was similar to that of momilactone A at a concentration of 30 μM, and it was higher than that of momilactone B at a concentration of 300 μM.[15] The inhibitory activity of momilactone A is higher than that of momilactone B in broad-leaf weed species. Also, the inhibitory activities of momilactones A and B differ in broad- and narrow-leaf plants.[15] In the present study, 10 kg of rice straw produced 27 mg of compound **5** (yield = 0.00027%); **5** had excellent bioactivity against blue-green algae (*M. aeruginosa*). Rice is the major crop produced in Korea, and farmers generally leave a large amount of rice straw in the fields during harvesting. On the basis of the bioassay (0.1 g/L of **5**), 0.011 megaton of rice straw, producing 30 kg of compound **5** per hectare, would theoretically be needed for inhibition of blue-green alga (*M. aeruginosa*) growth in paddy fields. Approximately 7 tons of rice straw per hectare is produced using the agricultural system recommended in Korea. On the basis of this study, the potential field production of compound **5** (∼19 g/ha) is less than that reported in a prior study;[18] however, this can be improved by changing the rice variety, cultivation method, extraction method, and other environmental conditions. Therefore, despite the low amount of compound **5** produced in this study, compound **5** could still be used to reduce interference in water-logged rice fields in a more ecofriendly manner. However, in the present study, we tested only for inhibition of blue-green algal growth by five compounds identified in rice straw. Further investigations are needed to evaluate the various bioactivities of the five compounds in rice straw under various cultivation conditions.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

CC, column chromatography; IR, infrared; NMR, nuclear magnetic resonance; COSY, correlated spectroscopy; HSQC, heteronuclear single-quantum coherence; HMBC, heteronuclaer multiple-bond coherence; ESI-FTMS, electrospray ionization Fourier transform mass; ODS, octadecyl silica; DW, dry weight; LSD, least significant difference; SD, standard deviation; TMS, tetramethylsilane

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