Flavusides A and B, Antibacterial Cerebrosides from the Marine-Derived Fungus *Aspergillus flavus*

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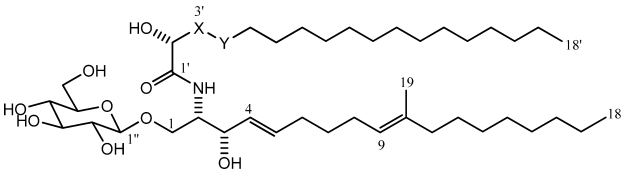
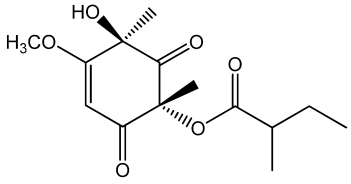
Flavusides A (1) and B (2), two new antibacterial cerebroside derivatives, and the previously described phomaligol A (3), kojic acid (4), methyl kojic acid (5), and dimethyl kojic acid (6) have been isolated from the extract of a marine isolate of the fungus *Aspergillus flavus*. The structure and absolute stereochemistry of two cerebrosides were assigned on the basis of NMR and Tandem FAB-MS/MS experiments. Compounds 1, 2, and 3 exhibited a mild antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. The minimum inhibitory concentration (MIC) values for each strain are as follows: compounds 1 and 2 showed 15.6 μg/ml for *S. aureus* and 31.2 μg/ml for methicillin-resistant *S. aureus* and multidrug-resistant *S. aureus*, and compound 3 exhibited 31.2 μg/ml for *S. aureus* and methicillin-resistant *S. aureus* and 62.5 μg/ml for multidrug-resistant *S. aureus*.

Key words flavuside; cerebroside; antibacterial activity; marine-derived fungus; *Aspergillus flavus*

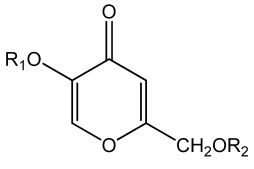
Marine microorganisms, particularly marine fungi, have recently gained prominence as an important source of biologically active secondary metabolites.[1] Among the marine fungi, those living in association with marine algae are a particularly promising source of novel natural products due to the special ecological niche in which they exist.[2] As part of a program to explore the bioactive metabolites produced by fungi isolated from marine habitats, our efforts have focused on microorganisms found in association with edible seaweed.[3] From the surface of the edible green algae, *Codium fragile* (Korean name: CheongGak), we have isolated a fungal strain *Aspergillus flavus*. When cultivated under saline conditions, the broth and mycelium of a 1 l cultivation of *A. flavus* were found to exhibit mild antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. This paper describes the isolation and structure elucidation of two antibacterial cerebrosides, flavusides A (**1**) and B (**2**), and identification of four known phomaligol A (**3**), kojic acid (**4**), methyl kojic acid (**5**), and dimethyl kojic acid (**6**).[4]



flavuside A (**1**) : X-Y = CH_2CH_2
B (**2**) : X-Y = CH = CH



phomaligol A (**3**)



kojic acid (**4**), R_1=R_2= H
5, R_1= Me, R_2= H
6, R_1= R_2 = Me

Flavuside A (**1**) was isolated as a colorless amorphous solid and analyzed to reveal a molecular formula of $C_{43}H_{81}NO_9$ by high resolution fast atom bombardment mass spectrometry (HR-FAB-MS) and ^{13}C-NMR methods. The IR spectrum of **1** showed bands characteristic of hydroxy (3333 cm^{-1}) and amide (1641 cm^{-1}) functionalities. Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra of **1**, including spectra from distortionless enhancement by polarization transfer (DEPT), correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC), and heteronuclear multiple bond coherence (HMBC) experiments, revealed signals that were ascribable to a monosaccharide (an anomeric proton at δ_H 4.11), an amide linkage (a nitrogenated methine proton at δ_H 3.81 and a carbonyl carbon at δ_C 173.7), and an aliphatic long chain (terminal methyl protons at δ_H 0.85 and methylene protons at δ_H 1.22—1.43), indicating that the compound was a glycosphingolipid. The ^{13}C-NMR spectrum revealed carbon resonances at δ_C 61.1 (CH_2), 70.0 (CH), 73.4 (CH), 76.5 (CH), 76.9 (CH), and 103.5 (CH), indicative of the presence of a β-glucopyranoside.[5] The coupling constant of the anomeric proton at δ_H 4.11 (d, J_{H1-H2}=7.8 Hz) and the chemical shift of the anomeric carbon (δ_C 103.5) further confirmed the β configuration of the glucose moiety (α-glucopyranoside: J_{H1-H2}= 3.7 Hz; δ_C 98.5).[6] The absolute configuration of the glucose moiety was found to be D (*vide infra*). The ^{1}H- and ^{13}C-NMR spectra showed the presence of typical disubstituted Δ^4 double bond [δ_H 5.39 (dd, J=15.3, 6.5 Hz, H-4), δ_C 131.0 (CH, C-4); δ_H 5.59 (dt, J=15.3, 6.0 Hz, H-5), δ_C 131.1 (CH, C-5)][7] and an additional trisubstituted double bond [δ_H 5.08 (t-like, J=6.2 Hz, H-9), δ_C 123.5 (CH, C-9); δ_C 134.9 (qC, C-10)] in a sphingosine. The large vicinal coupling constant of the olefinic protons (J_{H4-H5}=15.3 Hz) clearly indicated an *E* geometry for the double bond,[8] which was further supported from the chemical shift of the allylic carbons [δ_C 32.1 (C-6,

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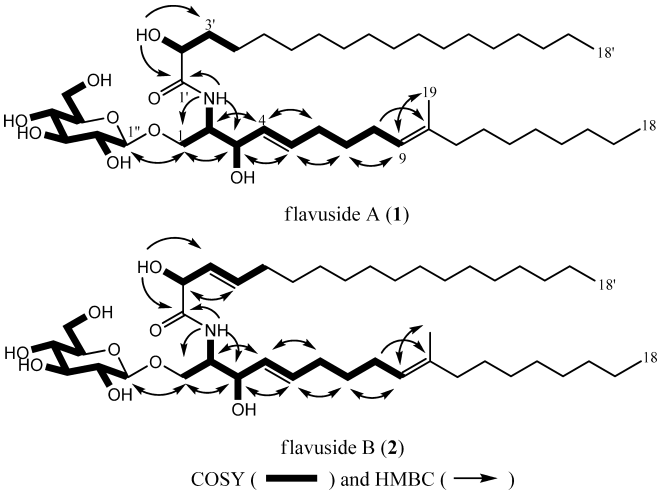

Fig. 1. Key COSY and HMBC Correlations of Compounds **1** and **2**

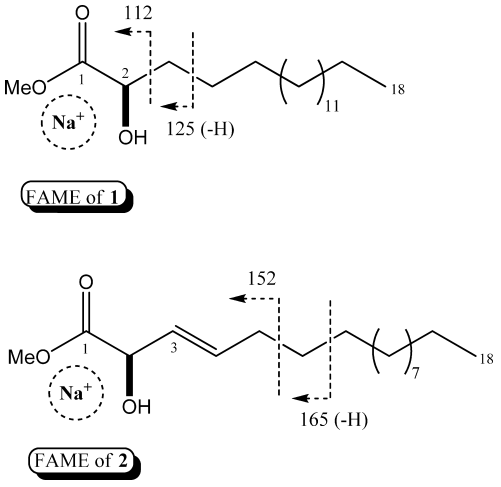

Fig. 2. Key FAB-CID Tandem Mass Fragmentations of the $[M+Na]^+$ Ions of the FAMEs Derived from **1** and **2**

C-8)].[7] The COSY correlations for H-1 through H-5 defined the 2-amino-1,3-dioxigenated-4-ene moiety (Fig. 1). The functional groups in **1** were identified and their connectivities elucidated using HMBC techniques. The key HMBC correlations, from H-1 to C-2, C-3, and C-1″, from 2-NH to C-1, C-2, C-3, and C-1′, from H-3 to C-1 and C-5, from H-5 to C-3, C-4, C-6, and C-7, from H_2-7 to C-5 and C-9, from H_2-8 to C-9 and C-10, from H-9 to C-7, C-8, and C-19, from H_3-19 to C-9 and C-10, from 2′-OH to C-1′, C-2′, and C-3′, showed the connections, C1-O-C1″ and C2-NH-C1′. Both connections were indicative of 1-*O*-glucopyransyl-2-amido-3,2′-dihydroxy-10-methyl-4,9-diene groups (Fig. 1). The spectral features revealed that compound **1** had the general structural components of cerebroside.[9] The chemical shifts of C-2 at 52.8 ppm and C-3 at 70.5 ppm supported a *threo* C2–C3 configuration, in agreement with the configuration of D-glucosyl-L-*threo*-ceramide (δ_C 53.7, 70.0).[10] The D-*erythro* isomers of these model compounds have been reported to display C-2/C-3 chemical shifts at δ_C 53.8/72.6.[10] Methanolysis of **1** yielded a fatty acid methyl ester (FAME), a long chain sphingosine base (LCSB), and an anomeric mixture of methyl glucoside. The FAME, methyl hydroxyoctadecanoate, and its molecular formula were established to be $C_{19}H_{38}O_3Na$ ($[M+Na]^+$ at *m*/*z* 337). The enhanced peak of the α-cleavage of alcohol at *m*/*z* 112 supported the position of the 2-hydroxy group in the fatty acid moiety (Fig. 2). The optical rotation of the ester ($[\alpha]_D$ −62, $CHCl_3$) identified it as the *R* isomer.[9,11] Therefore, the fatty acid moiety was deduced to be (2*R*)-2-hydroxyoctadecanoic acid from the foregoing data. The LCSB was peracetylated using acetic anhydride in pyridine, giving the desired triacetyl derivative. Triacetyl LCSB showed the same sign of optical rotation ($[\alpha]_D$ +4.2, $CHCl_3$) as did *threo*-triacetylsphingosine ($[\alpha]_D$ +8.43, $CHCl_3$) (the *erythro* epimer: $[\alpha]_D$ −12.9, $CHCl_3$).[12] The absolute configuration at C-2 of most natural cerebroside was all-2*S*.[9,13] The *threo* C2–C3 configuration, the specific rotation of the triacetyl LCSB, and the biogenetic pathway of the natural cerebroside suggested that **1** shared the 2*S*,2′*R*,3*S*-configuration at the asymmetric centers. The specific rotation of the methyl glucopyranoside ($[\alpha]_D$ +64, MeOH) indicated a D-configuration.[13] The D-configuration of the glucose moiety was further supported by comparison of the specific rotation of the anomeric mixture of glucose ($[\alpha]_D$ +51), derived from acid hydrolysis (10% aq. H_2SO_4) of methyl glucopyranoside, with that of the reported value (α/β-glucose: $[\alpha]_D$ +52.7).[14] On the basis of the above data, the structure of flavuside A (**1**) was defined as 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*E*,9*E*)-2-[(2′*R*)-2′-hydroxyoctadecanoylamino]-10-methyl-octadeca-4,9-dien-1,3-diol.

Flavuside B (**2**) was obtained in the form of a colorless amorphous solid. A molecular formula of $C_{43}H_{79}NO_9$, which gave five degrees of unsaturation, was established by HR-FAB-MS and ^{13}C-NMR methods. The general features of the UV, IR, and NMR spectra closely resembled those of flavuside A (**1**), except for the appearance of an additional disubstituted double bond [δ_H 5.43 (1H, dd, *J*=15.5, 5.5 Hz, H-3′), δ_C 129.0 (CH, C-3′); δ_H 5.67 (1H, dt, *J*=15.5, 6.7 Hz, H-4′), δ_C 130.9 (CH, C-4′)] in **2** (Table 1). The geometry of an additional double bond was presumed to be *E* due to the large vicinal coupling constant of the olefinic protons (J_{H4-H5}=15.5 Hz).[8] Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra of **2**, including the results from DEPT, COSY, HMQC, and HMBC experiments, suggested that **2** was a dehydrogenated derivative of **1**, and differed only in an additional double bond on the lipid base or the lipid amide units. The difference occurred in the fatty acid portion of **2**, determined by the appearance of methyl (2*R*,3*E*)-2-hydroxyoctadec-3-enoate ($[\alpha]_D$ −50, $CHCl_3$) at *m*/*z* 335 ($C_{19}H_{36}O_3Na$ $[M+Na]^+$) upon methanolysis of **2**.[9] The location of the new double bond was determined by FAB-collision induced dissociation (CID)-MS/MS analysis of the $[M+Na]^+$ ion at *m*/*z* 152. The enhanced peak corresponding to allylic cleavage at *m*/*z* 152 indicated that the double bond was located at C-3′ (Fig. 2). Methanolysis of **2** also yielded LCSB and a mixture of methyl α- and β-glucopyranosides. The data collected for LCSB, derived from **2**, were in agreement with those of **1**. The optical rotation of the mixture ($[\alpha]_D$ +64, MeOH) identified glucose as the D-isomer.[13] On the basis of these data, the structure of flavuside B (**2**) was established as 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*E*,9*E*)-2-[(2′*R*,3′*E*)-2′-hydroxyoctadec-3′-enoylamino]-10-methyl-octadeca-4,9-dien-1,3-diol. Compound **2** appears to be identical in its planar structure to the sphingosine A, (3′*E*,4*E*)-1-(β-D-glucopyranosyloxy)-3-

Table 1. ^{1}H- and ^{13}C-NMR Data for Flavusides A (**1**) and B (**2**)[a)]

Position	Flavuside A (**1**)		Flavuside B (**2**)	
	δ_H (mult, J)	δ_C (mult)	δ_H (mult, J)	δ_C (mult)
1	3.52, dd (10.5, 3.8)	68.7, CH_2	3.50, dd (10.2, 3.8)	68.6, CH_2
	3.92, dd (10.5, 5.6)		3.93, dd (10.2, 5.4)	
2	3.81, m	52.8, CH	3.79, m	52.9, CH
3	3.98, ddd (6.5, 6.5, 6.0)	70.5, CH	3.98, ddd (6.7, 6.7, 5.5)	70.5, CH
4	5.39, dd (15.3, 6.5)	131.0, CH	5.36, dd (15.3, 6.7)	130.9, CH
5	5.59, dt (15.3, 6.0)	131.1, CH	5.56, dt (15.3, 6.0)	130.9, CH
6	1.91, m	32.1, CH_2	1.91, m	32.1, CH_2
7	1.93, m	27.2, CH_2	1.93, m	27.3, CH_2
8	1.96, m	32.1, CH_2	1.95, m	31.3, CH_2
9	5.08, deformed t (6.2)	123.5, CH	5.09, deformed t (6.2)	123.5, CH
10		134.9, qC		134.9, qC
11	1.90, t (7.3)	39.0, CH_2	1.90, m	39.3, CH_2
12	1.33, m	27.4, CH_2	1.32, m	27.4, CH_2
13—16	1.23, m	28.7—31.3, CH_2	1.23, m	28.7—31.3, CH_2
17	1.23, m	22.1, CH_2	1.23, m	22.1, CH_2
18	0.85, dd (7.5, 5.5)	13.9, CH_3	0.84, dd (7.0, 6.5)	13.9, CH_3
19	1.53, s	15.7, CH_3	1.54, s	15.7, CH_3
2-NH	7.38, d (9.4)		7.38, d (9.4)	
3-OH	4.91, d (6.0)		4.94, d (5.5)	
1′		173.7, qC		172.0, qC
2′	3.81, m	71.0, CH	4.29, m	71.9, CH
3′	1.43, m	34.5, CH_2	5.43, dd (15.5, 5.5)	129.0, CH
4′	1.22, m	24.5, CH_2	5.67, dt (15.5, 6.7)	130.9, CH
5′—16′	1.22, m	28.7—31.3, CH_2	1.95, 1.23, m	31.6, 28.7—31.3, CH_2
17′	1.22, m	22.1, CH_2	1.23, m	22.1, CH_2
18′	0.85, dd (7.5, 5.5)	13.9, CH_3	0.84, dd (7.0, 6.5)	13.9, CH_3
2′-OH	5.51, d (5.1)		5.77, d (4.0)	
1″	4.11, d (7.8)	103.5, CH	4.11, d (7.8)	103.5, CH
2″	2.95, ddd (8.5,8.0,4.0)	73.4, CH	2.95, br dd (8.5, 8.0)	73.4, CH
3″	3.13, m	76.5, CH	3.13, m	76.5, CH
4″	3.02, m	70.0, CH	3.03, m	70.0, CH
5″	3.06, m	76.9, CH	3.06, m	76.9, CH
6″	3.44, m	61.1, CH_2	3.44, m	61.1, CH_2
	3.66, dd (11.3, 5.9)		3.66, dd (11.5, 5.5)	
2″-OH	4.96, d (4.0)		4.98, m	
3″-OH	4.93, d (5.6)		4.98, m	
4″-OH	4.90, d (5.1)		4.94, d (5.4)	
6″-OH	4.51, dd (5.9, 5.7)		4.52, t (5.5)	

a) Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C).

hydroxy-2-[(2′-hydroxyoctadecanyloxy)amino]-10-methyl-3′,4,9-octadecatriene, reported by Li *et al.*[15)] However, direct comparison of the stereostructures of **2** and sphingosine A remain unsolved because neither the full discussion for the sphingosine A nor key data, including $[\alpha]_D$ and MS, were presented in the published paper.[15)]

Compounds **1**, **2**, and **3** exhibited a mild antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. The minimum inhibitory concentration (MIC) values to each strain are as follows: compounds **1** and **2** exhibited 15.6 μg/ml for *S. aureus* and 31.2 μg/ml for methicillin-resistant *S. aureus* and multidrug-resistant *S. aureus*, and compound **3** showed 31.2 μg/ml for *S. aureus* and methicillin-resistant *S. aureus* and 62.5 μg/ml for multidrug-resistant *S. aureus*.

Cerebrosides are glycosphingolipids, consisting of a ceramide and a single sugar residue (glucose or galactose) at C-1. The hydrophobic ceramide portion involves a sphingoid base and an amide-linked fatty acyl chain. These amphipathic molecules have been reported to exhibit antitumor/cytotoxic, anti-HIV-1, neuritogenic, antihepatotoxic, immunosuppressive, immunomodulatory, cyclooxigenase-2 inhibitory, antifungal, antimicrobial, and antifouling activities.[13)] Because cerebrosides have been reported to exhibit diverse biological activity, a detailed investigation of the biological activities of compounds **1** and **2** would be of interest.

The known phomaligol A (**3**), kojic acid (**4**), methyl kojic acid (**5**), and dimethyl kojic acid (**6**) were also obtained in this investigation. They were identified by inspecting their NMR spectra and comparing these data with literature values.[4)]

Experimental

General Optical rotation was determined on a Perkin Elmer model 341 polarimeter. UV/visible spectra were measured on a Hitachi U-2001 UV/Vis spectrometer. IR spectra were recorded on a Bruker Fourier transform (FT)-IR model IFS-88 spectrometer. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP 400 NMR spectrometer, using TMS or solvent peaks [DMSO-d_6: ^{1}H (δ 2.50) and ^{13}C (δ 39.5)] as reference standard. FAB-mass spectra were recorded with a JMS-700 Mstation mass spectrometer (JEOL, Tokyo, Japan) using a MS-MP9020D data system. HPLC was performed on a YOUNG LIN-ACME HPLC system using a re-

versed-phase analytical column (Gemini C18, 4.6×250 mm, 5 μm) with UV detection.

Fungal Isolation and Culture The fungal strain, *Aspergillus flavus*, was isolated from the surface of the edible green algae, *Codium fragile* (Korean name: CheongGak), collected in GeoMun Island, Yeosu, Korea and identified based on 18S ribosomal RNA (rRNA) analyses (SolGent Co., Ltd., Daejeon, Korea), identity of 99%. A voucher specimen is deposited at Pukyong National University with the code MFA500. The fungus was cultured in 2.8-l Fernbach flasks (10×1 l) at first, and then additionally cultured two times more in culture medium consisting of soytone (0.1%), soluble starch (1.0%), and seawater (100%). The cultures were incubated at 29 °C for 20 d on the static condition.

Extraction and Isolation Each culture was filtered through cheesecloth to yield broth and mycelium residue, and the resulted broth and mycelium were extracted with EtOAc and CH_2Cl_2–MeOH (1 : 1) to afford broth extract (1.9 g) and mycelium extract (2.7 g), respectively. Both extracts exhibited antibacterial activity against *S. aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*. So, we combined both extracts, and subjected to Si gel flash chromatography. Elution was performed with CH_2Cl_2–MeOH (stepwise, 0—100% MeOH) to yield eight fractions. Fractions 2 and 7, which exhibited antibacterial activity against *S. aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus*, were separated by medium-pressure liquid chromatography (MPLC) [octadesyl silica (ODS)] using a H_2O–MeOH gradient elution to afford crude compound **3** and compounds **1** and **2**, respectively. They were further purified by recycling HPLC (Gemini C18, 4.6×250 mm, 5 μm, 1 ml/min) utilizing a 30 min gradient program of 50 to 100% MeOH in H_2O to furnish **1** (16.5 mg), **2** (12 mg), and **3** (15 mg), respectively. Compounds **4** (105 mg), **5** (15 mg), and **6** (21 mg) were isolated from fraction 5 by the same chromatographic method above.

Flavuside A (**1**): was obtained as a colorless amorphous solid; $[\alpha]_D^{20}$ +5 (c=0.55, MeOH); IR (neat) ν_{max} 3333, 2921, 2851, 1733, 1641, 1541, 1468, 1278, 1248, 1079, 1029, 966, 721 cm^{-1}; UV λ_{max} (MeOH) (log ε) 199 (4.1) nm; ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (DMSO-d_6, 100 MHz): see the Table 1; LR-FAB-MS m/z: 778 $[M+Na]^+$ (100), 760 $[M+Na-H_2O]^+$ (0.4), 496 $[M+Na-$fatty acid$]^+$ (5), 396 (5), 348 (2), 244 (0.3), 203 (0.1), and 185 (0.3); HR-FAB-MS m/z: 778.5823 $[M+Na]^+$ (Calcd for $C_{43}H_{81}NO_9Na$, 778.5809) (Δ +1.8 ppm).

Flavuside B **(2)**: was obtained as a colorless amorphous solid; $[\alpha]_D^{20}$ −8.3 (c=0.4, MeOH); IR (neat) ν_{max} 3327, 2923, 2853, 1739, 1651, 1538, 1467, 1378, 1241, 1078, 1032, 966, 721 cm^{-1}; UV λ_{max} (MeOH) (log ε) 199 (4.1) nm; ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (DMSO-d_6, 100 MHz): see the Table 1; LR-FAB-MS m/z: 776 $[M+Na]^+$ (100), 758 $[M+Na-H_2O]^+$ (0.7), 522 (0.1), 496 $[M+Na-$fatty acid$]^+$ (3), 346 (2), 348 (2), 244 (0.5), 203 (1), and 185 (0.3); HR-FAB-MS m/z: 776.5644 $[M+Na]^+$ (Calcd for $C_{43}H_{79}NO_9Na$, 776.5653) (Δ −1.1 ppm).

Phomaligol A (**3**), kojic acid (**4**), and methyl kojic acids (**5**, **6**): spectroscopic data were virtually identical to those reported in the literature.[4)]

Methanolysis of 1 A solution of **1** (10.0 mg) in 9% HCl in MeOH (2.0 ml) was refluxed at 90 °C for 10 h (N_2 atmosphere). The reaction mixture was neutralized with Ag_2CO_3 and filtrated. The residue, obtained by removal of the solvent, was partitioned into MeOH (10 ml) and hexane (10 ml) to give the hexane phase and the MeOH phase. The hexane layer was evaporated under N_2, and purified by Si gel flash chromatography. Elution was performed with hexane–EtOAc (stepwise, 0—100% EtOAc) yielded the FAME of **1** (1.0 mg): $[\alpha]_D$ −62 (c=0.1, $CHCl_3$); FAB-MS m/z 337 $[M+Na]^+$ (100), 321 (0.17), 307 (0.15), 293 (0.22), 279 (0.28), 265 (0.24), 251 (0.22), 237 (0.24), 223 (0.25), 209 (0.22), 195 (0.20), 181 (0.16), 167 (0.22), 153 (0.1), 139 (0.11), 125 (0.35), 112 (0.62). The MeOH layer was concentrated, and the residue was partitioned into EtOAc and H_2O. The EtOAc portion was dried, and peracetylated by using acetic anhydride (0.5 ml) in pyridine (1 ml) at r.t. for 10 h (N_2 atmosphere). The reaction mixture was dried, and subjected to Si gel flash chromatography [hexane–EtOAc (stepwise, 0—100% EtOAc)] to yield triacetyl LCSB of **1** (3.1 mg): $[\alpha]_D$ +4.2 (c=0.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ_H 0.87 (3H, t, J=6.7 Hz, H_3-18), 1.25 (14H, br s), 1.57 (3H, s, H_3-19), 2.00—2.15 (6H, m), 2.01, 2.04, 2.07 (each 3H, s, three Ac), 3.99 (1H, m), 4.19 (1H, m), 4.32 (1H, m), 4.83—4.87 (1H, m), 5.40 (1H, t, J=7.0 Hz), 5.46 (1H, dd, J=16.0, 9.5 Hz), 5.97 (1H, dt, J=16.0, 7.0 Hz). The H_2O portion was applied to a Diaion HP-20 column (Mitsubishi Kasei, 20 ml of resin, 10×250 mm). The column was eluted with distilled water (100 ml) and MeOH (100 ml), successively. The elute with MeOH was concentrated to afford methyl glucopyranoside (2.2 mg). The optical rotation recorded for methyl glucopyranoside isolated in this study was $[\alpha]_D$ +64 (c=0.22, MeOH), which showed methyl D-glucopyranoside in **1** (ref 13): $[\alpha]_D^{20}$ +70.8). The obtained methyl D-glucopyranoside was refluxed with 10% aq. H_2SO_4 (1 ml) for 2 h, and the reaction mixture was neutralized with $NaHCO_3$ and concentrated *in vacuo*. The reaction solid was dissolved in H_2O (10 ml), and then purified by Diaion HP-20 column, as described above, to yield D-glucose (1.1 mg) as a colorless syrup: $[\alpha]_D$ +51 (c=0.1, H_2O) [ref. 14): $[\alpha]_D^{20}$ +52.7].

Methanolysis of 2 The methnolysis of **2** was carried out similarly to **1**, and furnished a FAME (methyl-2-hydroxyocadec-3-enoic acid, 1.0 mg), triacetyl LCSB, and methyl glucopyranoside. The optical rotation and FAB-MS of FAME were $[\alpha]_D$ −50 (c=0.06, $CHCl_3$); FAB-MS m/z 335 $[M+Na]^+$ (100), 317 (0.30), 305 (0.12), 291 (0.15), 277 (0.18), 263 (0.2), 249 (0.21), 235 (0.18), 221 (0.14), 207 (0.12), 191 (0.16), 177 (0.16), 165 (0.26), 152 (0.76). The physicochemical data for methyl glucopyranoside and triacetyl LCSB were identical with those of **1**.

Antibacterial Assay The *in vitro* antibacterial activity of the extract and purified samples were evaluated by a conventional 2-fold serial dilution method using *S. aureus*, methicillin-resistant *S. aureus*, and multidrug-resistant *S. aureus* as indicator strains. A 5 ml suspension containing 10^5 cells per ml was used as inoculum of the test organism. The MIC values were determined after the inoculation for 18 h at 37 °C.[16)]

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