Novel Halogenated Metabolites from the Malaysian *Laurencia pannosa*[1]

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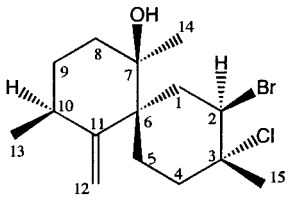
Received December 29, 2000

In connection with our chemotaxonomic studies of Malaysian species of the red algal genus *Laurencia*, the chemical composition of *Laurencia pannosa* Zanardini was examined. Two halogenated sesquiterpenoids, named pannosanol (**1**) and pannosane (**2**), have been isolated along with a halogenated C_{15}-acetogenin, (3*Z*)-chlorofucin (**3**). The structures of these compounds were determined from their spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, 2D NMR, and MS). Pannosanol and pannosane are novel halometabolites with an unusual rearranged chamigrane framework. Antibacterial activities of these metabolites against marine bacteria are also described.

Species of the red algal genus *Laurencia* (Rhodomelaceae, Ceramiales) are known to be prolific sources of a variety of halogenated secondary metabolites, such as C_{15}-acetogenins and C_{15}-, C_{20}-, and C_{30}-terpenoids.[2] Although the roles of these halogenated metabolites have not been clearly investigated, it is suggested that these metabolites function as chemical defense substances against marine herbivores.[3,4] Moreover, some halogenated metabolites have been shown to possess antibacterial activities against terrestrial bacteria.[5−9] More recently, we reported on the antibacterial potentials of halogenated compounds from *Laurencia* against marine bacteria isolated from the algal habitats.[10,11]

In the course of our chemical and biological studies of *Laurencia* species from the Malaysian waters, we reported chemical compositions of *L. snackeyi* (Weber-van Bosse) Masuda,[12] *L. similis* Nam et Saito,[13] *L. majuscula* (Harvey) Lucas,[10] and an unrecorded *Laurencia* sp.[10] As part of the chemical analyses of the Malaysian *Laurencia* species, we examined a sample of *L. pannosa* Zanardini that was collected at Pulau Talang-Talang Kecil, Kuching, Sarawak. This sample contained two novel halogenated sesquiterpenoids, pannosanol (**1**) and pannosane (**2**), with an unusual rearranged chamigrane skeleton, together with a new halogenated C_{15}-acetogenin, (3*Z*)-chlorofucin (**3**). In this paper we describe the isolation and structure elucidation of these compounds and their antibacterial activities against marine bacteria.



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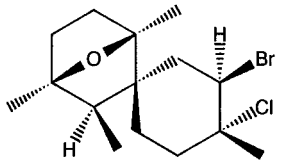

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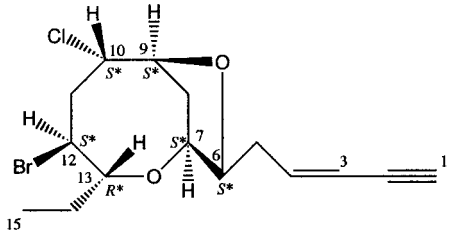
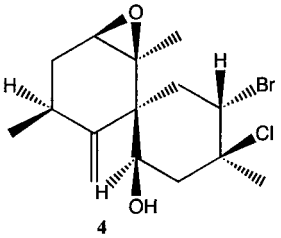


3: 3*Z*
5: 3*E*



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Results and Discussion

The partially dried specimens of *Laurencia pannosa*, collected at Pulau Talang-Talang Kecil, Kuching, Sarawak, were extracted with methanol. A combination of column and thin-layer chromatography of the methanol extract yielded pannosanol (**1**) (0.30% of dry algae weight), pannosane (**2**) (0.09%), and (3*Z*)-chlorofucin (**3**) (0.11%).

Pannosanol (**1**) analyzed for $C_{15}H_{24}BrClO$ by HREIMS and ^{13}C NMR data. Its IR spectrum showed the presence of a hydroxyl group at ν_{max} 3450 cm^{-1} and an exo-methylene group at ν_{max} 1621 and 910 cm^{-1}. The presence of the exo-methylene group was also supported by the typical signals in the ^{1}H and ^{13}C NMR spectra (Table 1) [δ_H 5.34 and 5.01 (1H, each s); δ_C 152.2 (C) and 113.1 (CH_2)]. Furthermore, the ^{1}H NMR spectrum revealed signals due to a secondary methyl group at δ_H 1.10 (3H, d, J = 6.3 Hz), two tertiary methyl groups at δ_H 1.21 (3H, s) and 1.75 (3H, s), a halomethine group at δ_H 4.18 (1H, dd, J = 13.2 and 3.4 Hz), and a hydroxyl proton at δ_H 1.82 (1H, s; D_2O exchangeable). The tertiary nature of the hydroxyl group was evident from the ^{1}H NMR spectrum of **1** with the addition of D_2O, which showed no signal change except for the signal at δ_H 1.82.

Assignments of the carbons bearing hydrogen(s) were established by the HSQC spectrum (Table 1). The ^{1}H−^{1}H COSY spectrum as well as the above-mentioned data showed the presence of partial structural units **1a**−**1h** in pannosanol (**1**) (Figure 1). All elements implied by the molecular formula of **1** have now been defined and are comprised in the structural units. The chemical shift values

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10.1021/np0006317 CCC: $20.00
Published on Web 04/27/2001

Table 1. ^{13}C NMR (100 MHz, DEPT), ^{1}H NMR (400 MHz), and HMBC Data[a] for Pannosanol (**1**)

C[b]	^{13}C (δ)	^{1}H (δ)	multiplicity, J (Hz)	HMBC correlations
1	40.7	1.96	dd, J = 13.2, 13.2 (Ha)	C-2, C-3, C-5, C-6, C-7, C-11
		2.68	ddd, J = 13.2, 3.4, 3.4 (Hb)	C-2, C-3, C-5, C-6, C-11
2	61.2	4.18	dd, J = 13.2, 3.4	C-1, C-3, C-15
3	72.9			
4	39.5	2.24	ddd, J = 13.7, 3.4, 3.4 (Ha)	C-2, C-3, C-5, C-6, C-15
		2.43	ddd, J = 13.7, 10.3, 3.4 (Hb)	C-3, C-5, C-15
5	25.1	1.69	m (Ha)	C-1, C-3, C-6
		2.03	m (Hb)	C-1, C-3, C-4, C-6
6	53.1			
7	75.7			
8	34.2	1.12	m	C-9, C-14
		1.79	m	C-9, C-14
9	35.9	1.45	m	C-7, C-8, C-10
		1.83	m	C-7, C-8, C-10
10	32.8	2.42	m	C-8, C-11, C-12, C-13
11	152.2			
12	113.1	5.01	s (Ha)	C-6
		5.34	s (Hb)	C-6
13	19.9	1.10	d, J = 6.3	C-10, C-11, C-13
14	23.2	1.21	s	C-6, C-7
15	24.6	1.75	s	C-2, C-3, C-4
		1.82	s (OH)	

[a] Measured in chloroform-d_1. [b] Assignment was made by the HSQC spectrum.

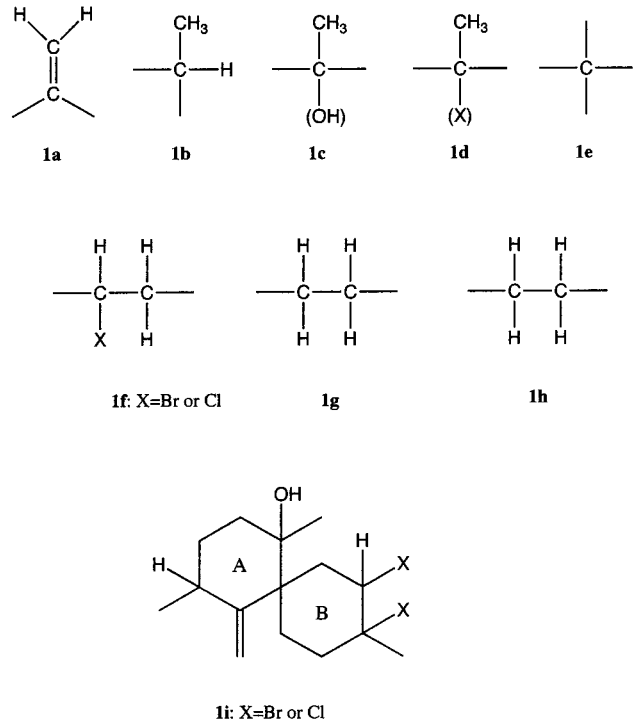

Figure 1. Partial structural units and planar structure for pannosanol (**1**).

of two tertiary methyl groups at δ_H 1.21 (**1c**) and 1.75 (**1d**) suggested that each of these methyls is attached to a carbon bearing an oxygen atom (OH) and a halogen atom (Br or Cl), respectively. Furthermore, in the ^{13}C NMR spectrum, the chemical shift value of the methine carbon (δ_C 61.2) in the unit **1f** indicated that a halogen atom is attached to this carbon.

Confirmation of the partial structural units and their connectivity was made with the aid of the HMBC spectrum (Table 1). A long-range correlation between the exo-methylene protons (**1a**) and a quaternary carbon (δ_C 53.1) in **1e**, the latter of which in turn showed cross-peaks to a methylene in **1f**, a methylene in **1g**, and a tertiary methyl (δ_H 1.21) in **1c**, was observed. Furthermore, long-range correlations between a methyl (δ_H 1.75) in **1d** and a quaternary carbon (δ_C 72.9) in **1d**, a methine carbon (δ_C 61.2) in **1f**, and a methylene carbon (δ_C 39.5) in **1g** established connection of unit **1d** with units **1f** and **1g**, leading to a six-membered ring (B-ring). On the other hand, long-range correlations between a secondary methyl (δ_H 1.10) in **1b** and a quaternary carbon (δ_C 152.2) in **1a** and a methylene carbon (δ_C 35.9) in **1h**, whose methylene protons showed correlation with a quaternary carbon (δ_C 75.7) in **1c**, confirmed connection of unit **1b** with units **1a** and **1h**, leading to another spiro-fused six-membered ring (A-ring). Thus, the planar structural formula **1i** should be assigned for pannosanol. The positions of the bromine and chlorine atoms were established by the halogen-induced ^{13}C isotope shifts in the ^{13}C NMR spectrum.[14−16] The quaternary carbon at C-3 (δ_C 72.9) showed an isotope shift of 1.14 ppb with relative intensities of about 2.86:1 induced by ^{35}Cl and ^{37}Cl. Hence the chlorine atom is linked to C-3 and the bromine atom to C-2, leading to the planar formula **1** for pannosanol.

The relative stereochemistry of **1** was determined by the NOESY spectrum as well as the coupling constants in the ^{1}H NMR spectrum. The methine proton on C-2 showed the coupling constant, J = 13.2 and 3.4 Hz, which is a typical axial proton on the B-ring in a chair conformation. Hence the bromine atom on C-2 is equatorial. The axial configuration of the methyl group (H_3-15) at C-3 was confirmed by observation of NOEs between H_3-15/Ha_{ax}-1 and H_3-15/Hb_{ax}-5. Furthermore, a NOE between Ha-12 and Hb_{ax}-4 established the stereochemistry around the spiro carbon at C-6 as shown in formula **1j** (Figure 2). NOEs between H-10/Hb_{eq}-1 and H_3-13/Hb-12 revealed an A-ring chair conformation with an equatorial methyl group (β) at C-10 as shown in formula **1k** (Figure 2). The equatorial configuration of the methyl group (H_3-14) (α) at C-7 was determined from NOEs between H_3-14/Ha_{ax}-1 and H_3-14/Hb_{ax}-5. Consequently, the structure of pannosanol must be represented by formula **1**, which has the relative configuration of 2*R**, 3*R**, 6*R**, 7*R**, and 10*S**.

Pannosane (**2**), $C_{15}H_{24}BrClO$, was shown to be isomeric with pannosanol (**1**). The spectral features of **2** were very similar to **1**. Since the IR spectrum revealed no absorptions due to hydroxyl and carbonyl groups, an oxygen atom was assumed to be involved in ether linkages. The ^{1}H NMR

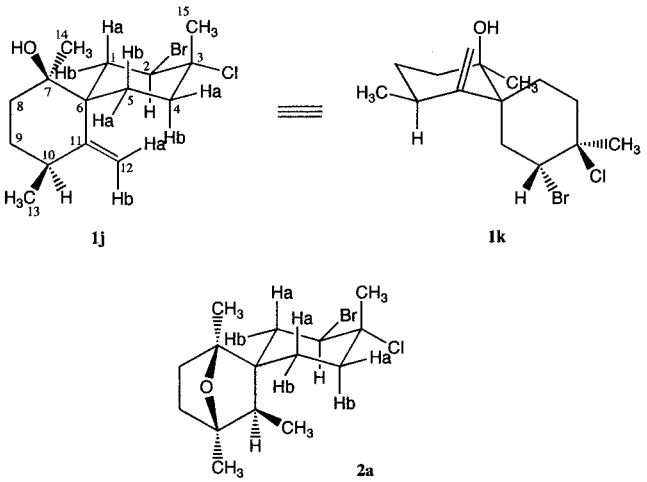

Figure 2. Stereochemistry of pannosanol (**1**) and pannosane (**2**).

spectrum indicated the presence of a secondary methyl at δ_H 1.09 (3H, d, J = 7.8 Hz), a tertiary methyl at δ_H 1.69 (3H, s) which is attached to a carbon bearing a halogen atom, and two tertiary methyls at δ_H 1.28 (3H, s) and 1.27 (3H, s), both of which are attached to a carbon bearing an ethereal oxygen atom. The $^1H-^1H$ COSY spectrum showed that **2** consisted of the same partial structural units (**1f**, **1g**, and **1h**, Figure 1) as in pannosanol (**1**). The planar structure of pannosane was confirmed by the connection of these fragments revealed by the HMBC spectrum (Table 2) as well as the halogen-induced ^{13}C isotope shifts in the ^{13}C NMR spectrum. The HMBC data together with the isotope shift of C-2 (δ_C 62.7), which was shifted 2.12 ppb with relative intensities of about 1:1, showed that pannosane has the same cyclohexane ring (B-ring) as pannosanol (**1**). Furthermore, the spiro carbon (δ_C 50.8) showed correlations with a tertiary methyl (δ_H 1.27) and a secondary methyl (δ_H 1.09), indicating that the spiro carbon is adjacent to these two methyl groups. In addition, since C-11 (δ_C 51.1) showed long-range correlations with the remaining tertiary methyl (δ_H 1.28), H_2-9, H_2-5, and H_2-1, the ether link must be located between C-7 and C-10, thus leading to planar formula **2** for pannosane. The relative stereochemistry was defined by the NOESY spectrum. NOEs were observed between Ha_{ax}-1/H_3-15, Hb_{ax}-5/H_3-15, Ha_{ax}-5/H_3-14, Hb_{ax}-4/H_3-12, and H_3-12/H_3-13. Thus the relative stereochemistry of pannosane was assigned as in formula **2a** (Figure 2).

Pannosanol (**1**) and pannosane (**2**) are the second examples of halogenated rearranged chamigrane-type sesquiterpenoids from the genus *Laurencia*. The first halogenated rearranged chamigrene derivative (**4**) was isolated from an undescribed *Laurencia* species collected at Rio Mar near Vero Beach, Florida.[17] A possible biogenetic pathway for rearranged chamigrenes is shown in Figure 3.

The third halogenated metabolite, **3**, analyzed for $C_{15}H_{20}BrClO_2$ by HREIMS. Its IR spectrum showed a band due to a terminal acetylene group at ν_{max} 3210 cm^{-1}. In addition, since the IR spectrum revealed no absorptions ascribable to hydroxyl and carbonyl functionalities, two oxygen atoms were assumed to be involved in ether linkages. The presence of a pent-2-en-4-ynyl moiety, which is frequently encountered in C_{15}-acetogenins found in various *Laurencia* species,[2] was readily recognized by the ^1H NMR spectrum (Table 3) [δ_H 3.11 (1H, d, J = 2.0 Hz), 5.57 (1H, dd, J = 10.7, 2.0 Hz), and 6.20 (1H, ddd, J = 10.7, 7.3, 7.3 Hz)]. The J value (10.7 Hz) for H-3 and H-4 as well as the chemical shift value (δ_H 3.11) of the acetylenic proton indicated the geometry of the double bond at C-3 to be *Z*.[18] Detailed analysis of the $^1H-^1H$ COSY spectrum permitted a partial structure **3a** to be assigned (Figure 4).

In the ^{13}C NMR spectrum (Table 3), the chemical shift values of the methine carbons at C-6 (δ_C 84.1), C-7 (δ_C 71.4), C-9 (δ_C 79.3), and C-13 (δ_C 83.0) indicated that the oxygen atoms are attached to these carbons. Moreover, the remaining substituents at C-10 and C-12 were verified to be halogen atoms based upon the chemical shifts of the pertinent carbons at δ_C 62.2 and 52.4, respectively. The ^1H NMR spectrum showed no signals around δ_H 3.5−2.9 due to methine protons on a 1,2-disubstituted oxirane ring[19] and near δ_H 2.7 due to methylene protons on a 1,3-disubstituted oxetane ring.[20,21] Hence two ether rings must be formed by bonding between C-6 and C-9 and between C-7 and C-13, leading to a planar formula **3b** for compound **3**. The formula **3b** was confirmed by the HMBC spectrum (Table 3), in which a cross-peak between H-13 and C-7 unambiguously indicated the link of C-7 and C-13. The positions of the bromine and chlorine atoms were also confirmed by the halogen-induced ^{13}C isotope shifts in the ^{13}C NMR spectrum. The methine carbon at C-12 (δ_C 52.4) showed an isotope shift of 1.22 ppb with relative intensities of about 1:1 induced by ^{79}Br and ^{81}Br. Hence, the bromine atom is linked to C-12 and the chlorine atom to C-10, leading to the planar formula **3**.

The gross structure of compound **3** was the same as chlorofucin (**5**), which has previously been found in *Laurencia snyderae* Dawson collected from La Jolla, California,[22] and in the Vietnamese *Laurencia pannosa*.[23] A detailed comparison of the ^{13}C and ^1H NMR data of both compounds revealed that compound **3** is isomeric with respect to the double bond at C-3 of chlorofucin. However, judging from the sign and the value of optical rotation for (3*Z*)-chlorofucin (**3**) (chlorofucin, $[\alpha]^{20}_D$ +12.0° ($CHCl_3$)[22]), the possibility that **3** has the enantiomeric structure of chlorofucin cannot be ruled out.

The antibacterial activity of the crude extract from *Laurencia pannosa* was tested against 13 species of marine bacteria isolated from algal habitats in the Malaysian waters. Antibacterial activity was seen against three species, *Chromobacterium violaceum*, *Proteus mirabilis*, and *Vibrio cholerae*. Hence, the antibacterial activities of the isolated halometabolites (**1**, **2**, and **3**) were tested against these three species. Pannosanol (**1**), which is the major metabolite of this species, showed antibacterial activity against all of the tested bacteria. The minimum inhibitory concentration (MIC) values of **1** were 60 μg/disk against *Proteus mirabilis* and 100 μg/disk against *Chromobacterium violaceum* and *Vibrio cholerae*. On the other hand, pannosane (**2**) and (3*Z*)-chlorofucin (**3**) showed activity only against *Chromobacterium violaceum*. The MIC values of **2** and **3** were 60 and 100 μg/disk, respectively.

The possibility that these halogenated compounds are involved in protecting *L. pannosa* against bacterial intrusion is substantial when we look at the distribution of these compounds within the algal thallus. Halogenated secondary metabolites of *Laurencia* are reported to be synthesized and/or stored in "*corps en cerise*", intracellular refractive globular inclusions, which are exclusively present in a superficial cortical layer and trichoblast cells.[24] These intracellular inclusions are present in all the investigated *Laurencia* species including the algae under study that produce halogenated secondary metabolites. The exclusive distribution of "*corps en cercise*" in the superficial cortical layer and trichoblast cells could be suggestive of

Table 2. ^{13}C NMR (100 MHz, DEPT), ^{1}H NMR (400 MHz), and HMBC Data[a] for Pannosane (**2**)

C[b]	^{13}C (δ)	^{1}H (δ)	multiplicity, J (Hz)	HMBC correlations
1	45.6	1.96	dd, J = 13.2, 13.2 (Ha)	C-2, C-3, C-5, C-11
		2.07	ddd, J = 13.2, 4.4, 2.9 (Hb)	C-2, C-3, C-5, C-6, C-7, C-11
2	62.7	4.44	dd, J = 13.2, 4.4	C-1, C-3, C-15
3	71.6			
4	41.4	2.28	m (Ha)	C-2, C-5, C-6, C-15
		2.29	m (Hb)	C-2, C-5, C-6, C-15
5	26.0	1.49	m (Ha)	C-3, C-4, C-6, C-7, C-11
		1.71	m (Hb)	C-1, C-3, C-6, C-7, C-11
6	50.8			
7	87.7			
8	34.6	1.45	m (Ha)	C-9
		2.01	m (Hb)	C-6, C-9, C-10, C-14
9	39.2	1.61	m (Ha)	C-8, C-10, C-11
		1.63	m (Hb)	C-8, C-10, C-11, C-13
10	85.7			
11	51.1	1.68	m	C-1, C-5, C-9, C-12
12	14.1	1.09	d, J = 7.8	C-6, C-10, C-11
13	18.9	1.28	s	C-9, C-10, C-11
14	18.2	1.27	s	C-6, C-7, C-8
15	23.6	1.69	s	C-2, C-3, C-4

[a] Measured in chloroform-d_1. [b] Assignment was made by the HSQC spectrum.

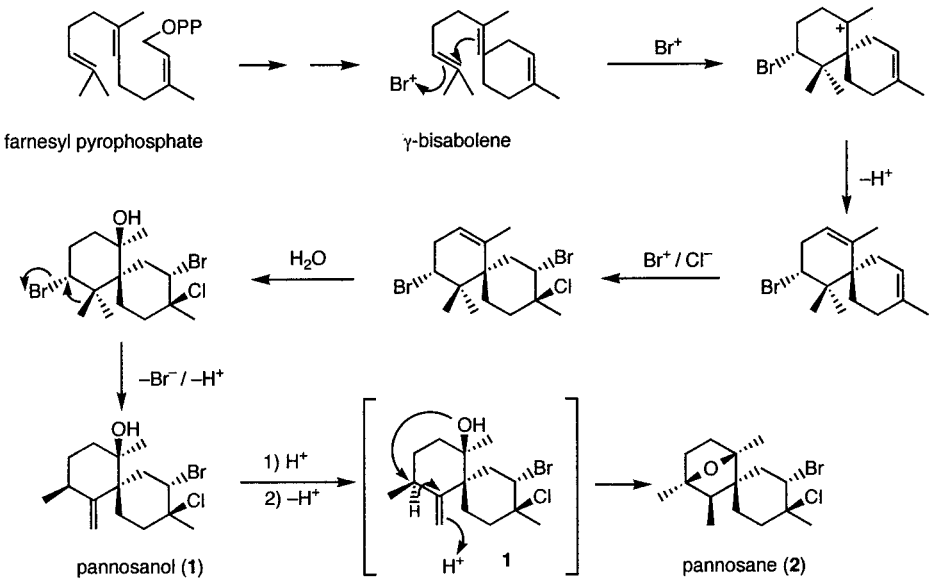

Figure 3. Possible biogenetic pathway of rearranged chamigrane derivatives.

their importance as barricades to withstand any bacterial intrusion.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a JASCO A-102 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured in $CDCl_3$ with TMS as the internal standard by using a JEOL-JNM-EX-400 spectrometer. EIMS and HREIMS were obtained on a JEOL JMS-DX-300 spectrometer. Optical rotations were measured on a JASCO DIP-140 polarimeter. Si gel (Merck, Kieselgel 60, 70-230 mesh) was used for column chromatography. Si gel plates (Merck, Kieselgel 60 F_{254S}) were used for preparative TLC.

Plant Material. A sample of *L. pannosa* Zanardini was collected at Pulau Talang-Talang Kecil (1°53′50″N, 109°45′59″E), Kuching, Sarawak, Malaysia, on May 26th, 1998. The voucher specimens (voucher nos. 089277, 089278, 089279) are deposited in the Herbarium (SAP) of the Graduate School of Science, Hokkaido University.

Extraction and Isolation. The partially dried alga (41 g) was extracted with MeOH. The MeOH solution was concentrated in vacuo and partitioned between Et_2O and H_2O. The Et_2O solution was washed with water, dried over anhydrous Na_2SO_4, and evaporated to leave a dark green oil (550 mg). The extract was then fractionated by Si gel column chromatography with a step gradient (hexane and EtOAc). The fraction eluted with hexanes−EtOAc (9:1) was further submitted to preparative TLC with hexanes−EtOAc (3:1) to give pannosane (**2**) (37 mg) and (3*Z*)-chlorofucin (**3**) (46 mg). In addition, the fraction eluted with hexanes−EtOAc (3:1) was further submitted to preparative TLC with hexanes−EtOAc (3:1) to give pannosanol (**1**) (122 mg).

Pannosanol (1): colorless oil; $[\alpha]^{24}_D$ +4.97° (*c* 0.52, $CHCl_3$); IR (neat) ν_{max} 3450, 1621, 1295, 1190, 1105, 1083, 1052, 1030, 978, 910, 810 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; LREIMS *m/z* (rel int) 320, 318, 316 $[M - H_2O]^+$ (1:4:3), 239, 237 $[M - H_2O - Br]^+$ (2:6), 201 $[M - H_2O - Br - HCl]^+$ (33), 161 (47), 145 (31), 127 (34), 119 (100), 109 (34), 107 (20), 105 (42), 99 (81), 93 (29), 91 (26), 71 (29), 43 (75); HREIMS *m/z* 316.0584 (calcd for $C_{15}H_{24}{}^{79}Br^{35}ClO$, 316.0593, $M - H_2O$).

Pannosane (2): colorless oil; $[\alpha]^{23}_D$ −6.41° (*c* 0.53, $CHCl_3$); IR (neat) ν_{max} 1360, 1217, 1115, 1088, 1065, 863, 845, 797 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; LREIMS *m/z* (rel int) 338, 336, 334 $[M]^+$ (0.3:0.9:0.6), 257, 255 $[M - Br]^+$ (20:60), 219 $[M - Br - HCl]^+$ (99), 201 $[M - Br - HCl - 2H - O]^+$ (32), 161 (57), 127 (35), 125 (31), 109 (39), 98 (26), 97 (76), 69 (21), 43 (100); HREIMS *m/z* 334.0728 (calcd for $C_{15}H_{24}{}^{79}Br^{35}ClO$, 334.0699, M).

Table 3. ^{13}C NMR (100 MHz, DEPT), ^{1}H NMR (400 MHz), and HMBC Data[a] for (3*Z*)-Chlorofucin (**3**)

C[b]	^{13}C (δ)	^{1}H (δ)	multiplicity, *J* (Hz)	HMBC correlations
1	80.1	3.11	d, *J* = 2.0	C-3, C-4
2	82.1			
3	110.5	5.57	dd, *J* = 10.7, 2.0	C-2, C-5
4	141.2	6.20	ddd, *J* = 10.7, 7.3, 7.3	C-1, C-2, C-5
5	30.1	2.72	ddd, *J* = 7.3, 6.8, 6.3 (Ha)	C-3, C-4, C-6
		2.81	ddd, *J* = 7.3, 6.8, 6.3 (Hb)	C-3, C-4, C-6, C-7
6	84.1	3.80	ddd, *J* = 7.3, 6.8, 2.0	C-4, C-7
7	71.0	4.00	m	C-6, C-9
8	33.3	2.07	ddd, *J* = 6.3, 4.9, 3.4 (Ha)	C-9, C-10
		2.33	m (Hb)	C-6, C-7, C-10
9	79.3	4.31	ddd, *J* = 3, 5.9, 4.9	C-7, C-11
10	62.2	4.22	ddd, *J* = 10.4, 5.4, 4.9	C-8
11	38.4	2.50	ddd, *J* = 10.4, 4.9, 4.4 (Ha)	C-9, C-10, C-12, C-13
		3.29	ddd, *J* = 9.3, 6.8, 4.4 (Hb)	C-12, C-13
12	52.4	4.51	dd, *J* = 9.3, 9.3	C-10, C-11, C-13, C-14
13	83.0	3.99	ddd, *J* = 9.3, 4.4, 3.0	C-7, C-12
14	23.1	1.72	dqd, *J* = 11.8, 7.8, 3.0 (Ha)	C-13, C-15
		1.99	dqd, *J* = 11.8, 7.8, 4.4 (Hb)	C-15
15	11.9	1.08	dd, *J* = 7.8, 7.8	C-14

[a] Measured in chloroform-d_1. [b] Assignment was made by the HSQC spectrum.

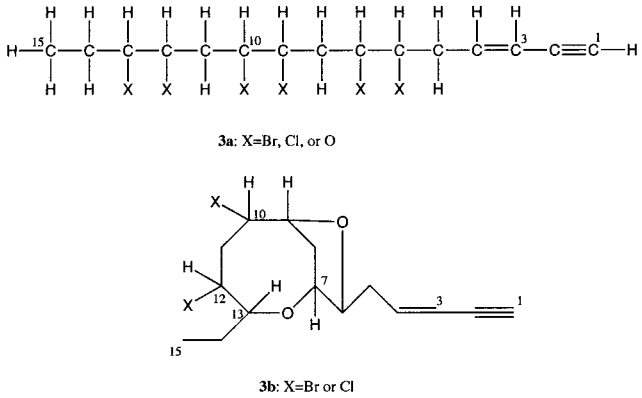

Figure 4. Partial and planar structures for (3*Z*)-chlorofucin (**3**).

(3*Z*)-Chlorofucin (3): colorless oil; $[\alpha]^{24}_D$ −11.3° (*c* 0.58, $CHCl_3$); IR (neat) ν_{max} 3210, 1220, 1180, 1130, 1101, 1042, 928, 898 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 3; LREIMS *m*/*z* (rel int) 350, 348, 346 $[M]^+$ (2:7:5), 313, 311 $[M - Cl]^+$ (7:7), 247, 245 $[M - HCl - C_5H_5]^+$ (15:15), 227 (35), 225 (29), 147 (23), 145 (28), 133 (22), 121 (100), 117 (45), 109 (32), 107 (49), 105 (48), 95 (22), 93 (33), 91 (38), 89 (35), 81 (60), 79 (60), 69 (46), 67 (45), 65 (68), 55 (42), 41 (59); HREIMS *m*/*z* 346.0310 (calcd for $C_{15}H_{20}{}^{79}Br\,ClO_2$, 346.0335, M).

Measurement of ^{13}C NMR Isotope Shifts. The ^{13}C NMR spectra in the bilevel complete decoupled mode were obtained with a JEOL JNM-EX-400 spectrometer. The operating frequency was 100.4 MHz. The spectra were obtained using a 2500 Hz spectral window and acquisition time of 52 s, and the number of points acquired was 131K. The FID was zero-filled to 262K data points (digital resolution was 0.0095 Hz/Pt). The standard Lorentzian−Gaussian resolution enhancement procedure was used before Fourier transformation to achieve a better separation of $^{37}Cl/^{35}Cl$ and $^{81}Br/^{79}Br$ splittings. In the spectrum of pannosanol (**1**), the signal of δ_C 72.9 ppm (C-3) showed an isotopic shift of 1.14 ppb (0.115 Hz) and a peak intensity ratio of 2.86:1, while the isotope shift of the signal for C-2 could not be obtained. On the other hand, in the spectrum of pannosane (**2**), the signal of δ_C 62.6 ppm (C-2) showed an isotopic shift of 2.12 ppb (0.214 Hz) and a peak intensity ratio of 1.0:1.0. The isotope shift of the signal for C-3, however, could not be detected. In addition, in the spectrum of (3*Z*)-chlorofucin (**3**), the signal of δ_C 51.6 ppm (C-12) showed an isotopic shift of 1.22 ppb (0.122 Hz) and a peak intensity ratio of 1.0:1.0, while the isotope shift of the signal for C-10 could not be obtained.

Antibacterial Bioassay. The antibacterial bioassay for the crude extract was carried out using 13 species of marine bacteria isolated from an algal bed at Penang Island, Malaysia.[25,26] These bacteria are *Chromobacterium violaceum*, *Clostridium fallax*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium cellobioparum*, *Escherichia coli*, *Enterobacter aerogenes*, *Flavobacterium helmiphilum*, *Shigella flexneri*, *Proteus mirabilis*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*. Since the antibacterial activities by the crude extract were observed against three species, *Chromobacterium violaceum*, *Proteus mirabilis*, and *Vibrio cholerae*, the bioassay for the isolated halogenated compounds was done against these three species. One loopful of each organism was precultured in 20 mL of peptone water (3% NaCl) overnight. The turbidity of the culture was adjusted to an optical density (OD) McFarland 0.5.[27,28] Then 0.1 mL of the precultured bacterial suspension was used to seed Nutrient Agar plates (3% NaCl). Paper disks (Whatman, 6 mm) impregnated with the crude extract or various amounts of the respective isolated compound were placed on the seeded agar plates, and the diameters of the inhibitory zones were measured after the plates were incubated at 28 °C for 24 h.

Acknowledgment. This study was supported in part by a Grant-in-Aid for Scientific Research (No. 09839001) from the Ministry of Education, Science, Sports and Culture of Japan and also by the Sasakawa Scientific Research Grant from the Japan Science Society (No. 12-300).

References and Notes

(1) Part 4 in the series Chemical Compositions of *Laurencia* Species from the Malaysian Waters. Part 3, see ref 10.
(2) Erickson, K. L. In *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, 1983; Vol. V, pp 131−257.
(3) Hay, M. E.; Fenical, W.; Gustafson, K. *Ecology* **1987**, *68*, 1581−1591.
(4) Kurata, K.; Taniguchi, K.; Agatsuma, Y.; Suzuki, M. *Phytochemistry* **1998**, *47*, 363−369.
(5) Waraszkiewicz, S. M.; Erickson, K. L. *Tetrahedron Lett.* **1974**, 2003−2006.
(6) Wratten, S. J.; Faulkner, D. J. *J. Org. Chem.* **1977**, *42*, 3343−3349.
(7) Carter, G. T.; Rinehart, K. L.; Li, L. H.; Kuentzel, S. L.; Connor, J. L. *Tetrahedron Lett.* **1978**, 4479−4482.
(8) Caccamese, S.; Azzolina, R.; Duesler, E. N.; Paul, I. C.; Rinehart, K. L. *Tetrahedron Lett.* **1980**, *21*, 2299−2302.
(9) König, G. M.; Wright, A. D. *Planta Medica* **1997**, *63*, 186−187.
(10) Vairappan, C. S.; Daitoh, M.; Suzuki, M.; Abe, T.; Masuda, M. *Phytochemistry*, in press.
(11) Vairappan, C. S.; Suzuki, M.; Abe, T.; Masuda, M. *Phytochemistry*, submitted.
(12) Masuda, M.; Takahashi, Y.; Okamoto, K.; Matsuo, Y.; Suzuki, M. *Eur. J. Phycol.* **1997**, *32*, 293−301.
(13) Masuda, M.; Kawaguchi, S.; Takahashi, Y.; Okamoto, K.; Suzuki, M. *Botanica Marina* **1999**, *42*, 199−202.
(14) Sergeyev, N. M.; Sandor, P.; Sergeyeva, N. D.; Raynes, W. T. *J. Magn. Reson. (Ser. A)* **1995**, *115*, 174−182.
(15) Raynes, W. T.; Sergeyev, N. M.; Sandor, P.; Grayson, M. *Magn. Reson. Chem.* **1997**, *35*, 141−143.
(16) Suzuki, M.; Nakano, S.; Takahashi, Y.; Abe, T.; Masuda, M. *Phytochemistry* **1999**, *51*, 657−662.

(17) Bittner, M. L.; Silva, M.; Paul, V. P.; Fenical, W. *Phytochemistry* **1985**, *24*, 987−989.
(18) Suzuki, M.; Kurosawa, E. *Bull. Chem. Soc. Jpn.* **1987**, *52*, 3791−3792.
(19) Tori, K.; Komeno, T.; Nakagawa, T. *J. Org. Chem.* **1964**, *29*, 1136−1141.
(20) Irie, T.; Izawa, M.; Kurosawa, E. *Tetrahedron* **1970**, *26*, 851−870.
(21) Pretsch, E.; Bühlmann, P.; Affolter, C. In *Structure Determination of Organic Compounds*; Springer: Berlin, 2000; p 205.
(22) Howard, B. M.; Schulte, G. R.; Fenical, W. *Tetrahedron* **1980**, *36*, 1747−1751.
(23) Suzuki, M.; Takahashi, Y.; Matsuo, Y.; Masuda, M. *Phytochemistry* **1996**, *41*, 1101−1103.
(24) Young, D. N.; Howard, B. M.; Fenical, W. *J. Phycol.* **1980**, *16*, 182−185.
(25) Vairappan, C. S.; Suzuki, M. *Phycol. Res.* **2000**, *48*, 55−61.
(26) Unpublished data.
(27) Hindler, J. A.; Gonzalez, A. H.; Drake, T. A. *J. Clin. Microbiol.* **1990**, *28*, 1271−1275.
(28) Sonnenwirth, C. A.; Jarett, L. *Gradwohl's Clinical Laboratory Methods and Diagnosis*, 8th ed.; The C. V. Mosby Company: St Louis, 1980; pp 1959−1970.

NP0006317