

Article

A New Bromoallene-Producing Chemical Type of the Red Alga *Laurencia nangii* Masuda

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Abstract: Six populations of *Laurencia nangii* were found to produce three bromoallenes; dihydroitomanallene B (**1**), itomanallene B (**2**) and pannosallene (**3**). Prior to this report, *L. nangii* were only known to produce C₁₅-acetogenins with acetylene functionality. This could be regarded as a new chemical race of *L. nangii*. The compound structures were elucidated on the basis of spectroscopic analysis and comparison with those previously reported in literature. Compound **1**, dihydroitomanallene B, was isolated as a new compound representing a minor variation of itomanallene B (**2**).

Keywords: *Laurencia nangii*; C₁₅-acetogenin; bromoallene; chemical race

1. Introduction

The Red algal genus *Laurencia* (Rhodomelaceae, Ceramiales) is a prolific producer of halogenated secondary metabolites such as sesquiterpenes, diterpenes, triterpenes and C₁₅-acetogenins [1]. Species of this genus are known to produce characteristic sets of halogenated secondary metabolites [2]. However, some species have been reported to produce related or unrelated sets of halogenated metabolites in different populations that are geographically close or distant [3,4]. Several morphologically similar, but chemically distinct, populations have been found in *Laurencia nipponica* Yamada and *Laurencia majuscula* (Harvey) Lucas growing in Japan. Thus, the existence of different chemical types of the same species had been suggested as chemical races [2].

In an ongoing investigation pertaining to the chemical constituents of red algae genus *Laurencia* from the coastal waters of Borneo (Malaysia), we reported the chemical composition of *L. snackeyi* (Weber-van Bosse) Masuda [5], *L. similis* Nam *et al.* Saito [6], *L. nangii* (Masuda) [7], *L. majuscula* (Harvey) Lucas [8–11] and *Laurencia* species [12,13]. Recently, we collected and examined six populations of *L. nangii* from Tun Sakaran Marine Park, Sabah, Malaysia. These six populations contained different types of halogenated non-terpene metabolites that led us to suggest the presence of “chemical races” in *L. nangii* of North Borneo Island, Sabah. Each of these populations showed the presence of a new bromoallene [dihydroitomanallene B (**1**)] along with two known bromoallenes, itomanallene B (**2**) and pannosallene (**3**). The structure of the new compound, dihydroitomanallene B (**1**), was very similar to the known itomanallene B (**2**) and was elucidated based on spectral data. The structures of known metabolites **2–3** were determined based on the comparison of spectral data of published reports of Suzuki *et al.* [14] and Suzuki *et al.* [15]. In this paper, we report the discovery of a bromoallene-producing *L. nangii* and the structure of compound **1**. Compounds **1** and **2** were very labile in CDCl₃, therefore their spectroscopic data were taken in C₆D₆. Hence, this paper will describe the isolation and structure elucidation of compound **1** and the importance of bromoallenes as chemotaxonomical markers in the red alga *L. nangii*.

2. Results and Discussion

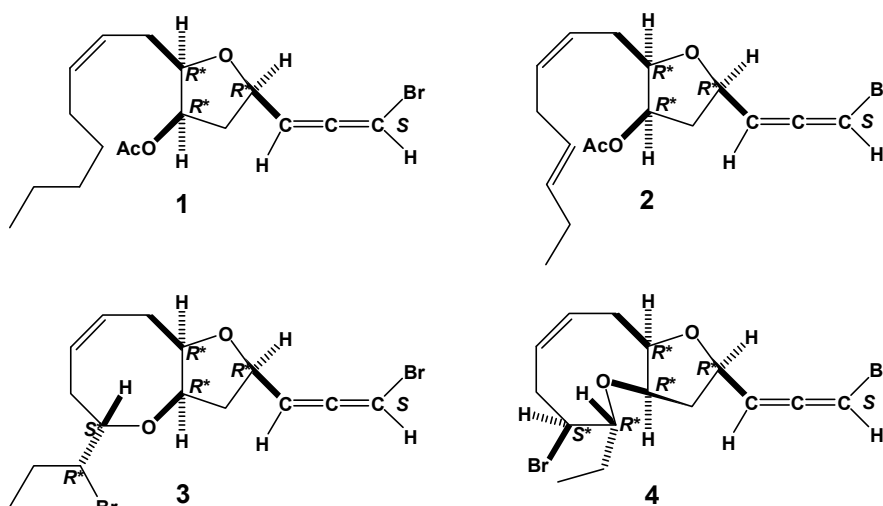
Compound **1** was isolated as a colorless oil, $[\alpha]_D^{25} + 64.01^\circ$ (CHCl₃). HR-MS gave a molecular formula of C₁₇H₂₅BrO₃. The ¹H- and ¹³C-NMR signals of **1** showed the presence of a typical terminal bromoallene moiety at δ H 5.66 (1H, dd, $J = 5.8, 2.0$ Hz) and 5.32 (1H, dd, $J = 5.8, 5.8$ Hz); δ C 201.5 (C), 102.6 (CH) and 73.5 (CH). The IR spectrum revealed the presence of an acetoxyl group without any hydroxyl groups at $\nu_{\max}$ 1,720 cm^{−1}, which was supported by a methyl signal at δ H 1.67 (3H, s) in the ¹H-NMR spectrum. Detailed ¹H and ¹³C-NMR data are given in Table 1. It is also important to note that data presented in Table 1 was taken in C₆D₆ because **1** was easily decomposed when spectra were taken in CDCl₃. However, data comparison of **2** with that of Suzuki *et al.* [14] was done in CDCl₃ since **2** was stable in this solvent. Chemical shift data of **1** and **2** taken in C₆D₆ are shown in Table 1.

The planar structure of **1** was readily determined as formula **1** (Figure 1) by detailed analysis of ¹H- and ¹³C-NMR, ¹H-¹H COSY, HSQC and HMBC spectral data. Moreover, the close resemblance between the C1–C10 ¹H and ¹³C-NMR data of **1** and **2**, together with co-existence of **1** and **2** in same alga indicated that **1** has the same chiral centers at C4, C6 and C7 as **2** and also the double bond at C9–C10 is in the Z-configuration. The bromoallenic moiety of **1** was also assigned as *S* from the strong positive rotation by application of Lowe’s rule [16].

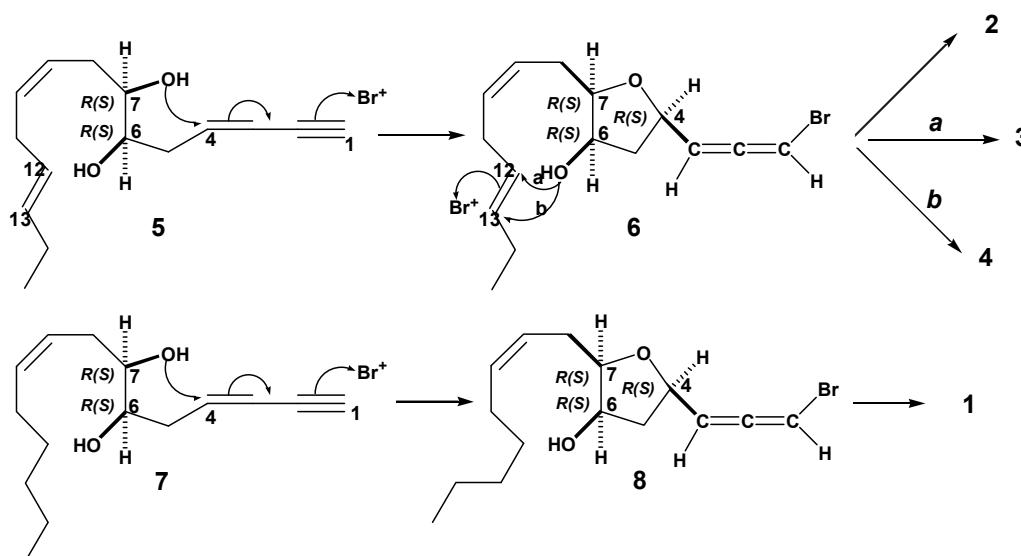
The co-existence of dihydroitomanallene B (**1**), itomanallene B (**2**) and pannosallene (**3**) in Malaysian *L. nangii*, and itomanallene B (**2**) and itomanallene A (**4**) in Japanese *Laurencia intricate* Lamouroux strongly suggested that these four bromoallenes would have the same absolute configurations at C4, C6 and C7. The halogenated C₁₅-acetogenins isolated from various *Laurencia* have been assumed to arise from common precursors, (6*R*,7*R*) or (6*S*,7*S*)-laurediol (**5**) [17]. Hence, dihydroitomanallene B (**1**) may arise from (6*R*,7*R*) or (6*S*,7*S*)-12,13-dihydrolaurediol (**6**) [18]. Bromonium ion-catalyzed cyclization between the hydroxyl group at C7 and C4 in (6*R*,7*R*) or (6*S*,7*S*)-laurediol (**5**) would give a monocyclic bromoallene (**6**) with (4*R*,6*R*,7*R*) or (4*S*,6*S*,7*S*)-configuration. As described by

Kikuchi *et al.* [19], the configurations between C12 and C13 of C₁₅-acetogenins are (12*R*,13*S*) or (12*S*,13*R*)-erythro, reflecting the (12*E*)-double bond in both precursors, (6*R*,7*R*)-laurediols and (6*S*,7*S*)-laurediols. Pannosallene (**3**) and itomanallene A (**4**) could be biosynthesized from **6** by (12*R*,13*R*)-bromonium ion-catalyzed cyclization, *via* route *a* and *b* respectively, as shown in Scheme 1. Compound **6** would further afford itomanallene B (**2**) via acetylation. Similarly, bromonium ion-catalyzed cyclization between the hydroxyl group C7 and C4 in (6*R*,7*R*) or (6*S*,7*S*)-12,13-dihydrolaurediol (**7**) would give a bromoallene (**8**), which would lead to the formation of dihydroitomanallene B (**1**) via acetylation. Thus, the absolute configurations of dihydroitomanallene B (**1**) and itomanallene B (**2**) would be 4*R*, 6*R* and 7*R* or 4*S*, 6*S* and 7*S* as in the case of pannosallene (**3**) and itomanallene A (**4**). The relative configuration between C3 and C4 remains unclear.

Figure 1. Bromoallene compounds **1**, **2**, **3** and **4**.



Scheme 1. Biogenesis pathway of compounds **1**, **2**, **3** and **4**.

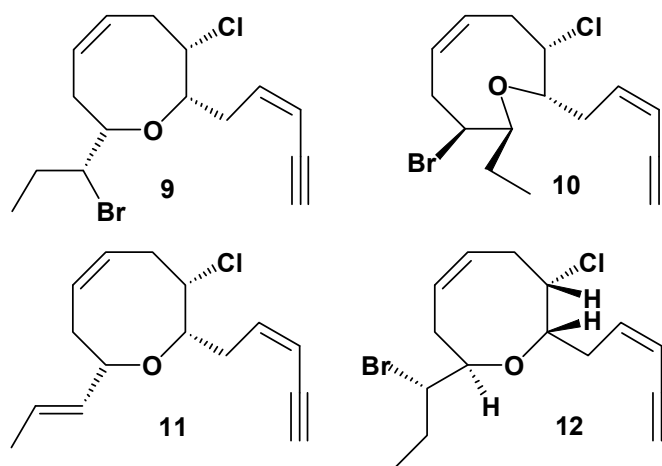


There are close to 50 species of *Laurencia* in this genus, that could be distinguished based on their morphological features and the type of halogenated metabolites they produce. Approximately 500 types of halogenated metabolites have been isolated and they are useful chemotaxonomic markers in

taxonomical classification of this genus [1,16,19]. In the Malaysian coastal waters, there are four major species of *Laurencia*; *L. snackeyi*, *L. majuscula*, *L. similis* and *L. nangii*. They are morphologically distinguishable and could be identified by the halogenated secondary metabolites they produce. *Laurencia snackeyi* produces halogenated snyderane sesquiterpenes, *L. majuscula* produces halogenated chamigranes, while, *L. similis* produces polybrominated indoles [5,6,8–10].

Information on the chemistry of *L. nangii* is very scarce and only two other publications are available, both reporting C₁₅-acetogenins with acetylenic functionalities as the halogenated metabolites produced [7,20]. Masuda originally described a type of *L. nangii* based on several specimens collected from Vietnam. It is a tropical alga with a wide distribution in the South East Asian waters, has been reported to be growing wild in Vietnam, Philippines, Indonesia and Malaysia [21]. During our routine field collection, we discovered six populations of *L. nangii* in Tun Sakaran Marine Park (South East of North Borneo Island) that produces these three bromoallenes. Compound **1**, dihydroitomanallene B, is a new unstable compound with minor chemical differences with itomanallene B (**2**). Itomanallene B (**2**) was first isolated from *L. intricate* by Suzuki *et al.* [14], while panasallene (**3**) was isolated from *Laurencia pannosa* Zanardini collected from Vietnam [15]. Both these specimen are morphologically different from *L. nangii*. Two other reports on *L. nangii*, reported C₁₅-acetogenins, *cis*-pinnatifidenyne (**9**), obtusenyne (**10**), 3(*Z*)-laurenyne (**11**) and *cis*-dihydrorhodophytin (**12**) (Figure 2) as its halogenated metabolite [7,19]. Masuda *et al.* also reported aplysiadiol as a constituent of *L. nangii*. However, upon reexamination, it was apparent that it was a contamination from *Laurencia* sp. that was found growing between thallus of *L. nangii*. To the best of our knowledge, this is the first report of a type of *L. nangii* that only produces bromoallene as its secondary metabolites. Hence, it is suggested this could be a new chemical race of *L. nangii*.

Figure 2. C₁₅-acetogenin compounds **9**, **10**, **11** and **12**.



3. Experimental

3.1. General

Optical rotations were measured on an AUTOPOL IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). ¹H-NMR (600 MHz) and ¹³C-NMR (150 MHz) spectra were recorded with a JEOL ECA 600, with TMS as internal standard. HR-ESI-TOFMS spectrum was

obtained with LCMS-IT-TOF (Shimadzu, Kyoto, Japan). Preparative TLC was performed with silica gel plate (Merck, Frankfurt, Germany; Kieselgel 60 F₂₅₄). Silica gel (Merck, Kieselgel 60, 70–230 mesh) was used for column chromatography. Analytical TLC was performed on Merck Kieselgel 60 F₂₅₄. Spots were visualized by UV light or by spraying with a 5% phosphomolybdic acid-ethanol solution.

3.2. Biological Material

The specimen of *L. nangii* was collected from Tun Sakaran Marine Park, Sabah (5°19'58"N, 115°12'02"E), between 12–28 October 2010. Collected specimens were transported at 4 °C, and air-dried at the laboratory. The voucher herbariums (MAR45943BOR) were made and deposited in the BORNEENSIS Collection of Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

3.3. Extraction and Isolation

The air-dried *Laurencia* (400 g) was extracted with MeOH (1 L) at room temperature for 7 days. The crude extract was evaporated under reduced pressure and the residue was partitioned between EtO₂ and H₂O. The EtO₂ fraction was further exposed to anhydrous sodium sulphate to remove moisture, and concentrated *in vacuo* to yield a dark green crude extract. The EtO₂ extract (800 mg) was chromatographed on a Si gel column using a hexane and EtOAc gradient of increasing polarity (hex:EtOAc; 9.5:0.5, 9:1, 8:2, 7:3, 6:4, 1:1) as eluant to yield six fractions. A portion of fraction 2 (85.8 mg) eluted with hexane/EtOAc (8:2) was submitted to repeated preparative TLC with CHCl₃ and toluene to yield compounds **1** (8.4 mg), **2** (5.2 mg) and **3** (6.8 mg).

3.4. Dihydroitomanallene B (1)

Colorless oil; $[\alpha]_D^{25} + 64.01^\circ$ (CHCl₃; *c* 0.39); IR $\nu_{\max}$ (CHCl₃) cm^{−1}: 2910, 1720, 1350, 1240, 1010, 800; LR-EIMS *m/z* (rel. int): 315, 313 (3:3) [M−CH₃CO]⁺, 275 (32), 273 (32), 247, 245 (16:16) [M−C₈H₁₅]⁺, 217 (18), 111 (25), 43 (100). HR-TOFMS *m/z* 356.0194 [M]⁺ (calcd. for C₁₇H₂₅⁷⁹BrO₃, 356.0187); ¹H-NMR and ¹³C-NMR spectral data: see Table 1.

Table 1. ¹H-NMR and ¹³C-NMR spectral data of compound **1** (recorded at 600/150 MHz in C₆D₆; δ in ppm, *J* in Hz).

C	¹³ C	1	¹³ C	2
		¹ H (<i>J</i> in Hz)		¹ H (<i>J</i> in Hz)
1	73.5	5.66 (dd, <i>J</i> = 5.8, 2.0 Hz, 1H)	73.6	5.67 (dd, <i>J</i> = 5.8, 2.0 Hz, 1H)
2	201.5	-	202.5	-
3	102.6	5.32 (dd, <i>J</i> = 5.8, 5.8 Hz, 1H)	102.6	5.31 (dd, <i>J</i> = 5.8, 5.8 Hz, 1H)
4	74.0	4.16 (dddd, <i>J</i> = 7.3, 5.8, 5.4, 2.0, 1H)	74.0	4.15 (dddd, <i>J</i> = 7.3, 5.8, 5.4, 2.0, 1H)
5	38.9	1.92 (ddd, <i>J</i> = 13.7, 5.4, 1.5 Hz, 1H)	38.9	1.90 (ddd, <i>J</i> = 13.7, 5.4, 1.5 Hz, 1H)
		1.67 (m, 1H)		1.65 (m, 1H)
6	73.9	5.08 (m, 1H)	73.9	5.04 (m, 1H)
7	81.8	3.47 (ddd, <i>J</i> = 7.3, 7.3, 3.4 Hz, 1H)	81.6	3.44 (ddd, <i>J</i> = 7.3, 7.3, 3.4 Hz, 1H)
8	27.5	2.54 (ddd, <i>J</i> = 13.7, 7.3, 3.4 Hz, 1H)	27.3	2.51 (ddd, <i>J</i> = 13.7, 7.3, 3.4 Hz, 1H)
		2.46 (ddd, <i>J</i> = 13.7, 7.3, 3.4 Hz, 1H)		2.43 (ddd, <i>J</i> = 13.7, 7.3, 3.4 Hz, 1H)
9	124.9	5.49 (m, 1H)	125.5	5.50 (m, 1H)

Table 1. Cont.

C	¹³ C	1 ¹ H (J in Hz)	¹³ C	2 ¹ H (J in Hz)
10	132.1	5.49 (m, 1H)	130.0	5.54 (m, 1H)
11	27.4	2.02 (m, 2H)	30.5	2.75 (br t, <i>J</i> = 6.8 Hz, 2H)
12	29.4	1.30 (m, 2H)	127.0	5.39 (m, 1H)
13	31.5	1.22 (m, 2H)	132.4	5.45 (m, 1H)
14	22.7	1.24 (m, 2H)	25.6	1.94 (m, 2H)
15	13.9	0.87 (t, <i>J</i> = 7.3 Hz, 3H)	13.7	0.92 (t, <i>J</i> = 7.3 Hz, 3H)
OAce	169.5	-	170.3	-
	20.4	1.67 (s, 1H)	20.4	1.66 (s, 1H)

4. Conclusions

As a part of our chemical investigation on the Bornean red algae genus *Laurencia*, a new chemical race of *L. nangii* is reported for the first time. A new compound with minor variation from itomanallene B (**2**) was isolated and identified as dihydroitomanallene B (**1**). A total of three bromoallenes [dihydroitomanallene B (**1**), itomanallene B (**2**) and pannosallene (**3**)] were isolated and identified from six populations of *L. nangii* collected from Tun Sakaran Marine Park, Semporna, Sabah, Malaysia. This finding has enriched our knowledge on the chemical constituents of Bornean red algae genus *Laurencia*. Since, chemical race populations of *L. nangii* have not been reported to date, this is the first such finding and suggests this species to consist of at least two chemical races; one that produces C₁₅ acetylenes and another that produces C₁₅ bromoallenes.

Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/1420-3049/17/2/2119/s1>.

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References and Notes

1. Suzuki, M.; Vairappan, C.S. Halogenated secondary metabolites from Japanese species of the red algal genus *Laurencia* (Rhodomelaceae, Ceramiales). *Curr. Top. Phytochem.* **2005**, 1–34.
2. Masuda, M.; Abe, T.; Suzuki, T.; Suzuki, M. Morphological and chemotaxonomic studies on *Laurencia composita* and *L. okamurae* (Ceramiales, Rhodophyta). *Phycologia* **1996**, 35, 550–562.
3. Masuda, M.; Abe, T.; Sato, S.; Suzuki, T.; Suzuki, M. Diversity of halogenated secondary metabolites in the red alga *Laurencia nipponica* (Rhodomelaceae, Ceramiales). *J. Phycol.* **1997**, 33, 196–208.
4. Abe, T.; Masuda, M.; Suzuki, T.; Suzuki, M. Chemical races in the red alga *Laurencia nipponica* (Rhodomelaceae, Ceramiales) *Phycol. Res.* **1999**, 47, 87–95.
5. Tan, K.L.; Matsunaga, S.; Vairappan, C.S. Halogenated chamigranes of red alga *Laurencia snackeyi* (Weber-van Bosse) Masuda from Sulu-Sulawesi Sea. *Biochem. Syst. Ecol.* **2011**, 39, 213–215.

6. Vairappan, C.S.; Ang, M.Y.; Ong, C.Y.; Phang, S.M. Biologically active polybrominated indoles in red algae, *Laurencia similis* from the coastal waters of Sabah (Rhodomelaceae, Ceramiales). *Malays. J. Sci.* **2004**, *23*, 119–126.
7. Vairappan, C.S.; Tan, K.L. C-15 Halogenated acetogenin with antibacterial activity against food pathogens. *Malays. J. Sci.* **2009**, *28*, 263–268.
8. Vairappan, C.S.; Daitoh, M.; Suzuki, M.; Abe, T.; Masuda, M. Antibacterial halogenated metabolites from the Malaysian *Laurencia* sp. *Phytochemistry* **2001**, *58*, 291–297.
9. Vairappan, C.S. Potent antibacterial activity of Malaysian red algal halogenated metabolites against human pathogenic bacteria. *Biomol. Eng.* **2003**, *20*, 255–259.
10. Vairappan, C.S.; Phang, S.M. Morphology and halochamigrene metabolites in Spratly Island species of *Laurencia majuscula* (Rhodomelaceae, Ceramiales). *Malays. J. Sci.* **2005**, *24*, 21–27.
11. Vairappan, C.S.; Sangeetha, P.A.; Tan, K.L.; Matsunaga, S. Role of secondary metabolites as defense chemicals against ice-ice disease bacteria in biofoulers at carrageenophyte farm. *J. Appl. Phycol.* **2010**, *22*, 305–311.
12. Vairappan, C.S.; Ishii, T.; Tan, K.L.; Suzuki, M.; Zhan, Z. Antibacterial activities of halogenated secondary metabolites from Bornean *Laurencia* spp. *Marine Drug.* **2010**, *8*, 1743–1749.
13. Vairappan, C.S.; Suzuki, M.; Okino, T.; Ishii, T.; Abe, T.; Masuda, M. Antibacterial secondary metabolites from Malaysian *Laurencia* sp. *Phytochemistry* **2008**, *69*, 2490–2494.
14. Suzuki, M.; Takahashi, Y.; Mitome, Y.; Itoh, T.; Abe, T.; Masuda, M. Brominated metabolites from an Okinawan *Laurencia intricate*. *Phytochemistry* **2002**, *60*, 861–867.
15. Suzuki, M.; Takahashi, Y.; Matsuo, Y.; Masuda, M. Pannosallene, a brominated C15 nonterpenoid from *Laurencia pannosa*. *Phytochemistry* **1996**, *41*, 1101–1103.
16. Lowe, G. The absolute configuration of allenes. *Chem. Commun.* **1965**, 411–413.
17. Kurosawa, E.; Fukuzawa, A.; Irie, T. *trans*- and *cis*-Laurediol, unsaturated glycols from *Laurencia nipponica* Yamada. *Tetrahedron Lett.* **1972**, *13*, 2121–2124.
18. Fukuzawa, A.; Honma, T.; Takasugi, Y.; Murai, A. Biogenetic intermediates, (3*E* and 3*Z*,12*Z*)-laurediols and (3*E* and 3*Z*)-12,13-dihydrolaurediols isolated from *Laurencia nipponica*. *Phytochemistry* **1993**, *32*, 1435–1438.
19. Kikuchi, H.; Suzuki, T.; Kurosawa, E.; Suzuki, M. The structure of notoryne, a halogenated C15 nonterpenoid with a novel carbon skeleton from the red alga *Laurencia nipponica* Yamada. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 1763–1775.
20. Masuda, M.; Abe, T.; Kogame, K.; Kawaguchi, S.; Phang, S.M.; Daitoh, M.; Sakai, T.; Takahashi, Y.; Suzuki, M. Taxonomic notes on marine algae from Malaysia. VIII. Three species of *Laurencia* (Rhodophyceae). *Bot. Mar.* **2002**, *45*, 571–579.
21. Masuda, M. A taxonomic study of the genus *Laurencia* (Ceramiales, Rhodophyta) from Vietnam. IV. *Laurencia nangii* sp. nov. *Cryptogamie Algal.* **1997**, *18*, 309–318.

Sample Availability: Not available.