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Structures of Laurenenyne-A and -B, Novel Halogenated Acetogenins from a Species of the Red Algal *Laurencia*[1)]

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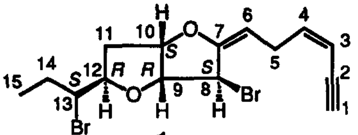
Abstract: Two novel halogenated acetogenins, laurenenyne-A and -B, have been isolated as an inseparable mixture from an undescribed *Laurencia* species collected in Japan. Their structures including the absolute configuration were determined on the basis of spectroscopic and chemical evidence.

Species of the red algal genus *Laurencia* are known to be prolific sources of a variety of halogenated secondary metabolites, particularly terpenoids and C_{15} acetogenins.[2,3)] Species discrimination in *Laurencia* is complicated by a high degree of morphological variation within individual species. Halogenated secondary metabolites are considered to be a useful taxonomic feature at the species level. Although species of the genus *Laurencia* produce several halogenated secondary metabolites in common, most synthesize at least one specific metabolite not found in the others.[4)] Chemical studies based on cultured and field-collected materials of several *Laurencia* species have revealed that synthesis of halogenated secondary metabolites is not affected by environmental factors.[5)] However, there are several instances in which a species of *Laurencia* produces different halogenated metabolites depending on the populations studied.[2)] Diverse secondary metabolites within such a species demand reexamination of the morphological features required for taxonomy.[2)]

In connection with taxonomic reevaluation of Japanese *Laurencia* species[6)] based upon morphological and chemical features as well as genetic affinities, we examined a species of *Laurencia* collected at Kamishima, Toba, Mie Prefecture, on the Pacific coast of central Japan on June 15, 1991. This species differs from all previously known species of the genus in Japanese waters. It is related to *L. tristicha* Tseng, Chang, E. Z. Xia et B. M. Xia reported from Hong Kong in having tristichous branches, but does not agree completely with their original description.[7)] The species under study seems not to have previously been described and a formal description will shortly be published elsewhere. This species contained novel halogenated C_{15} non-terpenoid compounds along with a known halogenated sesquiterpene, 2,10-dibromo-3-chloro-α-chamigrene.[8)] In this paper we describe the isolation and structure elucidation of these novel C_{15} non-terpenoids, named as laurenenyne-A and laurenenyne-B, which seem to be characteristic of this species.

A combination of column and thin-layer chromatography of methanol extracts has led to the isolation of laurenenyne-A (**1**) as the mixture with laurenenyne-B (**2**) in 4.3% yield based on the extracts. It was difficult to separate laurenenyne-A (**1**) from laurenenyne-B (**2**), since the sample gave a single spot on TLC (silica gel) and a broad peak in HPLC (ODS) under the solvent systems used.

The ^{1}H and ^{13}C NMR spectra of this mixture exhibited many doubled signals in a ratio of 3:1, indicating that both compounds were two inseparable isomers, or an equilibrium mixture of two stereoisomers. No spectral change in the ^{1}H NMR spectrum with varying temperature was observed. Furthermore, the ratio of the mixture in the methanol extracts changed with increase of laurenenyne-B (**2**) (2:5) during storage in a freezer (-18 °C) for about twenty days. These results indicate clearly that laurenenyne-A (**1**) and -B (**2**) exist as an inseparable mixture of isomers.



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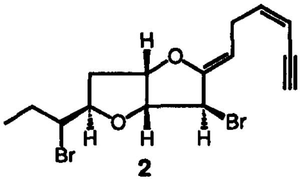

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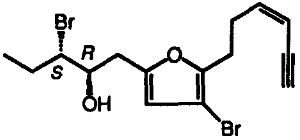

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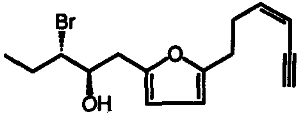

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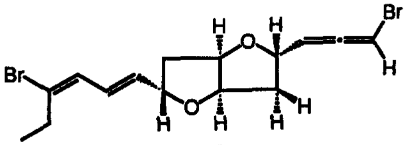

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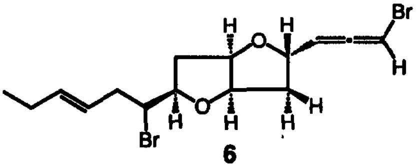

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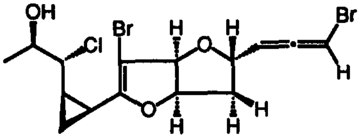

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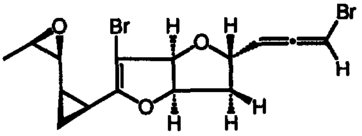

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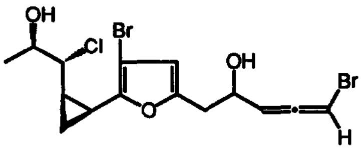

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Molecular formulae of **1** and **2** were established as $C_{15}H_{18}Br_2O_2$ on the basis of the high resolution mass spectrum. As mentioned above, the ^{1}H and ^{13}C NMR spectra of this mixture revealed two sets of signals assignable to laurenenyne-A (**1**) and laurenenyne-B (**2**) as shown in Table 1 and Table 2. The ^{1}H NMR and mass spectra of **1** showed the presence of a *cis*-2-penten-4-ynyl moiety [δ_H 3.15 (1H, d, J=2.0 Hz), 5.49 (1H, br d, J=10.7 Hz), and 5.94 (1H, br ddd, J=10.7, 7.3, and 7.3 Hz) and m/z 65 ($C_5H_5^+$)][9] in the molecule. These data were typical for *Laurencia* cyclic ethers having a C_{15} straight-chain skeleton.

Table 1. ^{13}C (100 MHz) and ^{1}H NMR (400 MHz) data for laurenenyne-A (**1**).

C	^{13}C$^{a)}$ δ	^{1}H$^{b)}$ δ, *J* (Hz)	^{1}H$^{c)}$ δ, *J* (Hz)
1	80.4	3.15, d, *J*=2.0	2.71, d, *J*=2.0
2	82.2		
3	108.6	5.49, br d, *J*=10.7	5.23, br d, *J*=10.7
4	142.6	5.94, br ddd, *J*=10.7, 7.3, 7.3	5.57, br ddd, *J*=10.7, 7.3, 7.3
5	28.0	2.98, br ddd, *J*=7.3, 7.3, 7.3	2.71, br ddd, *J*=7.3, 7.3, 7.3
		3.15, br ddd, *J*=7.3, 7.3, 7.3	3.05, br ddd, *J*=7.3, 7.3, 7.3
6	100.0	4.94, dd, *J*=7.3, 7.3	4.71, dd, *J*=7.3, 7.3
7	156.1		
8	45.0	4.90, s	4.65, s
9	89.8	4.73, d, *J*=2.9	4.22, d, *J*=2.9
10	84.1	5.12, dd, *J*=4.4, 2.9	4.55, dd, *J*=4.4, 2.9
11	37.1	2.33, ddd, *J*=14.6, 8.8, 4.4	1.64, ddd, *J*=14.6, 8.3, 4.4
		2.59, dd, *J*=14.6, 2.9	2.30, dd, *J*=14.6, 2.9
12	84.0	4.26, ddd, *J*=9.8, 8.8, 2.9	3.92, ddd, *J*=8.8, 8.3, 2.9
13	60.5	3.83, ddd, *J*=9.8, 8.8, 2.9	3.76, ddd, *J*=9.8, 8.8, 2.9
14	28.3	1.62, m	1.55, m
		2.04, m	1.95, m
15	11.4	0.99, dd, *J*=7.3, 7.3	0.96, dd, *J*=7.3, 7.3

a) Assignment was made with the aid of HSQC spectrum. b) Measured in chloroform-d_1.
c) Measured in toluene-d_8.

Table 2. ^{13}C (100 MHz) and ^{1}H NMR (400 MHz) data for laurenenyne-B (**2**).

C	^{13}C$^{a)}$ δ	^{1}H$^{b)}$ δ, *J* (Hz)	^{1}H$^{c)}$ δ, *J* (Hz)
1	80.1	3.13, d, *J*=2.0	2.80, d, *J*=2.0
2	82.1		
3	108.7	5.49, br d, *J*=10.7	5.29, br d, *J*=10.7
4	142.6	5.94, br ddd, *J*=10.7, 7.3, 7.3	5.66, br ddd, *J*=10.7, 7.3, 7.3
5	26.7	2.98, br ddd, *J*=7.3, 7.3, 7.3	2.94, br ddd, *J*=7.3, 7.3, 7.3
		3.15, br ddd, *J*=7.3, 7.3, 7.3	3.25, br ddd, *J*=7.3, 7.3, 7.3
6	100.7	4.71, dd, *J*=7.3, 7.3	4.35, dd, *J*=7.3, 7.3
7	154.9		
8	48.7	4.63, s	4.19, s
9	84.2	4.67, d, *J*=2.9	4.15, d, *J*=2.9
10	84.9	5.19, dd, *J*=4.4, 2.9	4.58, dd, *J*=4.4, 2.9
11	37.0	2.36, ddd, *J*=14.6, 8.8, 4.4	1.64, ddd, *J*=14.6, 8.3, 4.4
		2.57, dd, *J*=14.6, 2.9	2.28, dd, *J*=14.6, 2.9
12	84.2	4.27, ddd, *J*=9.8, 8.8, 2.9	3.90, ddd, *J*=8.8, 8.3, 2.9
13	60.5	3.79, ddd, *J*=9.8, 8.8, 2.9	3.72, ddd, *J*=9.8, 8.8, 2.9
14	28.1	1.62, m	1.55, m
		2.04, m	1.95, m
15	11.4	1.00, dd, *J*=7.3, 7.3	0.98, dd, *J*=7.3, 7.3

a) Assignment was made with the aid of HSQC spectrum. b) Measured in chloroform-d_1.
c) Measured in toluene-d_8.

The ^{1}H-^{1}H COSY spectrum of **1** suggested the presence of the partial structural units **A**, **B**, and **C** (Fig.1) in the molecule. All the elements implied by the molecular formula are comprised in the partial structures. Since the IR spectrum showed the absence of hydroxyl and carbonyl functions, two oxygen atoms were assumed to be involved as an ether linkage. Furthermore, an intense absorption at ν_{max} 1681 cm^{-1} indicated the presence of a vinyl ether group which was further supported by the observation of a quaternary carbon atom at δ_C 156.1 in the ^{13}C NMR spectrum of **1**. Therefore, the substituent at C-7 in the unit **C** must be not a bromine atom but an oxygen atom. The chemical shift (δ_C 60.5) of the methine carbon at C-13 in the unit **A** suggested that a bromine atom is attached to this carbon. Moreover, in the unit **A**, substituents at C-9, C-10,

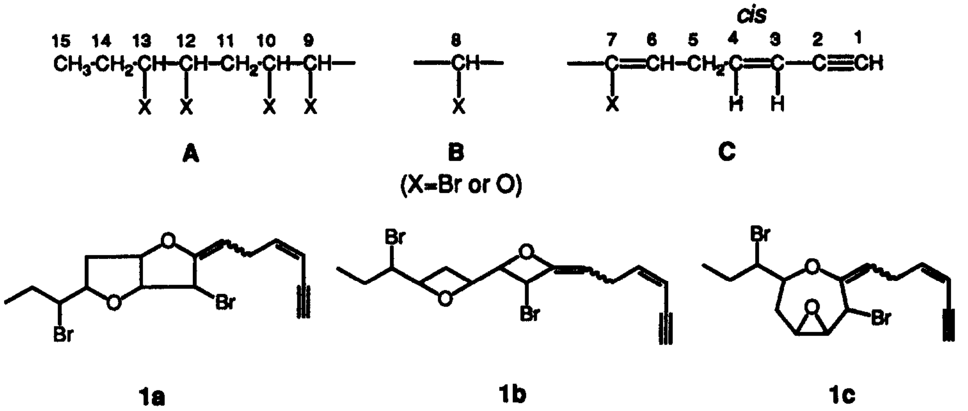


Fig. 1. Partial structural units and possible structures for **1**.

and C-12 are ethereal oxygen atoms based upon the chemical shifts of the pertinent carbons (δ_C 89.8, 84.1, and 84.0, respectively). In consequence, the remaining bromine atom could be attributed to the substituent at C-8 in the unit **B**. Since laurenenyne-A (**1**) has six degrees of unsaturation, **1** has to contain two oxide rings. In view of the above data, three possible planar structures **1a**, **1b**, and **1c** (Fig. 1) were assigned for laurenenyne-A. The latter two structures **1b** and **1c** , however, could be ruled out because of the presence of oxetane ring or oxirane ring, for which there was no evidence. The planar formula **1a** should therefore be assigned as the structure for laurenenyne-A.

The structure of laurenenyne-B (**2**) was also deduced from the spectral data. The ^{1}H-^{1}H COSY spectrum of **2** also indicated the presence of the same partial structures **A**, **B**, and **C** in the molecule. The ^{1}H NMR spectrum (Table 2) of **2** was very similar to that of laurenenyne-A (**1**). A comparison of the spectral data of **1** and **2** suggested that both laurenenynes differ in double-bond geometry at C-6 and C-7. A distinct difference was, however, observed in the signals due to the H-6. The signal of the H-6 in **1** was observed at δ_H 4.94, while that of **2** was observed at δ_H 4.71. This difference of the chemical shifts of the H-6 was explicable through the electronic influence of the ether oxygen atom at C-7. The lower chemical shift of the H-6 in **1** might be ascribed to the deshielding effect of the oxygen atom at C-7, thus establishing that laurenenyne-A possesses *E*-configuration at C-6 and laurenenyne-B *Z*-configuration at C-6. Similarly, the signal of the H-8 (δ_H 4.90) in **1** was also observed in lower field region than that (δ_H 4.63) in **2**, probably reflecting the influence of the conjugated enyne moiety which is situated close to the H-8 in **1**. Similar differences in the chemical shifts of the H-6 and the H-8 were also seen in the spectrum measured in toluene-d_8 (Table 1 and Table 2). The NOE difference spectra did not provide unambiguous data for the assignment of the double-bond geometry at C-6 and C-7 in **1** and **2**.

The relative stereochemistries in laurenenyne-A (**1**) and -B (**2**), excluding that at C-13, were defined by the NOE difference spectra as well as the coupling constants in the ^{1}H NMR spectra. In the NOE difference experiments, irradiation of the H-9 at δ_H 4.73 induced an intense positive NOE of the H-10, a weak NOE of the H-8, and no NOE of the H-12. A weak negative NOE of the H-9 was observed on irradiation of the H-8 at δ_H 4.90. Furthermore, irradiation of the H-12 at δ_H 4.25 revealed no NOE of the H-9. These NOE data indicate unambiguously the relative configurations at C-8, C-9, C-10, and C-12. The relative configurations were supported by the coupling constants in the ^{1}H NMR spectra. The splitting patterns of the protons at C-8, C-9, C-10, C-11, and C-12 were very similar to those of C_{15} bromoallenes with a 2,6-dioxabicyclo[3.3.0]octane skeleton, **5** and **6**, which have been isolated from *L. okamurae* Yamada[10] and *L. nipponica* Yamada,[11] respectively. The ^{1}H NMR spectrum of **5**, in which the two side chains at C-4 and C-9 both had an *exo*-configuration, showed the coupling constants with $J_{5\beta,6}$=$J_{7,8\beta}$=0 Hz, $J_{5\alpha,6}$=5.5 Hz, $J_{6,7}$=4.5 Hz, and $J_{7,8\alpha}$=4.5 Hz. On the other hand, the ^{1}H NMR spectrum of **6**, in which one of the side chain at C-9 had an *endo*-configuration, showed the coupling constants with $J_{5\beta,6}$=0 Hz, $J_{5\alpha,6}$=5.0 Hz, $J_{6,7}$=5.0 Hz, $J_{7,8\beta}$=3.5 Hz, and $J_{7,8\alpha}$=7.5 Hz. In laurenenyne-A and -B, the coupling constants between H-8 and H-9 and between H-10 and Hα-11 were observed as 0 Hz, indicating the *trans*-relationship between H-8 and H-9 and the *exo*-configuration of the bromopropyl side chain at C-12. Furthermore, the *cis*-relationship between H-9 and H-10 was proved by the *J* values between the pertinent protons (2.9 Hz in **1** and **2**).

Pure laurenenyne-A (**1**) and -B (**2**) were very unstable compounds and during storage in a freezer (-18 °C) for three days decomposed into a dark brown oily substance, from which an isomeric alcohol **3**[12] was obtained in about 30% yield. The IR spectrum of **3** showed the presence of a hydroxyl group at ν_{max} 3595 and 3566 cm^{-1} and an end acetylene group at ν_{max} 3300 and 2094 cm^{-1}. Furthermore, the IR spectrum revealed absorptions at ν_{max} 1606 and 1570 cm^{-1}, which suggested the existence of a furan ring since **3** had no aromatic ring. The ^{1}H NMR (Table 3), ^{13}C NMR (Table 3), and ^{1}H-^{1}H COSY spectra of **3** indicated the presence of the partial structures **D**, **E**, **F**, and **G** (Fig. 2) in the molecule. The chemical shift (δ_C 63.8) of the methine carbon at C-13 in the unit **D** suggested the same substitution as in the case of laurenenyne-A (**1**) and -B (**2**). In the ^{1}H-^{1}H COSY spectrum measured in benzene-d_6, the H-12 at δ_H 3.70 (m) was coupled to the proton at δ_H 1.65 (d, *J*=5.9 Hz) which was exchangeable with a deuterium, thus confirming that the substituent at C-12 is a hydroxyl group. Hence the remaining oxygen atom joins to the olefinic carbon atom in the unit **E** and one of the olefinic carbon atom in the unit **F**, and further the bromine atom is attached to another carbon atom in the unit **F**. Since compound **3** has six degrees of unsaturation, the remaining three degrees of unsaturation had to be satisfied by a furan ring as judged from the structural units **E** and **F**. Thus, in order to confirm the structure of **3**, we measured ^{1}H-detected heteronuclear multiple-bond ^{1}H-^{13}C correlation spectrum (HMBC).[13] In the HMBC spectrum (Table 3) the H_2-5 showed cross peaks to C-3, C-4, C-6, and a quaternary carbon at δ_C 151.1. The H_2-6 revealed couplings to C-4 ,C-5, and two quaternary carbons at δ_C 151.1 and δ_C 97.0. Furthermore, the H_2-11 showed cross peaks to a quaternary carbon at δ_C 150.6 and a methine carbon at δ_C 111.4 as well as C-12 and C-13. The proton at δ_H 6.14, which was adjacent to the methine carbon at δ_C 111.4, showed cross peaks to

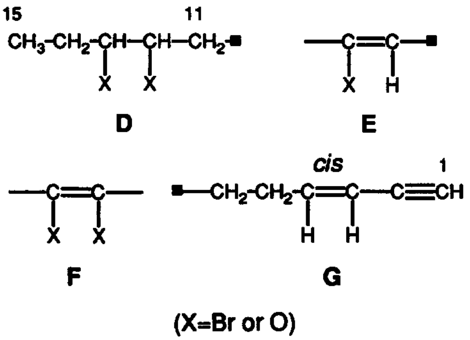


Fig. 2. Partial structural units for **3**. (■ : quaternary carbon atom)

Table 3. ^{13}C (100 MHz) and ^{1}H NMR (400 MHz) and HMBC data for compound 3.

C	$^{13}C^{a)}$ δ	$^{1}H^{b)}$ δ, *J* (Hz)		long range correlations
1	80.0	3.10,	d, *J*=2.0	
2	81.9			H-3, H-4
3	109.5	5.49,	br d, *J*=10.7	H-1, H_2-5
4	143.6	6.00,	br ddd, *J*=10.7, 7.3, 7.3	H_2-5, H_2-6
5	28.5	2.67,	m	H-3, H-4, H_2-6
6	25.2	2.75,	m	H-4, H_2-5
7	151.1			H_2-5, H_2-6, H-9
8	97.0			H_2-6
9	111.4	6.14,	s	H_2-11
10	150.6			H-9, H_2-11
11	32.9	2.89, 2.97,	dd, *J*=15.1, 7.3 dd, *J*=15.1, 3.9	H-9, H-12, H-13, OH
12	72.9	~4.0 ,	$m^{c)}$	H_2-11, H-13, OH
13	63.8	~4.0 ,	$m^{d)}$	H_2-11, H_2-14, H_3-15, OH
14	26.9	1.86, 1.98,	m m	H-13, H_3-15
15	12.4	1.09,	dd, *J*=7.3, 7.3	H_2-14, H-13
OH		2.17,	d, *J*=5.4	

a) Assignment was made with the aid of HSQC spectrum. b) Measured in chloroform-d_1.
c) δ 3.70 (m) in benzene-d_6. d) δ 3.65 (ddd) in benzene-d_6.

C-11 and two quaternary carbons at δ_C 151.1 and δ_C 150.6. The above results unambiguously indicate that the bromine atom is attached to C-8 to lead formula 3 for the isomeric compound.

The absolute configuration of the secondary hydroxyl group in 3 was established by the application of the advanced Mosher's method.[14] Compound 3 was transformed to (*R*)- and (*S*)-MTPA esters, **3a** and **3b**, respectively. The Δδ values (δ_S-δ_R) obtained from these MTPA esters are shown in Fig. 3. The systematic arrangement of the values except for those of the protons due to the conjugated enyne group showed the *R*-configuration at C-12. The negative sign of the Δδ values for the protons of the conjugated enyne group suggested that compound 3 takes a conformation in which these protons are located near to the MTPA ester group and affected by the anisotropy of the aromatic ring, though we have no evidence for the conformation. Similar phenomena on the reversal of the sign of the Δδ values were reported by Kusumi and his co-workers.[15]

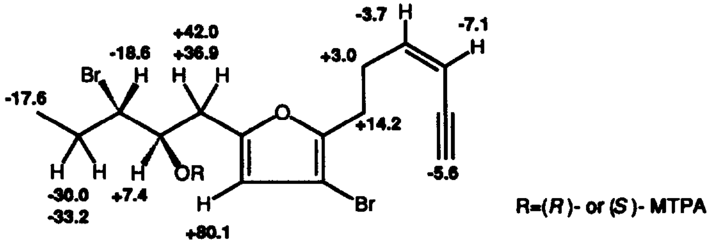
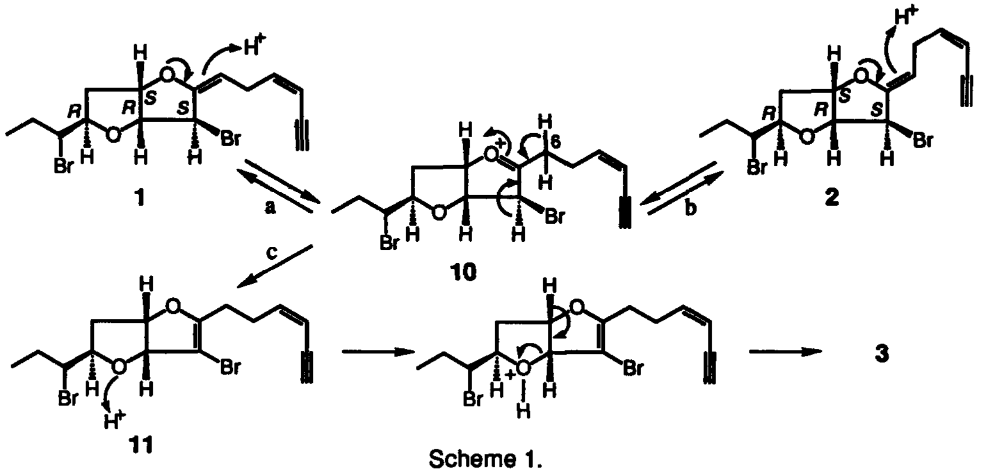


Fig. 3. Δδ values (δ_S- δ_R) of MTPA esters are shown in Hz (400 MHz).

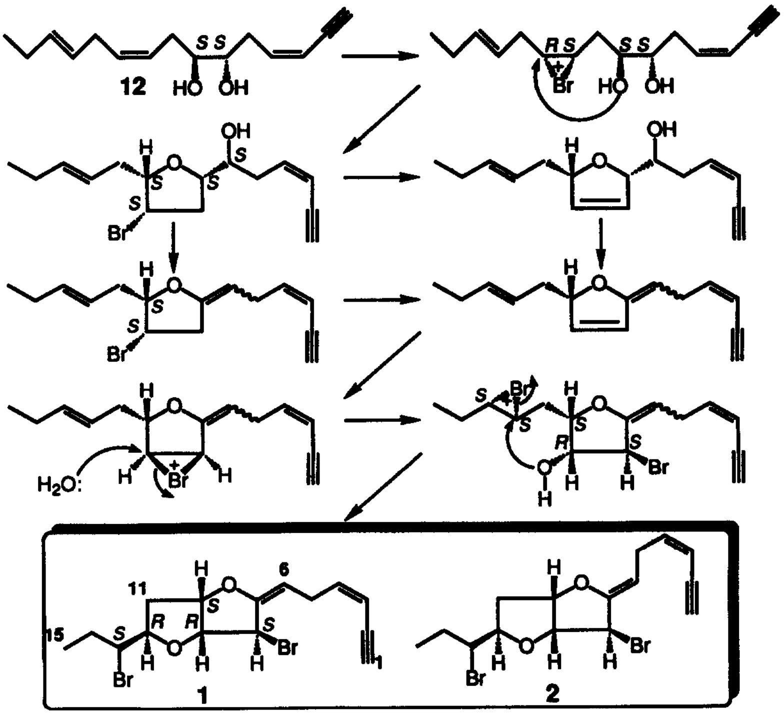


Scheme 1.

Although we have no chemical evidence for interpretation of the isomerization reaction, the mechanism of the isomerization reactions of laurenenyne-A (**1**) and -B (**2**) could be explained by assuming that hydrogen bromide, which derived from both laurenenynes **1** and **2**, may serve as a catalytic initiator as shown in Scheme 1. Protonation of the double bond at C-6 and C-7 in both **1** and **2** may give an oxonium ion **10** from which **1** and **2** may be regenerated by elimination of the proton at C-6 (path a and path b). On the other hand, elimination of the proton at C-8 (path c) may afford a vinyl bromide **11**, which in turn will give rise to a furan derivative **3** via protonation to an ethereal oxygen atom followed by elimination of the H-10. Moreover, elimination of the bromine atom at C-8 in the oxonium ion **10** may yield another furan derivative **4**. Similar isomerization reaction has been encountered in bromoallene **7**[16] with a chlorohydrin moiety. During storage in a freezer (-18 °C) the crystalline bromoallene **7** was changed into a brown oil which gave a furan derivative **9**,[17] while okamurallene (**8**) and its congeners [16] were very stable. Treatment of laurenenynes with deuterated sulfuric acid in methanol at room temperature did not afford any furan derivative.

Since the stereochemistry at C-12 in laurenenyne-A (**1**) and -B (**2**) was retained in the isomeric alcohol **3**, the absolute configuration at C-12 in **1** and **2** was concluded to be the same *R* as that of **3**. Hence the remaining chiral centers at C-8, C-9, and C-10 were assigned as *S*, *R*, and *S*, respectively, based upon the relative stereochemistry discussed above. The absolute configuration of C-13 was deduced from biogenetical viewpoint. Many halogenated C_{15} non-terpenoids isolated from various *Laurencia* species contained (12*R*,13*S*)- or (12*S*,13*R*)-configuration,[2,3] and were suggested to arise from (6*S*,7*S*)- or (6*R*,7*R*)-laurediol,[18] reflecting the (12*E*)-double bond in both precursors, whereas two metabolites from Japanese *L. pinnata*, laurepinnacin and isolaurepinnacin,[19] had (12*R*,13*R*)- and (12*S*,13*S*)-configuration, respectively. Assuming that laurenenyne-A and -B are also biosynthesized from (6*S*,7*S*)- or (6*R*,7*R*)-laurediol via (12*S*,13*S*)-bromonium ion, the absolute configurations at C-12 and C-13 may be *R* and *S*, respectively. A possible biogenetic pathway from (6*S*,7*S*)-laurediol (**12**) is shown in Scheme 2.

Consequently, the structures, including the absolute configuration, of laurenenyne-A and laurenenyne-B must be represented by formulae **1** and **2**, respectively. Laurenenyne-A and-B are the first example of halogenated C_{15} acetogenins from *Laurencia* species with 2,6-dioxabicyclo[3.3.0]octane framework having a bromopropyl and a C_6-enyne side chains as well as a vinyl ether moiety at C-7 and characterize the undescribed species under study.



Scheme 2.

EXPERIMENTAL

The IR spectra were measured on a JASCO IR-700 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ solution, unless otherwise stated, with TMS as the internal standard by using a JEOL JNM-EX-400 spectrometer. The low and high resolution EI mass spectra were recorded on a JEOL JMS-DX303 spectrometer. Optical rotations were measured on a JASCO DIP-140 polarimeter in $CHCl_3$ solution. Silica gel (Merck, Kieselgel 60, 70-230 mesh) was used for column chromatography. Silica-gel plate (Merck, Kieselgel 60 F_{254S}) was used for preparative thin-layer chromatography.

Isolation. A sample of an undescribed species of genus *Laurencia* was collected at Kamisima, Toba, Mie Prefecture, on June 15, 1991. The dried alga (100 g) was soaked in methanol and left for three days. The methanol solution was concentrated *in vacuo* and partitioned between ether and water. The ethereal solution was washed with water, dried over anhydrous Na_2SO_4, and evaporated to leave a green oil (840 mg). The extracts were fractionated by silica-gel column chromatography with a step gradient (hexane, benzene, and ethyl acetate). The fraction eluted with hexane was further chromatographed on a thin-layer plate with hexane to give a colorless oil (80 mg), whose spectral data were coincident with those of 2,10-dibromo-3-chloro-α-chamigrene.[4] The fraction eluted with hexane-benzene (1:1) was submitted to thin-layer chromatography with benzene to yield a mixture of laurenenyne-A (**1**) and laurenenyne-B (**2**) (36 mg).

Laurenenyne-A (**1**): Colorless oil ;$[\alpha]_D^{20}$ -151° (*c* 0.450);[20] IR ($CHCl_3$),[21] ν_{max} 3300, 2094, 1681, 1310, 1284, 1265, 1144, 1110, 1056, 976, 882, 863, 840 and 821 cm^{-1}; ^1H and ^{13}C NMR, Table 1; LR-EIMS (70eV),[21] *m/z* (rel intensity) 392, 390, 388 (0.6:1.1:0.6; M^+), 349, 347, 345 (1.2:2.4:1.2), 327, 325, 323 (0.9:1.9:1.1; M^+-C_5H_5), 311, 309 (11:12; M^+-Br), 269, 267 (17:18; M^+-C_3H_6Br), 159, 157 (20:20),115 (38), 109 (26), 105 (29), 91 (43), 81 (59), 77 (49), 65 (36), 53 (51), 53 (51), 43 (35), 41 (100) and 39 (63); HR-EIMS,[21] *m/z* 389.9653. Calcd for $C_{15}H_{18}{}^{79}Br^{81}BrO_2$, 389.9653 [M].

Laurenenyne-B (**2**): Colorless oil;$[\alpha]_D^{20}$ -168° (*c* 0.550);[20] ^1H and ^{13}C NMR, Table 2.

Compound **3**: Colorless oil;$[\alpha]_D^{13}$ +29.8° (*c* 0.375); IR ($CHCl_3$), ν_{max} 3678, 3596, 3566, 3300, 2394, 1606, 1570, 1287, 1109, 1039 and 854 cm^{-1}; ^1H and ^{13}C NMR, Table 3; LR-EIMS, *m/z* 392, 390, 388 (0.2:0.3:0.2; M^+), 327, 325, 323 (17:34:19; M^+-C_5H_5), 175, 173 (94:100; M^+-$C_9H_{12}BrO$), 113 (49), 71 (76), 65 (90), 51 (38), 43 (70), 41 (54) and 39 (61); HR-EIMS, *m/z* 389.9679. Calcd for $C_{15}H_{18}{}^{79}Br^{81}BrO_2$, 389.9653 [M].

Preparation of (*R*)- and (*S*)-MTPA esters of **3**. (*R*)- and (*S*)-MTPA esters were prepared by using (+)- and (-)-MTPA instead of (+)- and (-)-MTPA chloride, respectively.[22] A solution of **3** (1.1 mg) in dry methylene chloride (0.5 ml) was treated with (+)-MTPA (2.1 mg), dicyclohexylcarbodiimide (10 mg), 4-dimethylaminopyridine (10 mg) and trimethylamine (2.5 μl), and the mixture was allowed to stand at room temperature with stirring for 4 h and evaporated to dryness. The residue was chromatographed on a silica-gel plate with hexane-ethyl acetate (10:1) to afford pure (*R*)-MTPA ester **3a** (0.6 mg). (*S*)-MTPA ester **3b** (0.7 mg) was also prepared by treatment of **3** (1.1 mg) with (-)-MTPA according to the same procedure as in the case of (*R*)-MTPA ester **3a**.

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12) A monobromo compound **4** was also obtained as a minor product; **4**; pale yellow oil; $[\alpha]_D^{20}$ +39.0° (*c* 0.143); ^{1}H NMR, δ 1.09 (3H, dd, *J*=7.3 and 7.3 Hz, H_3-15), 1.85 (1H, m, H-14), 2.00 (1H, m, H-14), 2.23 (1H, d, *J*=5.5 Hz, OH), 2.67 (2H, m, H_2-5), 2.71 (2H, m, H_2-6), 2.92 (1H, dd, *J*=15.1 and 7.3 Hz, H-11), 3.00 (1H, dd, *J*=15.1 and 3.9 Hz), 3.11 (1H, d, *J*=1.9 Hz, H-1), ca. 4.0 (2H, m, H-12 and H-13), 5.49 (1H, br d, *J*=10.7 Hz, H-3), 5.95 (1H, d, *J*=2.9 Hz, H-8), 6.01 (1H, br ddd, *J*=10.7, 7.3 and 7.3 Hz, H-4) and 6.05 (1H, d, *J*=2.9 Hz, H-9); LR-EIMS, *m/z* 312, 310 (0.4:0.4; M^+), 247, 245 (23:24; M^+-C_5H_5), 159 (10), 145 (8), 113 (12), 95 (100), 91 (9), 81 (9), 71 (19), 65 (16), 43 (19), 41 (14) and 39 (14); HR-EIMS, *m/z* 310.0565. Calcd for $C_{15}H_{19}{}^{79}BrO_2$, 310.0568 [M].

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20) The optical rotations of laurenenyne-A (**1**) and -B (**2**) were estimated on the basis of $[\alpha]_D$ values of the mixtures with a different ratio.

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