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Fourteen Chamigrane Derivatives from a Red Alga, *Laurencia nidifica*

Junji Kimura,* Noboru Kamada, and Yoshikazu Tsujimoto

Department of Chemistry, College of Science and Engineering, Aoyama Gakuin University, 6-16-1 Chitosedai, Setagaya-ku, Tokyo 157-8572

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The red algae genus *Laurencia* is well known as a source of halogenated sesquiterpenes. From *Laurencia nidifica*, we isolated fourteen chamigrane derivatives: Two of them are new, accompanied by ten known compounds, and two artifacts. The structures were confirmed by NMR and mass spectroscopy, and compared with spectral data in the literature.

The marine genus *Laurencia* is widely distributed. Chemical investigations of *Laurencia* for secondary metabolites have been lively since 1970.[1] Noteworthy constituents have been chamigrane derivatives, which are halogenated terpenes possessing unique structures compared with their terrestrial counterparts. Some possess attractive physiological activities.

We had an opportunity to collect *L. nidifica*; we reexamined lipid extracts, and isolated two new sesquiterpenes having a chamigrane skeleton as well as ten known chamigrane derivatives and two artifacts.

Freeze-dried alga was soaked in MeOH for 1 d. The MeOH extract was partitioned between $CHCl_3$ and H_2O, and the organic layer was subjected to silica-gel flash chromatography using a stepwise gradient of hexane/ethyl acetate. The first fraction was subjected to repeated HPLC on silica with hexane to yield 2,10-dibromo-3-chloro-α-chamigrene (**1**)[2—4] and nidificene (**2**).[5] Similarly, two new compounds (**3** and **4**) were isolated from the second fraction together with deoxyprepacifenol (**5**)[6,7] and 2,10-dibromo-3-chloro-7,8-epoxychamigrane (**6**).[2] The third fraction afforded nidifidienol (**7**),[8] prepacifenol (**8**),[7,9] prepacifenol epoxide (**9**),[10,11] and pacifenol (**10**).[12] The final fraction yielded johnstonol (**11**)[11,13] and 2,10-dibromo-3-chlorochamigran-7-en-9-ol (**12**) (Fig. 1).[7,14]

The two new compounds (**3** and **4**) have a chami-

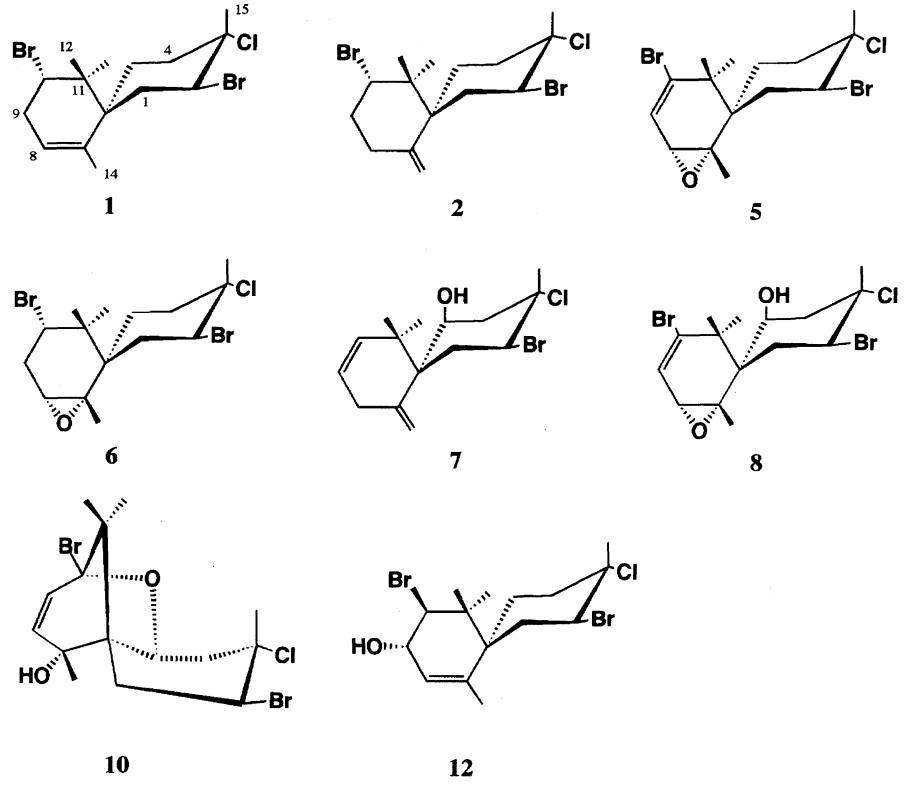
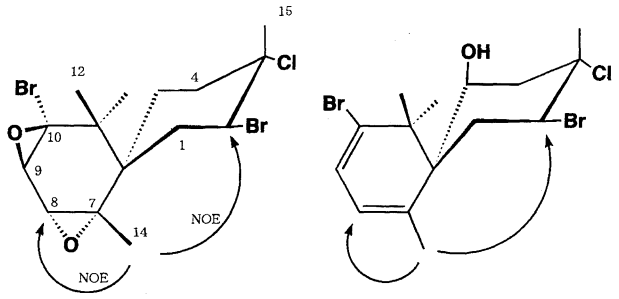


Fig. 1. Known chamigrane derivatives.

grane skeleton with compositions of $C_{15}H_{21}Br_2ClO_2$ and $C_{15}H_{21}Br_2ClO$. Compound **3** displayed a molecular mass of 425.9595 by HREIMS. The ^{1}H NMR spectrum showed characteristic peaks of δ = 3.08 (1H, s), 3.63 (1H, s), and 4.59 (1H, dd, J = 4.6, 12.7 Hz). The ^{13}C NMR data of **3** revealed the presence of five quaternary carbons resonating at δ = 71.3 (C-3), 48.0 (C-6), 60.5 (C-7), 76.3 (C-10), and 45.7 (C-11). These peaks and the coupling constants are similar to that of known compound **9**,[11] and it is clear that the **3** dose not have the C-5 hydroxy group of **9**, i.e., 2,10-dibromo-3-chloro-7,8 : 9,10-diepoxychamigrane (IUPAC; 2,8-dibromo-9-chloro-2,3 : 4,5-diepoxy-1,1,5,9-tetramethylspiro[5,5]undecane). The stereochemistry of **3** was proved by NOE data and by a comparison with spectral data in the literature.[11] The irradiation of δ = 1.47 (CH_3-14) increased the peaks of 3.08 (H-8) and 4.59 (H-2). Furthermore, the peaks at δ = 71.3 (C-3) and 62.6 (C-2) are characteristic of vicinal chloro and bromo carbons of chamigrane derivatives.[15] Hence, **3** possesses the same stereochemistry as that of **9** (Fig. 2).

The ^{1}H NMR spectrum of **4** showed two coupled olefinic protons at δ = 6.20 (d, J = 5.6 Hz) and 5.59 (br.d, J = 5.6 Hz), and HREIMS displayed a molecular mass of 409.9568 ($C_{15}H_{21}{}^{79}Br_2{}^{35}ClO$). Analogous to **3**, the ^{13}C NMR peaks at δ = 51.5 and 45.5 are *spiro* and quaternary carbon-bearing geminal methyl groups in chamigranes, and the peaks at δ = 136.5, 123.6, 126.6, and 140.6 are olefinic carbons. After a detailed examination and comparison with the spectral data in the literature, it is clear that **4** is 2,10-dibromo-3-chloro-chamigrane-7,9-dien-5-ol (IUPAC; 4,8-dibromo-3-chloro-3,7,7,11-tetramethylspiro[5,5]undeca-8,10-dien-1-ol); also, the stereochemistry of **4** was determined as follows. 1) The ^{13}C-chemical shifts for C-3 at δ = 71.4 and for C-2 at 62.8 imply that the chlorine is axial and the bromine is equatorial,[15] and that H-2 is axial by NOE enhancements of δ = 4.90 (H-2) and 5.59 (H-8) in response to the irradiation of 2.05 (CH_3-14). 2) The hydroxy group attached to C-5 is axial because H-5 is clearly equatorial, based on the coupling constants (after a D_2O exchange, the H-5 signal at δ = 4.28 is apparent doublets of doublets having J = 3.4 and 6.7 Hz).[16]



3 **4**

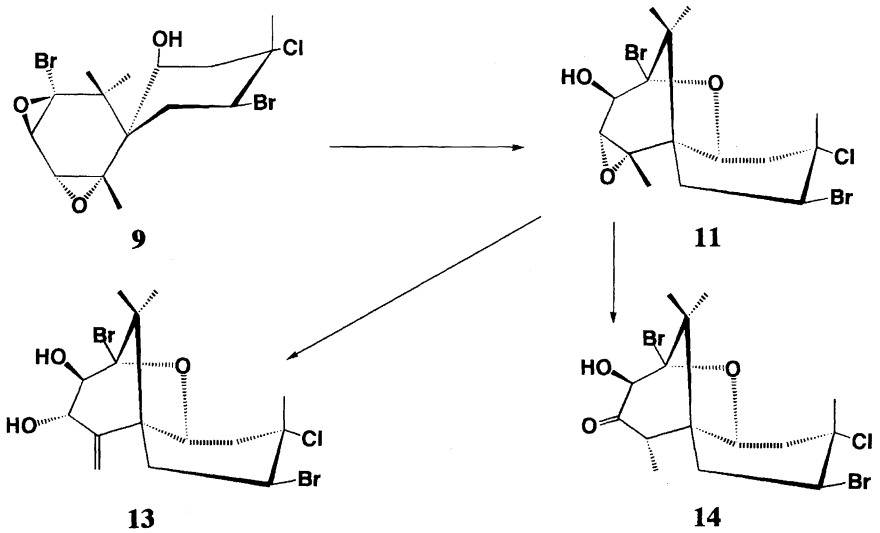
Fig. 2. New chamigrane derivatives.

The structure of **9** was determined based on ^{1}H- and ^{13}C-NMR spectral data in comparison with the literature (Fig. 3).[11] It was known that compound **9** is comparatively unstable and can be converted to **11**.[6] Compound **9**, which was left for a few days in a refrigerator, was subjected to HPLC, again. Two artificial compounds (**13** and **14**) besides **11** were obtained. The ^{1}H NMR spectrum of **13** agreed with that of pacifenediol.[6,10] The ^{1}H NMR spectrum of **14** showed characteristic absorption for a doublet methyl group at δ = 1.39, and for a broad doublet methine-bearing hydroxy group at 4.03. The remaining ring protons matched those of **13** (Fig. 3).

Thus, the 5-hydroxy group of **9** was located at C-10 arising from the cleavage of an epoxy ring. The product, johnstonol (**11**), was further converted to artificial compounds, **13** (pacifenediol) and **14**, by hydrogen transfer.

These chamigranes, containing some acetylated functions, were screened for antiviral activities to Herpes 1-type (HSV-1) (Table 1).

As shown in Table 1, nidificene (**2**) and nidifidienol (**7**) have good activity against HSV-1. The *exo*-methylene group might be a factor in the antiviral activity.



9 **11**

13 **14**

Fig. 3. Possible production of artifacts.

Table 1. Antiviral Activities to HSV-1

Compound	1	2	3	4	5	6	7
IC_{50} ($\mu g\,ml^{-1}$)	> 100	1.5	13.0	> 100	> 100	> 100	2.4
Compound	**9**	**10**	**11**	**11-Ac**	**12**	**12-Ac**	**13**
IC_{50} ($\mu g\,ml^{-1}$)	> 100	7.6	93.5	63.7	> 100	> 100	9.5

Experimental

General Procedures: NMR-spectra were measured on a JEOL instrument operating at 270 MHz for ^1H and 28 MHz for ^{13}C respectively, and the solvent was $CDCl_3$. The optical rotation was determined in $CHCl_3$ on a Perkin Elmer 341 digital polarimer. The mass spectra were obtained with a JEOL SX-102 mass spectrometer. HPLC was performed on a Shimadzu LC-10 apparatus equipped with a RI detector using a GL Science column (Inertsil prep-SIL, 10.0×250 mm). The solvents were distilled prior to use.

Determination of Antiviral Effect by Plaque Reduction Assay:[17] Assays were carried out in confluent Vero cell monolayers in 12-well tissue-culture plates. Vero cell monolayers were inoculated with a F-strain of Herpes simplex virus type 1 dilute in an Eagle minimal essential medium (MEM) containing 0.1% bovine serum albumin (BSA) to yield about 50 plaques per well, and were left for 1 h at 37 °C to allow the viruses to adsorb. The cells were then overlaid with MEM containing 0.1% BSA and 0.8% agarose, with or without the addition of the drug, and incubated at 37 °C. Plaques were visually inspected in agarose overlaid with neutral red on the third day after infection. By reference to the number of plaques observed in virus control monolayers (untreated cultures), the concentration of the test drug which inhibited the plaque numbers by 50% (IC_{50}) was calculated.

Extraction and Separation: The red alga was collected at a depth of about 0.5 m at Pupukea, Oahu in 1996. It was identified by Prof. I. A. Abbot as *Laurencia nidifica*. The freeze-dried sample (200 g) was soaked in MeOH (6 L) for 1 d; after solvent removal, the residue (26.6 g) was obtained. The extract was partitioned between $CHCl_3$ and H_2O (400 mL each), and the aqueous layer was reextracted two times with $CHCl_3$ (400 mL). The combined $CHCl_3$ extract residue (3.97 g) was subjected to silica-gel flash chromatography (Wakogel LP-40, 9.5×7.0 cm) using a stepwise gradient of hexane/ethyl acetate (Fr. 1 ; 100 : 2.5), (Fr. 2 ; 100 : 5), (Fr. 3 ; 100 : 10), then (Fr. 4 ; 100 : 40). Fr. 1 (65 mg) was subjected to repeated HPLC on silica gel to afford 2,10-dibromo-3-chloro-α-chamigrene (**1**, 28 mg) and nidificene (**2**, 2 mg). Similarly, two new chamigrane derivatives (**3**, 1 mg and **4**, 2 mg) were obtained from Fr. 2 (504 mg) together with deoxyprepacifenol (**5**, 61 mg) and 2,10-dibromo-3-chloro-7,8-epoxychamigrane (**6**, 23 mg). Also, nidifidienol (**7**, 102 mg), prepacifenol (**8**, 348 mg), 2,10-dibromo-3-chloro-7,8-epoxychamigrane (**9**, 417 mg), and pacifenol (**10**, 449 mg) were isolated from Fr. 3 (1300 mg). The final fraction (Fr. 4 ; 556 mg) afforded johnstonol (**11**, 106 mg) and 2,10-dibromo-3-chlorochamigran-7-en-9-ol (**12**, 112 mg). All other compounds besides **3** and **4** were identified by a detailed comparison of their ^1H and ^{13}C NMR and mass spectral data with the corresponding literature values.

2,10-Dibromo-3-chloro-7,8 : 9,10-diepoxychamigrane (3): Colorless oil, $[\alpha]_D$ = +91° ($CHCl_3$, c = 0.05); ^1H NMR ($CDCl_3$, ppm) δ = 4.59 (H-2, dd, J = 4.6, 12.7 Hz), 3.63 (H-9, s), 3.08 (H-8, s), 2.10—2.40 (H-1, 4, 5, m), 1.71 (CH_3-15, s), 1.47 (CH_3-14, s), 1.36, 0.98 (CH_3-12, 13, s); ^{13}C NMR ($CDCl_3$, ppm) δ = 76.3 (C-10), 71.3 (C-3), 62.6 (C-2), 60.5 (C-7), 56.6, 55.4 (C-8, 9), 48.0 (C-6), 45.7 (C-11), 40.4, 37.9, 25.8 (C-1, 4, 5), 26.7, 24.0, 23.1, 22.2 (C-12, 13, 14, 15). HREIMS Found: m/z 425.9595. Calcd for $C_{15}H_{21}{}^{79}Br_2{}^{35}ClO_2$: M, 425.9596.

2,10-Dibromo-3-chlorochamigrane-7,9-dien-5-ol (4): Colorless oil, $[\alpha]_D$ = −36° ($CHCl_3$, c = 0.12); ^1H NMR ($CDCl_3$, ppm) δ = 6.20 (H-9, d, J = 5.6 Hz), 5.59 (H-8, br.d, J = 5.6 Hz), 4.90 (H-2, dd, J = 4.7, 13.3 Hz), 4.28 (H-5, m), 2.45 (H-4, dd, J = 6.7, 15.2 Hz), 2.28 (H-4, dd, J = 3.6, 15.0 Hz), 2.37 (H-1, dd, J = 13.3, 14.0 Hz), 2.21 (H-1, dd, J = 4.6, 14.0 Hz), 2.00 (CH_3-14, s), 1.84 (CH_3-15, s), 1.48, 1.02 (CH_3-12, 13, s); ^{13}C NMR ($CDCl_3$, ppm) δ = 140.6, 136.5 (C-7, 10), 126.6, 123.6 (C-8, 9), 74.3 (C-5), 71.4 (C-3), 62.8 (C-2), 51.5 (C-6), 48.4, 36.4 (C-1, 4), 45.5 (C-11), 30.0, 25.7, 23.1, 22.1 (C-12, 13, 14, 15). HREIMS Found: m/z 409.9568. Calcd for $C_{15}H_{21}{}^{79}Br_2{}^{35}ClO$: M, 409.9647.

Isolation of Johnstonol (11), Pacifenediol (13), and 14: Part of crude prepacifenol epoxide (**9**, 26 mg) was subjected to HPLC with 20% ethyl acetate in hexane to afford johnstonol (**11**, 14 mg), pacifenediol (**13**, 8 mg), and **14** (3 mg).

14: $[\alpha]_D$ = −12° ($CHCl_3$, c = 0.01); ^1H NMR ($CDCl_3$, ppm) δ = 4.65 (H-2, dd, J = 4.6, 11.2 Hz), 4.21 (H-5, dd, J = 5.4, 12.0 Hz), 4.03 (H-8, br.d), 2.94 (H-7, q, J = 6.9 Hz), 2.89 (OH-9, d, J = 3.3 Hz), 2.69 (H-4a, dd, J = 5.4, 15.0 Hz), 2.40 (H-4b, dd, J = 12.0, 15.0 Hz), 2.34 (H-1a, dd, J = 11.2, 15.8 Hz), 2.17 (H-1b, dd, J = 4.6, 15.8 Hz), 1.80 (CH_3-15, s), 1.50 (CH_3-13, s), 1.39 (CH_3-14, d, J = 6.9 Hz), 1.12 (CH_3-12, s); ^{13}C NMR ($CDCl_3$, ppm) δ = 208.5 (C-8), 107.4 (C-10), 82.1 (C-9), 76.1 (C-5), 69.1 (C-3), 58.2 (C-2), 52.2 (C-7), 51.0 (C-6), 48.3 (C-11), 46.0 (C-4), 36.6 (C-1), 33.3 (C-15), 28.2 (C-13), 19.6 (C-12), 12.2 (C-14). HREIMS Found: m/z 441.9633. Calcd for $C_{15}H_{21}{}^{79}Br_2{}^{35}ClO_3$: M, 441.9721.

Acetylation of 11 and 12: Acetylation of **11** or **12** was carried out with acetic anhydride and pyridine by the usual method. The acetylated product was quantitatively obtained by preparative TLC (solvent; 20% ethyl acetate in hexane). Acetylated product of **11**: ^1H NMR ($CDCl_3$, ppm) δ = 5.30 (H-8, s), 4.68 (H-5, dd, J = 4.6, 13.7), 4.29 (H-2, dd, J = 3.0, 12.7 Hz), 2.95 (H-8, s), 2.57 (H-4a, dd, J = 4.6, 14.5 Hz), 2.14 (CH_3CO-, s), 2.08—2.28 (H-1, 4b, m), 1.73 (CH_3-15, s), 1.48 (CH_3-14, s), 1.28, 1.15 (CH_3-12, 13, s).

The acetylated product of **12** was identified by a comparison with spectral data in the literature.[14]

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