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Halogenated diterpenoids from the red alga *Laurencia nipponica*

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Abstract

Chemical compositions of three collections of the red alga *Laurencia nipponica* from the western part of the Sea of Japan were studied. One of them contained a series of the previously known sesquiterpenoids. Another one gave C_{15} bromoallene ethers, predominantly. Finally, two new halogenated diterpenes, 15-bromoparguer-9(11)-ene-16-ol and 15-bromoparguer-7-ene-16-ol, were isolated from the third collection of the same species. Structures of these diterpenoids were established by 1D and 2D NMR (^{1}H–^{1}H COSY, DEPT, HMQC, HMBC and NOESY) along with molecular calculations for conformations having lowest energies and mass spectroscopy. Diversity and variability of halogenated secondary metabolites in *L. nipponica* were discussed.

Keywords: *Laurencia nipponica*; Rhodophyceae; Rhodomelaceae; Red alga; Diterpenoid; Halogenated metabolite; Chemical structure; Chemical diversity

1. Introduction

The red algae belonging to the genus *Laurencia* are known to be extremely rich sources of different secondary metabolites (Erickson, 1983; Blunt et al., 2003). Over 50 compounds have been isolated from *Laurencia nipponica* only. However, in spite of the fact that *Laurencia* is one of the most studied genus of marine algae in respect of secondary metabolites until now, new studies on these algae frequently lead to the isolation of new natural products, especially terpenoids and C_{15}-acetogenins.

Taking into consideration that representatives of the same species of this genus from different localities may contain different sets of chemical compounds, we decided to study several samples of the red alga *L. nipponica*, collected in Troitsa and Posyet Bays of the Peter the Great Bay southward Vladivostok. The marine red alga *L. nipponica* growing near Japan is known to have several morphologically similar, but chemically distinct populations which can be referred as chemical races (Masuda et al., 1997; Abe et al., 1999). Up to date there were no reports on natural products of this species populations growing in the Russian waters.

2. Results and discussion

Our investigation of the population of *L. nipponica* collected in August 1998 (see Fig. 1, point 1) used different kinds of chromatography followed by NMR spectroscopy (^{1}H–^{1}H COSY, HMBC, HMQC, NOESY) and GC-MS. As a result, a number of previously known compounds including laurediol diacetate

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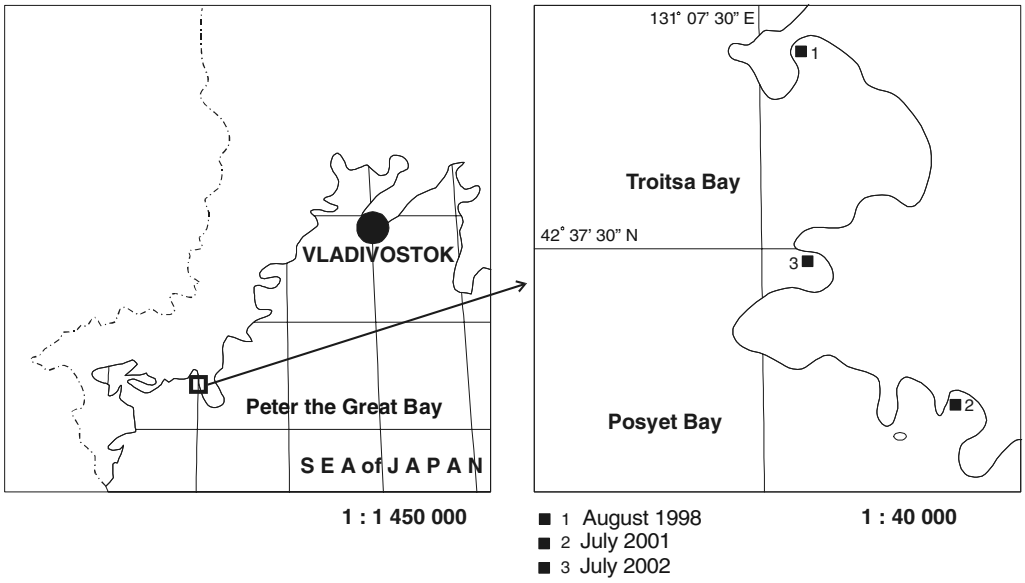
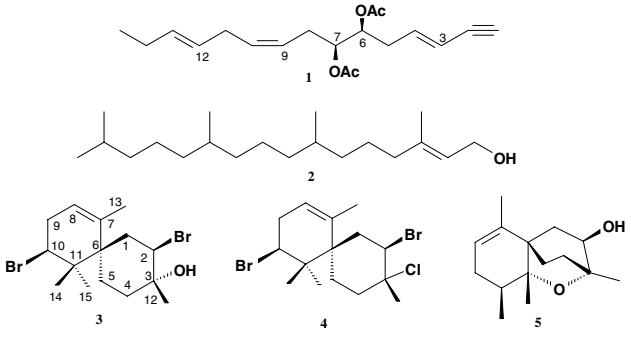
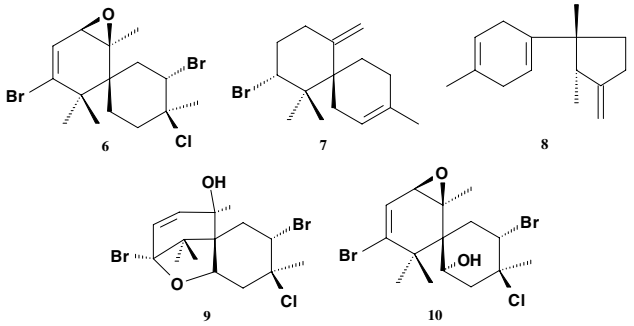


Fig. 1. Geographical localities of the *Laurencia nipponica* collections.

(**1**) (Kurosawa et al., 1972), phytol (**2**) (Sims and Pettus, 1976), 2,10-dibromo-3-hydroxy-α-chamigrene (**3**), 2,10-dibromo-3-chloro-α-chamigrene (**4**) (Suzuki et al., 1983), spironippol (**5**) (Fukuzawa et al., 1981) have been isolated and identified. In addition, two new C_{15} bromoallenes, whose structures and absolute configurations now are under investigation, have been found.

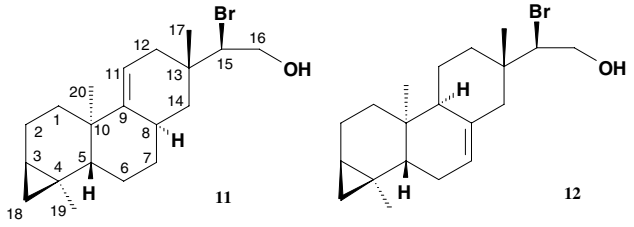


In continuing of our studies, we have collected the same species from Posyet Bay in July 2001 (see Fig. 1, point 2). From that collection, we have isolated deoxyprepacifenol (**6**) (Watanabe et al., 1989), 10-bromo-β-chamigrene (**7**) (König and Wright, 1997), isodihydrolaurene (**8**) (Suzuki et al., 1982) with pacifenol (**9**) (Sims and Fenical, 1971) and prepacifenol (**10**) (Sims and Fenical, 1973) as major constituents.



The identification of prepacifenol has been confirmed by its X-ray diffraction analysis. The structures of other sesquiterpene compounds have been established on the basis of NMR spectra and GC-MS.

In July 2002 (see Fig. 1, point 3) we have collected this alga again in Troitsa Bay (another coast) and isolated only three individual substances, phytol (**2**) and two new pargueranes (**11**, **12**). But surprisingly, this collection of the alga did not show the presence neither sesquiterpenes, nor C_{15} bromoallenes. Herein we report structure elucidation of **11** and **12**.



The red alga *L. nipponica* (division Rhodophyceae), collected from the depth of 1.5–2 m in the Troitsa Bay near Experimental Marine Station of our Institute in July 29, 2002 was extracted with ethanol. A column chromatography of the ethanol soluble materials on LH-20 and silica gel followed by HPLC gave 15-bromoparguer-9(11)-ene-16-ol (**11**) (0.002%) and 15-bromoparguer-7-ene-16-ol (**12**) (0.001%, dry weight).

Compound **11**, a colourless oil, $[\alpha]_D^{25} - 9.1^\circ$ ($CHCl_3$; *c* 0.33), was found to be $C_{20}H_{31}BrO$ by interpretation of its DEPT spectrum as well as on the basis of HREIMS. The ^1H NMR spectrum (Table 1) had three signals in high field at δ_H −0.03 (1H, *dd*, *J* = 5.9, 3.9 Hz), 0.40 (1H, *dd*, *J* = 8.3, 4.0 Hz) and 0.61 (1H, *dt*, *J* = 9.3, 6.0 Hz). These are typical values of the cyclopropane proton signals in spectra of parguerane compounds. The presence of three methyl groups and a trisubstituted double bond was indicated by the ^1H and ^{13}C NMR spectra (δ_H 1.02, 0.97, 0.95 (3H, each, *s*), 5.23 (1H, *dt*, *J* = 6.1, 1.9 Hz), δ_C 25.0, 24.3, 18.0, 147.2, 114.5). Moreover, the ^1H NMR spectrum showed the ABX pattern for β-hydroxy-α-bromo-ethyl side chain [3.84 (1H, *dd*, *J* = 12.5, 8.8 Hz), 3.88 (1H, *br dd*, *J* = 12.5, 4.4), 4.36 (1H, *dd*, *J* = 8.8, 3.5 Hz)]. Assignments were carried out using ^1H–^1H COSY, HSQC and HMBC spectra.

The relative stereochemistry of **11** was established by NOESY spectrum (Table 1). The NOE correlations between H-15/H-8, H_3-20, H_3-17 and H_β-16/(H^α)-14, H-8, H_3-17 allow to assume the 3*R**, 4*S**, 5*S**, 8*R**, 10*R**, 13*R**, 15*S** relative configuration under condition of the equatorial position of CH_3-17 (see Fig. 2). The ^1H NMR and ^{13}C NMR spectra of **11** were very similar to those of the 15-bromoparguer-9(11)-ene-16-ol recently isolated from the Australian red alga *Laurencia filiformis* (Rochfort and Capon, 1996). However, only 19 of 20 carbon atoms in ^{13}C NMR spectra of these compounds showed the same chemical shifts while the remaining signals, which were assigned to C-15, were differing from each other in 3.1 ppm. Moreover, differences (0.2 ppm) in chemical shifts of one angular methyl group (CH_3-17 or CH_3-20, since these resonances were noted as interchangeable by Rochfort and Capon) and in the signals of protons at C-14 (about 0.2 ppm) were observed in ^1H NMR spectra of **11** and the parguerane derivative isolated from *L. filiformis*. We suggest that **11** may be an epimer at C-15 of 15-bromoparguer-9(11)-ene-16-ol isolated from *L. filiformis* or even differs from that by stereochemistry at both C-13 and C-15.

Compound **12**, a colourless oil, $[\alpha]_D^{25} + 55^\circ$ ($CHCl_3$; *c* 0.14) was determined to have the molecular formula

Table 1
^{13}C NMR (75.5 MHz, DEPT), ^1H NMR (300 MHz), HMBC and NOESY spectroscopic data[a] for 15-bromoparguer-9(11)-ene-16-ol (**11**)

C[b]	^{13}C (δ)	^1H (δ)	Multiplicity, *J* (Hz)	HMBC (H/C)	NOESY (H/H)
1	31.2	1.60 (H_α)	*m*	5, 10	11
		0.92 (H_β)			11, 18β
2	19.5	1.81(H_β)	*m*	1, 4, 10	
		2.00 (H_α)	*m*	1, 18	20
3	19.4	0.61	*dt*, *J* = 9.3, 6.0	1, 18, 19	19
4	16.4				
5	50.2	1.12	*dd*, *J* = 3.6, 13.1		18β
6	25.5	1.57			
		1.83			
7	35.9	1.83			
		0.95			
8	30.7	2.25		9	15, 16, 20
9	147.2				
10	37.6				
11	114.5	5.23	*dt*, *J* = 6.1, 1.9	8, 10, 12, 13	1
12	39.3	1.83 (H_α)			
		2.37 (H_β)			17
13	35.6				
14	41.9	1.28 (H_β)			
		1.81 (H_α)			16
15	70.1	4.36	*dd*, *J* = 8.8, 3.5	13, 16, 17	8, 17, 20
16	64.6	3.84 (H_α)	*dd*, *J* = 12.5, 8.8	15	
		3.88 (H_β)	*br dd*, *J* = 12.5, 4.4		8, 14α, 17
17	25.0	1.02	*s*	12, 13, 14, 15	12β, 15, 16
18	21.5	−0.03 (H_β)	*dd*, *J* = 5.9, 3.9	4, 5, 19	1β, 5
		0.40 (H_α)	*dd*, *J* = 8.3, 4.0	4, 5, 19	19
19	24.3	0.97	*s*	3, 4, 5, 18	3, 18α
20	18.0	0.95	*s*	1, 5, 9, 10	2α, 8, 15

[a] Measured in chloroform-d_1.
[b] Assignments were made with the aid of the ^1H–^1H COSY and HSQC spectra.

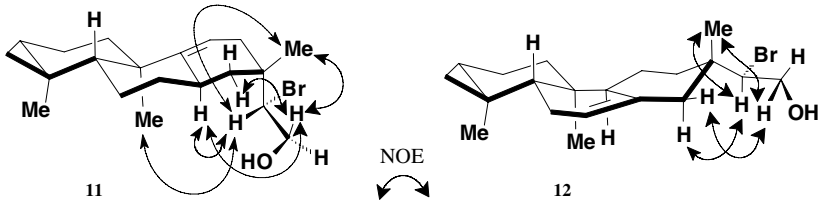


Fig. 2. The key NOE correlations for compounds **11** and **12**.

$C_{20}H_{31}BrO$ (HR-EIMS). The presence of cyclopropane protons was readily proved by typical signals in the ^1H NMR spectrum δ_H 0.03 (1H, *dd*, $J = 9.0$, 3.4 Hz), 0.33 (1H, *dd*, $J = 9.1$, 4.0 Hz) and 0.66 (1H, *m*) (Table 2). The ^1H and ^{13}C NMR spectra indicated the presence of three methyl groups and one trisubstituted double bond [δ_H 0.93, 0.97, 0.82, (3H, each, *s*), 5.34 (1H, *br d*, $J = 5.9$ Hz), δ_C 19.4, 24.7, 19.5, 121.2, 136.3]. Detailed analysis of the ^1H and ^{13}C NMR, ^1H–^1H COSY, DEPT, HSQC, HMBC and NOESY spectra indicated that compound contains a 7(8) double bond and its relative configuration could be presented by formula **12**. The sequences of hydrogen and carbon atoms of rings A, B and C were established by ^1H–^1H COSY and HMBC. The long ranged COSY correlation H-7/H-9, long-range correlations H_3-17/H^{α}-12, H^{α}-14 and H^{β}-12/H^{β}-14 suggest the axial position of CH_3-17 and the chair conformation of the ring C. Moreover, the axial position of CH_3-20 was proven by the ^1H–^1H COSY long-range correlation between H_3-20/H^{β}-1, H^{β}-5 as well as syn diaxial position CH_3-19 and CH_3-20 was confirmed by their long-range COSY correlation. The 3*R**, 4*S**, 5*S**, 9*R**, 10*R**, 13*R**, 15*S** relative configurations were assumed on the basis of NOE correlations between H-15/H-14$^{\alpha}$, H_3-17 and $H_{\beta}16$/(H^{β})-14, H_3-17 at axial position of CH_3-17 (see Fig. 2).

Strain-energy minimised conformations for **11** and **12** have been determined by the semiempirical molecular calculation (AM1 method) using CS MOPAC Rro®, implementation of CS Chem3D Rro®, CambridgeSoft Corporation (http://www.camsoft.com). The lowest energy conformations (see Fig. 3) are in a good agreement

Table 2
^{13}C NMR (74.5 MHz, DEPT), ^1H NMR (300 MHz), HMBC and NOESY spectroscopic data[a] for 15-bromoparguer-7-ene-16-ol (**12**)

C[b]	^{13}C (δ)	^1H (δ)	Multiplicity, J (Hz)	HMBC (H/C)	NOESY (H/H)
1	30.3	0.96 (H_{α})	*m*		
		1.07 (H_{β})			18β
2	19.3	1.68 (H_{β})	*ddd*, $J = 13.4$, 5.2,		
		1.93 (H_{α})	3.2		20
3	20.6	0.66	*m*		19
4	15.1				
5	38.6	1.54	*m*	4	18β
6	27.1	2.00	*m*		
		2.15	*m*	10	
7	121.2	5.34	*br d*, $J = 5.9$		
8	136.3				
9	50.3	1.34	*m*		20, 14α
10	32.4				
11	24.6	1.10 (H_{β})1,60 (H_{α})	*mm*		17
12	37.2	1.39 (H_{α})	*td*, $J = 13.1$, 4.3		
		1.76 (H_{β})	*dm*, $J = 13.2$		
13	39.6				
14	46.9	1.92 (H_{β})	*m*	7, 8, 9, 13	16
		2,12 (H_{α})	*m*	7, 8	15, 9
15	76.6	4.07	*dd*, $J = 9.3$, 2.8	12, 16	17, 14α
16	63.9	3.80 (H_{α})	*dd*, $J = 12.7$, 3.7		
		3.94 (H_{β})	*dd*, $J = 12.4$, 2.8		17, 14β,
17	19.4	0.93	*s*	12, 13, 14, 15	11β, 15, 16
18	19.7	0.03 (H_{β})	*dd*, $J = 9.0$, 3.4	2, 5, 19	1β, 5
		0.33 (H_{α})	*dd*, $J = 9.1$, 4.0	2, 4, 5, 19	19
19	24.7	0.97	*s*	3, 4, 5	3, 18α
20	19.5	0.82	*s*	1, 5, 9, 10	2α, 9

[a] Measured in chloroform-d_1.
[b] Assignment were made with the aid of the ^1H–^1H COSY and HSQC spectra.

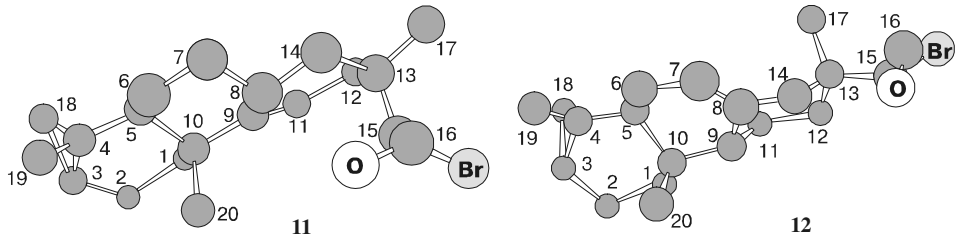


Fig. 3. Strain-energy minimised conformations of compounds **11** and **12**.

with the long ranged COSY correlations and observed NOEs and hence with proposed structures for metabolites **11** and **12**.

The natural products having the parguerane carbon skeleton were first described in result of an investigation on secondary metabolites of the sea hare *Aplysia dactylomela* who feed on red algae such as *Laurencia* spp. (Schmitz et al., 1982). Some later structures and absolute configurations of parguerene type compounds isolated from red alga *Laurencia obtusa* were elucidated by X-ray analysis (Suzuki et al., 1989). Recently, several new brominated parguerane diterpenes have been isolated from the same species (Takeda et al., 1990) as well as from *L. filiformis* (Rochfort and Capon, 1996) and *L. saitoi* (Kurata et al., 1998). These studies are addressed to the relative stereochemistry of the tetracyclic carbon skeleton of isolated parguerenes with the exception of that at C-15. Our study represents the next case of parguerane isolation. We have found two new porguerane diterpenoids, one of them having a new double bond position (Δ^7). Previously, parguerane derivatives were not isolated from *L. nipponica.*

The intracellular inclusions "*corps en cerise*" are considered sites of synthesis and/or storage of halogenated compounds whose importance as chemical defence substances is undeniable. It is well known that there is a great variability in morphological features within species belonging to the genus *Laurencia* including content of "*corps en cerise*", which may be connected with chemical diversity and variability of secondary metabolites in *Laurencia* spp. Moreover, Masuda et al. (1997) revealed a great diversity of halogenated secondary metabolites within one species, namely *L. nipponica*, even in morphologically similar populations from sympatric locality. At least two types of chemical races were found. One of them was characterised by prepacifenol (Sims and Fenical, 1973) and related compounds as specific end products of halogenated sesquiterpenoid biosynthesis. Another chemical type accumulated C_{15} bromoethers such as laureatin (Suzuki and Kurosawa, 1987).

Our results show that Russian populations of the same species contain similar chemical races and one additional chemical race which is characterised by biosynthesis of diterpenoids of parguerane series.

3. Experimental

3.1. General

^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz); $CDCl_3$, TMS as int. standard (coupling constant, J in Hz); GLC-MS: HP-5MS capillary column (30.0 m × 250 μm × 0.25 μm) at 150 → 230 °C, helium, 70 eV. Optical rotation ($CHCl_3$). CC: silica-gel (KSK, Russia, 0.125–0.250 mm), LH-20 (Sephadex). HPLC: ULTRASPHERA™ Si (5μ, 250 × 4.6 mm) and. SERVA Zorbax-ODS® (5μ, 4.6 × 150 mm). TLC: silica-gel plate (Sorbfil, Russia, 5-17 μm). All known metabolites were identified by comparison of their spectral data with those of the authentic specimens. Yields are based on the weight of the dry alga.

3.2. Collection

A samples of *L. nipponica* were collected in Troitsa Bay of the Peter the Great Bay, Sea of Japan, Russia, 1.5–2 m, in August 1998 and July, 2002, Posyet Bay of the Peter the Great Bay, Sea of Japan in July 2001. A voucher specimen is deposited in the Herbarium of the Pacific Institute of Bioorganic Chemistry.

3.3. Extraction and isolation

Fresh alga collected in July 2002 (dry weight 140 g) was exhaustively extracted EtOH. The EtOH solution was evaporated to a dark green oil (917 mg) The extract was chromatographed on LH-20 ($CHCl_3$–MeOH) and then fractionated by silicagel CC with step-wise gradient (hexane–EtOAc). The fraction eluted with hexane–EtOAc (10:1) was further submitted to HPLC on silicagel (hexane–EtOAc, 25:1) to yield, 15-bromoparguer-9(11)-ene-16-ol (3.3 mg; 0.002% based on dry alga) and fraction with 15-bromoparguer-7-ene-16-ol (3.4 mg), which was purified by reverse phase HPLC (MeOH–H_2O, 90:10) to obtain 15-bromoparguer-7-ene-16-ol (1.4 mg, 0.001%).

3.4. 15- Bromoparguer-9(11)-ene-16-ol (*11*)

Oil; $[\alpha]_D^{25}$ − 9.1° ($CHCl_3$; *c* 0.33); ^{1}H and ^{13}C NMR, see Table 1; GLC-MC *m/z* (%) 366/368 [M^+] (17), 351/

353 (61), 337/339 (28), 324/326 (14), 311/313 (61), 286 (14), 269 (8), 243 (28), 227 (22), 211 (14), 187 (39), 161 (39), 145 (47), 119 (64), 105 (94), 91 (100), 79 (67), 67 (42), 51 (42). HR-MS *m/z*: 366.1576. Calc. for $C_{20}H_{31}{}^{79}BrO$, 366.1558 [M].

3.5. 15- Bromoparguer-7-ene-16-ol (*12*)

Oil; $[\alpha]_D^{25} + 55^\circ$ ($CHCl_3$; *c* 0.14); ^1H and ^{13}C NMR, see Table 2; GLC-MC *m/z* (%) 366/368 [M^+] (25), 351/353 (72), 337/339 (26), 324/326 (14), 311/313 (47), 286 (16), 269 (14), 243 (33), 227 (20), 213 (15), 199 (36), 185 (28), 173 (30), 159 (45), 145 (35), 131/133 (45), 119 (61), 105 (82), 91 (100), 79 (64), 67 (34), 55 (37). HR-MS *m/z*: 366.1550. Calc. for $C_{20}H_{31}{}^{79}BrO$, 366.1558 [M].

Acknowledgements

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