Cuparene-Derived Sesquiterpenes from the Chinese Red Alga *Laurencia okamurai* YAMADA

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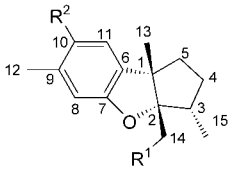
Four new cuparene-derived sesquiterpenes, laureperoxide (**1**), 10-bromoisoaplysin (**2**), isodebromolaurinterol (**3**), and 10-hydroxyisolaurene (**4**), together with seven known, related sesquiterpenes, **5**–**11**, have been isolated from the red alga *Laurencia okamurai*. Their structures were determined on the basis of detailed spectroscopic analyses and comparison with known compounds. Compounds **8**–**11** were shown for the first time to represent true natural products, and the full ^{1}H- and ^{13}C-NMR assignments of **5** are reported for the first time.

Introduction. – Red algae of the genus *Laurencia* (Ceramiales, Rhodomelaceae), an established rich source of halogenated terpenes and C_{15}-cyclic ether enynes [1], can be collected from tropical and subtropical waters. *Laurencia* species have invariably produced an astonishing variety of structurally unusual secondary metabolites [2]. Although the roles of these metabolites have not been clearly elucidated, it was suggested that they function as chemical defense substances against marine herbivores [3]. Moreover, some halogenated metabolites have been reported to possess diverse biological activities [4–7].

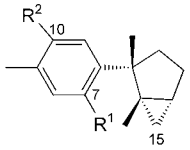
The chemistry of the cosmopolitan *Laurencia okamurai* YAMADA, mainly containing cuparane type sesquiterpenes and acetogenins, is quite varied. In contrast to other *Laurencia* species, two characteristics were observed in previous studies [8]: *1*) the dominance of one major metabolite associated with several minor components difficult to separate, and *2*) a marked variability of the constituents depending on the site of collection.

In the course of our ongoing program toward the isolation of biologically active compounds from Chinese marine organisms [9], we examined constituents of *L. okamurai* collected off the coast of Nanji Island, Zhejiang Province, P. R. China. From this species, we now report the isolation and characterization of four new sesquiterpenes named laureperoxide (**1**), 10-bromoisoaplysin (**2**), isodebromolaurinterol (**3**), and 10-hydroxyisolaurene (**4**), along with seven known, related compounds, aplysinol (**5**) [10], isoaplysin (**6**) [11], debromolaurinterol (**7**) [10], debromolaurinterol acetate (**8**) [12], laurinterol acetate (**9**) [12], debromoisolaurinterol acetate (**10**) [12], and isolaurinterol acetate (**11**) [12], all possessing a cuparane skeleton.

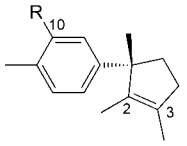
Results and Discussion. – The algal material, collected from Nanji Island (Zhejiang Province, China) was first extracted with acetone, and the residue was partitioned



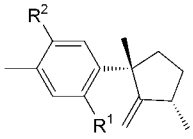
1 R^1 = OOH, R^2 = Br
2 R^1 = R^2 = Br
5 R^1= OH, R^2 = Br
6 R^1 = Br , R^2 = H



3 R^1 = H, R^2 = OH
7 R^1 = OH, R^2 = H
8 R^1 = OAc, R^2 = H
9 R^1 = OAc, R^2 = Br



4 R = OH
12 R = H



10 R^1 = OAc, R^2 = H
11 R^1 = OAc, R^2 = Br

between Et_2O and H_2O. Compounds **1**–**11** were obtained by repeated column chromatographic and RP-HPLC purification of the Et_2O soluble fraction. Compounds **8**–**11** were shown for the first time to be true natural products, and full ^{1}H- and ^{13}C-NMR assignment of **5** has been achieved. The new compounds **1**–**4** show considerable structural analogies with the co-occurring, known sesquiterpenes.

Laureperoxide (**1**) was isolated as an optically active colorless oil. Its molecular formula, $C_{15}H_{19}BrO_3$, was deduced by HR-EI-MS in combination with ^{13}C-NMR (DEPT) experiments. The dominant M^+ peak at *m*/*z* 326/328 in a 100 : 98 ratio in the HR-EI mass spectrum indicated the presence of one Br-atom. Further, the intense peaks at *m*/*z* 294 and 279 due to loss of O_2 and CH_2OOH suggested the presence of a hydroperoxy (OOH) moiety. This was confirmed by a downfield, broadened NMR *singlet* at δ(H) 8.74, which disappeared in the presence of D_2O [13][14]. Analysis of the ^{1}H- and ^{13}C-NMR spectra revealed the presence of three Me groups at δ(H) 1.13 (*d*, $J = 6.8$ Hz), 1.41 (*s*), and δ(H) 2.32 (*s*), an *AB* type CH_2 group bearing a heteroatom at δ(H) 4.26/4.30 (*AB*, $J = 11.8$ Hz), and a 1,2,4,5-tetrasubstituted phenyl ring at δ(H) 6.68, 7.16 (2*s*, 2×1 H). This implied, considering three degrees of unsaturation, a tricyclic sesquiterpene framework. These NMR data were strongly reminiscent of the sesquiterpene aplysinol (**5**) [10]. A comparison of overall ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*, resp.) revealed that **1** differs from **5** only by the presence of an OOH group, in agreement with a molecular-weight difference of 16 mass units. The location of the OOH group at C(14)[1]) was inferred from the downfield chemical shifts of the CH_2(14) resonances relative to those of **5** (*Tables 1* and *2*).

Finally, the relative configurations at C(1), C(2), and C(3) were suggested to be the same as in **5** on the basis of almost identical ^{13}C-NMR chemical shifts, and unequivocally confirmed by means of a NOESY experiment (*Figure*). The absolute configuration of **1** is tentatively assumed to be the same as that of **5**, since the optical rotations of the two compounds were found to be very similar ($[\alpha]_D^{20} = -48.0$ *vs.* -53.0 for **1** and **5**, resp.) [15]. Consequently, the structure of laureperoxide (**1**) was established as [(3*S**,3a*S**,8b*S**)-7-bromo-1,2,3,8b-tetrahydro-3,6,8b-trimethyl-3a*H*-benzo[*b*]cyclopenta[*d*]furan-3a-yl]methyl hydroperoxide.

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[1]) Arbitrary C-atom numbering.

Table 1. *^{1}H-NMR Data for Compounds* **1**–**5**. At 400 MHz in $CDCl_3$, δ in ppm, J in Hz.

	1	**2**	**3**	**4**	**5**
H_β–C(3)	1.97 (*m*)	2.15 (*m*)	1.08 (*m*)	–	1.83 (*m*)
H_α–C(4)	1.69 (*m*)	1.20 (*m*)	1.66 (*m*)	2.29 (*m*)	1.26 (*m*)
H_β–C(4)	1.17 (*m*)	1.72 (*m*)	1.94 (*m*)	2.29 (*m*)	1.68 (*m*)
H_α–C(5)	1.87 (*ddd*, J = 12.6, 6.0, 3.9)	1.69 (*m*)	1.40 (*ddd*, J = 11.9, 7.8, 4.1)	1.93 (*ddd*, J = 12.8, 8.2, 5.7)	1.65 (*m*)
H_β–C(5)	1.63 (*ddd*, J = 12.6, 11.8, 6.5)	1.89 (*dd*, J = 11.2, 5.6)	1.62 (*m*)	1.93 (*ddd*, J = 12.8, 9.2, 7.7)	1.86 (*m*)
H–C(7)	–	–	6.93 (*dd*, J = 7.8, 1.5)	6.74 (*dd*, J = 7.6, 1.6)	–
H–C(8)	6.68 (*s*)	6.67 (*s*)	7.05 (*d*, J = 7.8)	7.03 (*d*, J = 7.6)	6.66 (*s*)
HO–C(10)	–	–	4.56 (*s*)	4.59 (*s*)	–
H–C(11)	7.16 (*s*)	7.14 (*s*)	6.90 (*d*, J = 1.5)	6.65 (*d*, J = 1.6)	7.16 (*s*)
Me(12)	2.32 (*s*)	2.33 (*s*)	2.22 (*s*)	2.22 (*s*)	2.32 (*s*)
Me(13)	1.41 (*s*)	1.52 (*s*)	1.33 (*s*)	1.38 (*s*)	1.48 (*s*)
CH_2(14)[a]	4.26 (*d*, J = 11.8) 4.30 (*d*, J = 11.8)	3.55 (*d*, J = 11.2) 3.69 (*d*, J = 11.2)	1.23 (*s*)	1.39 (*q*, J = 1.2)	3.71 (*d*, J = 12.2) 3.85 (*d*, J = 12.2)
Me(15)[b]	1.13 (*d*, J = 6.8)	1.11 (*d*, J = 6.8)	0.43 (*dd*, J = 7.8, 5.0) 0.63 (*dd*, J = 5.0, 4.2)	1.71 (*q*, J = 1.2)	1.09 (*d*, J = 6.7)
HOO	8.74 (br. *s*)	–	–	–	–

[a]) Me Groups for **3** and **4**. [b]) CH_2 Group for **3**.

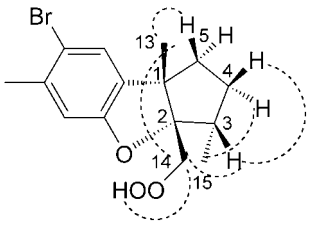

Figure. *Selected key NOESY correlations for compound* **1**

10-Bromoisoaplysin (**2**) was also obtained as a colorless oil. Both ^{13}C-NMR (DEPT) data and HR-EI-MS measurements supported the molecular formula $C_{15}H_{18}Br_2O$. The HR-EI mass spectrum showed the M^+ peak at m/z 372/374/376 in a 1:2:1 ratio, indicating the presence of *two* Br-atoms. The IR spectrum of **2** exhibited no OH and C=O absorptions, indicating that **2** was an ether. The spectral data of **2** were very similar to those of **1** and the co-occurring sesquiterpene isoaplysin (**6**) [11]. Careful comparison of the NMR data of **2** and **1** revealed a difference only in the substituents at C(14) (Br in **2**; OOH in **1**). Due to the replacement of the OOH group by a second Br-atom, the C(14) resonance of **2** was shifted significantly upfield from δ(C) 78.1 to 34.4, in accord with the proposed structure. All NMR data of **2** were unambiguously assigned by ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments, as reported in *Tables 1* and *2*. Once again, the relative configurations at C(1), C(2), and C(3) were deduced by NOESY correlations to correspond to those in **1**. From these

Table 2. *^{13}C-NMR Data of Compounds* **1**–**5**. At 100 MHz in $CDCl_3$; δ in ppm.

Position	**1**	**2**	**3**	**4**	**5**
1	55.3 (*s*)	55.6 (*s*)	48.0 (*s*)	54.7 (*s*)	54.7 (*s*)
2	99.7 (*s*)	97.9 (*s*)	29.3 (*s*)	137.3 (*s*)	100.3 (*s*)
3	42.7 (*d*)	43.8 (*d*)	23.6 (*d*)	132.0 (*s*)	42.4 (*d*)
4	31.6 (*t*)	31.5 (*t*)	25.7 (*t*)	35.9 (*t*)	31.7 (*t*)
5	42.3 (*t*)	42.7 (*t*)	38.2 (*t*)	41.5 (*t*)	42.5 (*t*)
6	135.5 (*s*)	135.7 (*s*)	148.7 (*s*)	149.1 (*s*)	136.4 (*s*)
7	158.0 (*s*)	158.1 (*s*)	119.1 (*d*)	118.6 (*d*)	158.4 (*s*)
8	111.0 (*d*)	110.8 (*d*)	130.5 (*d*)	130.5 (*d*)	110.8 (*d*)
9	137.4 (*s*)	137.3 (*s*)	120.3 (*s*)	120.4 (*s*)	137.1 (*s*)
10	115.0 (*s*)	114.8 (*s*)	153.3 (*s*)	153.5 (*s*)	114.8 (*s*)
11	126.4 (*d*)	126.1 (*d*)	113.4 (*d*)	106.9 (*d*)	126.4 (*d*)
12	23.2 (*q*)	23.2 (*q*)	15.2 (*q*)	15.2 (*q*)	23.1 (*q*)
13	23.1 (*q*)	22.7 (*q*)	26.2 (*q*)	24.2 (*q*)	22.9 (*q*)
14	78.1 (*t*)	34.4 (*t*)	18.2 (*q*)	10.3 (q)	64.0 (*t*)
15	13.7 (*q*)	13.6 (*q*)	15.8 (*t*)	14.3 (*q*)	13.8 (*q*)

data, the structure of **2** was identified as (3*S**,3a*S**,8b*S**)-7-bromo-3a-(bromomethyl)-2,3,3a,8b-tetrahydro-3,6,8b-trimethyl-1*H*-benzo[*b*]cyclopenta[*d*]furan.

Isodebromolaurinterol (**3**) has the molecular formula $C_{15}H_{20}O$, as established by HR-EI-MS, with *m*/*z* 216.1513 (M^+; calc. 216.1514). This formula was identical to that of debromolaurinterol (**7**) [10], which differs from **3** only in the position of the phenolic OH substituent. A NOESY experiment revealed significant cross-peaks between the 10-OH group and both H–C(11) and Me(12), clearly indicating that the OH group was located at C(10) of the aromatic ring. Detailed analysis of the 2D-NMR spectra (^{1}H,^{1}H-COSY, HMQC, HMBC, NOESY) of **3** allowed the unambiguous assignments of all ^{1}H- and ^{13}C-NMR signals (see *Tables 1* and *2*, resp.), as well as the determination of the relative configurations at C(1), C(2), and C(3). Thus, compound **3** was identified as 5-[(1*S**,2*R**,5*R**)-1,2-dimethylbicyclo[3.1.0]hex-2-yl]-2-methylphenol.

Compound **4** was shown to be isomeric with **3**, having the molecular formula $C_{15}H_{20}O$, as indicated by HR-EI-MS (*m*/*z* 216.1509 (M^+; calc. 216.1514)). However, the NMR data of these two compounds were somewhat different. Analysis of the 2D-NMR spectra (^{1}H,^{1}H-COSY, HMQC, HMBC) of **4** indicated the presence of the same 1,2,4-trisubstituted benzene ring, together with a 1,2,3-trimethylcyclopentenyl partial structure, suggested by the presence of two Me resonances at δ(H) 1.39 and 1.71 (2*q*, $J = 1.2$ Hz each), resembling those of isolaurene **12** [16]. 2D-NMR Experiments confirmed that **4** is the 10-OH derivative of isolaurene (**12**), its systematic name being 2-methyl-5-[(1*S**)-1,2,3-trimethylcyclopent-2-en-1-yl]phenol.

The red alga *L. okamurai* of Japanese origin was extensively studied by *Suzuki* and co-workers [10][11][17]. However, no phytochemical investigation of the *Chinese* species has been reported up to now. Although many *Laurencia* sesquiterpenes exhibit antibacterial and antifungal properties [18], the new compounds **1**–**4** were found to be inactive against the fungus *Cladosporium cucumerinum*. Further studies will be conducted with the new compounds to test their bioactivities, such as cytotoxic or anti-inflammatory properties, *etc*. We are also interested in understanding the true biological/ecological role of these metabolites in the life cycle of red algae.

This research was financially supported by the *National Marine 863 Project* (No. 2003AA624030, 2005AA624040), and the *National Natural Science Foundation for Outstanding Chinese Youths* (No. 30125044). We are indebted to Prof. *L.-D. Gu* (Shanghai Normal University, P. R. China), for the collection and identification of the algae.

Experimental Part

General. Reverse-phase high-performance liquid chromatography (RP-HPLC): *Agilent-1100* series apparatus with a *VWD-G1314A* detector (210 nm); semi-prep. *ODS-HG-5* column (5 μm; 10 mm (i.d.) × 25 cm). Column chromatography (CC): silica gel (200–300 and 400–600 mesh; *Qingdao Hai Yang Co.*). TLC: silica gel plates *G60 F-254* (*Yan Tai Zi Fu Co.*). UV Spectra: *Varian Cary-300-Bio* spectrophotometer; λ_{max} (log ε). Optical rotation: *Perkin-Elmer-241MC* polarimeter; in $CHCl_3$. IR Spectra: *Nicolet Magna FT-IR-750* spectrometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker DRX-400* spectrometer (at 400 and 100 MHz, resp.); referencing to residual $CDCl_3$ (δ(H) 7.26, δ(C) 77.0); δ in ppm, *J* in Hz. MS: *Finnigan-MAT-95* mass spectrometer.

Biological Material. The red algae were collected by hand along the coast of Nanji Island in the East China Sea, Zhejiang Province, P. R. China, in June 1999. A voucher specimen (No. MA99-01) was deposited at the Shanghai Institute of Materia Medica (SIBS-CAS) for inspection.

Extraction and Isolation. The fresh algae (500 g dry-weight) were exhaustively extracted with acetone (3 × 1000 ml). The acetone extract was concentrated *in vacuo* to give a residue (36.1 g), which was partitioned between Et_2O and H_2O. The org. extract was evaporated to yield a dark yellow oil (25.8 g), which was fractioned by CC (SiO_2; petroleum ether (PE)/Et_2O gradient): twelve fractions. The fraction eluted with PE/Et_2O 8.5 : 1.5 was re-chromatographed (SiO_2; PE/Et_2O gradient) to afford **2** (16.7 mg) and **6** (2.1 mg). The fraction eluted with PE/Et_2O 9 : 1 was purified by RP-HPLC (MeOH/H_2O 85 : 15) to afford **8** (2.8 mg), **9** (10.9 mg), **10** (1.1 mg), and **11** (1.1 mg). The fraction eluted with PE/Et_2O 7.5 : 2.5 was re-chromatographed (1. SiO_2, PE/Et_2O; 2. *Sephadex LH-20*, PE/$CHCl_3$/MeOH 2 : 1 : 1) and subjected to RP-HPLC (MeOH/H_2O 70 : 30) to afford pure **1** (9.7 mg), **3** (3.0 mg), **4** (6.7 mg), **5** (246.5 mg), and **7** (205.6 mg).

Laureperoxide (= *[(3*S**,3a*S**,8b*S**)-7-Bromo-1,2,3,8b-tetrahydro-3,6,8b-trimethyl-3a*H*-benzo[*b*]cyclopenta[*d*]furan-3a-yl]methyl Hydroperoxide*; **1**). Colorless oil. UV (MeOH): 207 (4.51), 232 (3.55), 299 (3.51). $[\alpha]_D^{20} = -48.0$ (c = 0.25, $CHCl_3$). IR (KBr): 3600, 3450, 3020, 1615, 1605, 1500, 1276, 1011, 987, 930. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-EI-MS: 326.0525 (M^+, $C_{15}H_{19}BrO_3^+$; calc. 326.0518).

10-Bromoisoaplysin (= *(3*S**,3a*S**,8b*S**)-7-Bromo-3a-(bromomethyl)-2,3,3a,8b-tetrahydro-3,6,8b-trimethyl-1*H*-benzo[*b*]cyclopenta[*d*]furan*; **2**). Colorless oil. UV (MeOH): 209 (4.35), 232 (3.53), 299 (3.48). $[\alpha]_D^{20} = -20.9$ (c = 0.42, $CHCl_3$). IR (KBr): 3540, 3001, 1620, 1595, 1500, 1287, 1003, 987. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-EI-MS: 371.9724 (M^+, $C_{15}H_{18}Br_2O^+$; calc. 371.9724).

Isodebromolaurinterol (= *5-[(1*S**,2*R**,5*R**)-1,2-Dimethylbicyclo[3.1.0]hex-2-yl]-2-methylphenol*; **3**). Colorless oil. UV (MeOH): 202 (4.78), 249 (3.51). $[\alpha]_D^{20} = 3.0$ (c = 0.11, $CHCl_3$). IR (KBr): 3350, 3025, 2988, 1620, 1575, 1490, 1351, 876. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-EI-MS: 216.1513 (M^+, $C_{15}H_{20}O^+$; calc. 216.1514).

10-Hydroxyisolaurene (= *2-Methyl-5-[(1*S**)-1,2,3-trimethylcyclopent-2-en-1-yl]phenol*; **4**). Colorless oil. UV (MeOH): 212 (4.31), 278 (3.63), 289 (3.48). $[\alpha]_D^{20} = 25.4$ (c = 0.34, $CHCl_3$). IR (KBr): 3319, 2924, 1620, 1581, 1519, 1408, 1244, 810. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-EI-MS: 216.1513 (M^+, $C_{15}H_{20}O^+$; calc. 216.1514).

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Received January 31, 2005