A Laurane Sesquiterpene and Rearranged Derivatives from the Chinese Red Alga *Laurencia okamurai* Yamada

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One new laurane sesquiterpenoid, 3β-hydroxyaplysin (**2**), and two novel rearranged sesquiterpenes, laurokamurenes A (**3**) and B (**4**), together with three known related compounds, 3α-hydroxydebromoaplysin (**1**), debromoaplysin (**6**), and laurinterol (**7**), have been isolated from the Chinese red alga *Laurencia okamurai*. Their structures, including relative stereochemistry, were determined on the basis of detailed interpretation of 2D NMR spectra and comparison with related known compounds.

Red algae of the genus *Laurencia* (Ceramiales, Rhodomelaceae) are found throughout the world, mostly in tropical and subtropical regions. Over the four decades since Irie's pioneering investigations on *Laurencia*,[1] a significant number of cuparene- and laurane-derived sesquiterpenoids have been isolated from the red algal genus *Laurencia*.[2] These sesquiterpenoids, mostly halogenated with bromine and a few even with iodine, comprise a class of metabolites characteristic of *Laurencia*.[3]

In the course of our ongoing program toward the isolation of biologically active compounds from Chinese marine organisms,[4−6] we have recently examined the red alga *Laurencia okamurai* Yamada, collected off the coast of Nanji Island, Zhejiang Province, China, resulting in the discovery of several cuparene-derived sesquiterpenoids.[7] Our continuing studies on the minor constituents of the same specimen led to the isolation of one additional new laurane sesquiterpenoid, 3β-hydroxyaplysin (**2**), and two novel sesquiterpenes, laurokamurenes A (**3**) and B (**4**), with an uncommon rearranged laurane skeleton, along with three known related compounds, 3α-hydroxydebromoaplysin (**1**),[8] debromoaplysin (**6**),[9] and laurinterol (**7**).[10] We report herein the isolation and structural elucidation of these new minor compounds.

The algal material of *L. okamurai* was extracted with acetone. The Et_2O-soluble portion of this extract was subjected to repeated chromatographic separations [normal-phase silica gel (200−300 and 400−600 mesh) column chromatography, Sephadex LH-20 column chromatography, and reversed-phase HPLC] to give three new sesquiterpenoids (**2**−**4**), respectively, along with three known related sesquiterpenes (**1**, **6**, and **7**).

The known sesquiterpenes were readily identified as 3α-hydroxydebromoaplysin (**1**), debromoaplysin (**6**), and laurinterol (**7**), by comparing their spectroscopic data with those reported in the literature.[8−10] Among them, compound **1** was recently reported as a synthetic intermediate derived in the course of synthesis of **6** and aplysin.[8] However, this is the first report to isolate it from a natural source. The ^{13}C NMR data of **1** are also reported for the first time.

Compound **2** was obtained as a colorless oil. Its molecular formula, $C_{15}H_{19}O_2Br$, was determined by HREIMS at *m*/*z* 310.0591 $[M]^+$ (calc 310.0568**)**, in combination with ^{13}C NMR (DEPT) experiments, suggesting the presence of six degrees of unsaturation. The dominant $[M]^+$ peaks at *m*/*z* 310 and 312 with intensities of 1/0.98 in the HREIMS spectrum indicated the presence of one bromine atom in the molecule. The presence of a hydroxyl group was evident from an IR absorption at 3380 cm^{-1}. The ^1H NMR spectrum revealed two *para*-disposed aromatic protons at δ 6.58 (s, 1H) and 7.17 (s, 1H), an aromatic methyl group at δ 2.31 (s, 3H), and three aliphatic methyl groups at δ 1.28 (s, 3H), 1.39 (s, 3H), and 1.40 (s, 3H), which implied, from the required degrees of unsaturation, a tricyclic sesquiterpenoid framework.

The NMR data of **2** were very similar to those of the co-occurring sesquiterpenes, 3α-hydroxydebromoaplysin (**1**)[8] and laurinterol (**7**).[10] Analysis of the 2D NMR spectra of **2** readily determined the presence of the same 1,2,4,5-tetrasubstituted benzene ring partial structure as in **7** in the molecule. Further, the $^1H-^1H$ COSY spectrum of **2** showed cross-peaks between H_2-4 (δ 1.63, m) and H_2-5 (δ 1.75, ddd, J = 12.3, 4.8, 3.5 Hz, H-5α; 2.02, ddd, J = 12.3, 10.8, 3.1 Hz, H-5β), indicating clearly the linkage between C-4 and C-5 in the molecule. Moreover, strong long-range correlations between H_3-13 and C-1 (δ 54.0), C-2 (δ 101.2), and C-5 (δ 40.8) observed in the HMBC experiment confirmed the connections between C-1 and C-2 and C-5. Furthermore, the correlations between H_3-15 and C-3 (δ 82.8) and C-4 (δ 37.0) confirmed the connectivity between C-3 and C-4. Additionally, the correlations between H_3-14 and C-1, C-2, and C-3 suggested the connections between C-2 and C-1 and C-3. The above observations, bearing in mind the presence of an oxygen-bearing quaternary carbon at δ 82.8 (C-3), indicated the presence of the same 1,2,3-trimethyl-3-hydroxycyclopentenyl moiety as in **1**.

Careful comparison of 1D and 2D NMR data ($^1H-^1H$ COSY, HMQC, and HMBC) of **2** and model compound **1** revealed that the differences between them were due to the nature of the substitution pattern of the benzene ring and the configuration at C-3, while the rest of the structure of **2** is the same as in **1**. The relative configuration at C-1, C-2, and C-3 was determined by NOESY experiments on **2**. Significant NOE correlations were observed between H_3-13 at δ 1.39 and H_3-14 at δ 1.28 and Hβ-5 at δ 2.02, while no correlation was observed between H_3-15 at δ 1.40 and H_3-13 and H_3-14. These observations indicated that H-5α and H_3-15 were α-oriented, while HO-3, H-5β, H_3-13, and H_3-14 were β-oriented. Detailed analysis of its 2D NMR spectra allowed unambiguous assignments of the ^1H and ^{13}C NMR data of **2** (Tables 1 and 2). Therefore, the structure of **2** was elucidated as 3β-hydroxyaplysin.

Compound **3** was isolated as a colorless oil. The molecular formula $C_{15}H_{19}OBr$, established by HREIMS at *m*/*z* 294.0614 $[M]^+$ (calc 294.0619), is identical to that of the co-occurring laurinterol (**7**).[10] The phenolic nature of **3** was shown by an IR band at 3520 cm^{-1} and UV absorptions at 202, 232, and 299 nm. The ^1H NMR spectrum revealed the presence of two *para*-disposed protons (δ 6.84, s and δ 7.20, s), one olefinic proton (δ 5.77, dd, J = 3.1, 1.7 Hz), an allylic methylene (δ 2.11, ddd, J = 11.0, 9.2, 1.7 Hz and

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Table 1. ^{1}H NMR Data for Compounds **1**−**4** and **6**[a]

position	1	2	3	4	6[b]
3			2.06 (m)	2.01 (m)	1.84 (m)
4	1.64 (m)	1.63 (m)	2.11 (ddd, 11.0, 9.2, 1.7)	2.01 (m)	1.17 (m)
			2.55 (ddd, 11.0, 8.5, 3.1)	2.41 (ddd, 11.5, 9.7, 2.9)	1.60 (m)
5	1.60 (m)	1.75 (ddd, 12.3, 4.8, 3.5)	5.77 (dd, 3.1, 1.7)	5.69 (br s)	1.60 (m)
	1.75 (m)	2.02 (ddd, 12.3, 10.8, 3.1)			1.78 (m)
7				7.21 (d, 7.9)	
8	6.57 (d, 1.2)	6.58 (s)	6.84 (s)	7.10 (d, 7.9)	6.54 (d, 1.6)
10	6.71 (dd, 7.5, 1.2)			7.10 (d, 7.9)	6.67 (dd, 8.4, 1.6)
11	6.93 (d, 7.5)	7.17 (s)	7.20 (s)	7.21 (d, 7.9)	6.93 (d, 8.4)
12	2.30 (s)	2.31 (s)	2.34 (s)	2.33 (s)	2.29 (s)
13	1.36 (s)	1.39 (s)	0.90 (s)	0.97 (s)	1.33 (s)
14	1.25 (s)	1.28 (s)	0.99 (s)	1.09 (s)	1.30 (s)
15	1.23 (s)	1.40 (s)	1.03 (d, 6.6)	0.99 (d, 6.6)	1.07 (d, 6.8)
OH[b]			5.34 (s)		

[a] Bruker DRX 400 MHz spectrometers; chemical shifts (ppm) referenced to $CDCl_3$ (δ_H 7.26). Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b] Phenol hydroxyl group in the case of **3**.

Table 2. ^{13}C NMR Data for Compounds **1**−**4**[a]

position	1	2	3	4
1	52.1 (C)	54.0 (C)	147.5 (C)	152.8 (C)
2	99.7 (C)	101.2 (C)	49.5 (C)	47.7 (C)
3	81.2 (C)	82.8 (C)	45.0 (CH)	45.8 (CH)
4	38.0 (CH_2)	37.0 (CH_2)	38.5 (CH_2)	37.8 (CH_2)
5	40.2 (CH_2)	40.8 (CH_2)	131.1 (CH)	126.1 (CH)
6	134.2 (C)	136.4 (C)	123.4 (C)	135.5 (C)
7	157.2 (C)	157.1 (C)	152.5 (C)	127.5 (CH)
8	110.1 (CH)	111.3 (CH)	111.1 (CH)	128.6 (CH)
9	138.5 (C)	137.1 (C)	138.1 (C)	136.1 (C)
10	122.0 (CH)	114.5 (C)	114.4 (C)	128.6 (CH)
11	122.7 (CH)	126.6 (CH)	132.0 (CH)	127.5 (CH)
12	21.7 (CH_3)	23.1 (CH_3)	22.8 (CH_3)	21.1 (CH_3)
13	23.8 (CH_3)	23.4 (CH_3)	20.6 (CH_3)	20.6 (CH_3)
14	15.8 (CH_3)	14.7 (CH_3)	26.2 (CH_3)	26.2 (CH_3)
15	22.3 (CH_3)	22.4 (CH_3)	14.3 (CH_3)	14.0 (CH_3)

[a] Bruker DRX 100 MHz spectrometers; δ values are reported in ppm referenced to $CDCl_3$ (δ_C 77.0) as internal standard. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

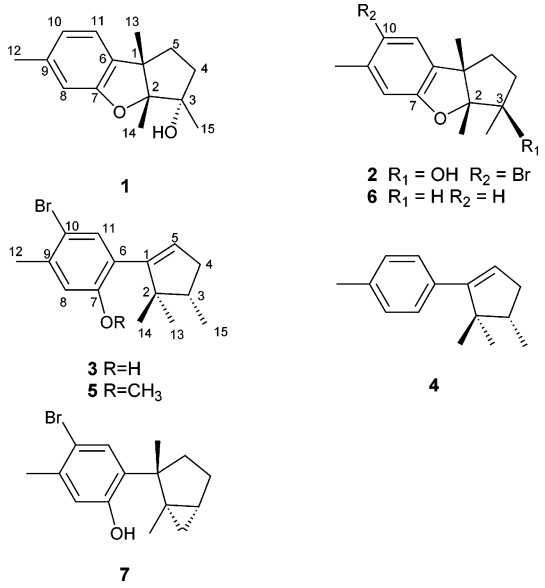

Figure 1. Structures of compounds **1**−**7**.

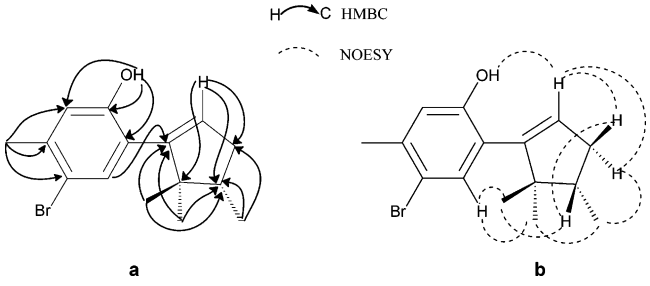

Figure 2. Selected key HMBC correlations (a) and NOESY correlations (b) for laurokamurene B (**3**).

δ 2.55, ddd, J = 11.0, 8.5, 3.1 Hz), an aromatic methyl (δ 2.34, s), and three aliphatic methyls (δ 0.90, s; δ 0.99, s; and δ 1.03, d, J = 6.6 Hz). The NMR data mentioned above were strongly reminiscent of those of **5**,[11] a synthetic product from the acid-catalyzed rearrangement of laurinterol methyl ether. A comparison of the NMR data of **3** and **5** revealed the only difference resided in the nature of the substituent at C-7 on the benzene ring (−OH in **3**; −OCH_3 in **5**). The position of the double bond at C-1 and C-5 and the geminal methyls at C-2 on the cyclopentenyl moiety were further confirmed by a HMBC experiment (Figure 2a). Finally the relative configuration of H_3-15 at C-3 was assigned as α by the observation of NOE correlations (Figure 2b) between H-3β (δ 2.06, m) and H_3-14 (δ 0.99, s) and between H_3-13 (δ 0.90, s) and H_3-15 (δ 1.03, d, J = 6.6 Hz).

Compound **4** was also isolated as a colorless oil. The compound was shown to be a hydrocarbon with the molecular formula $C_{15}H_{20}$ by HREIMS at m/z 200.1554 $[M]^+$ (calc 200.1565). The ^{1}H NMR spectrum exhibited two 2H doublets at δ 7.10 (d, J = 7.9 Hz, 2H) and 7.21 (d, J = 7.9 Hz, 2H), indicating the presence of a 1,4-disubstituted benzene ring. Further, the NMR data suggested the aliphatic portion of the molecule to be 2,2,3-trimethyl-$\Delta^{1(5)}$-cyclopentenyl, the same as that of **3**, in accord with the proposed structure **4**. All NMR data of **4** were unambiguously assigned by ^{1}H−^{1}H COSY, HMQC, and HMBC experiments as reported in Tables 1 and 2. Once again, the relative configuration of C-3 was deduced to be the same as that of **3** on the basis of the NOESY experiments.

A growing collection of brominated and nonbrominated sesquiterpenoids, which can be classified into more than 20 sesquiterpenoid skeletons, continue to be isolated from red algae of the genus *Laurencia* in recent years.[12] However, in most cases, three methyls in the aliphatic portion were located at either positions 1, 2, 3 (laurene type) or 1, 2, 2 (cuparene type). To the best of our knowledge, this is the first report of the isolation of sesquiterpenes with a 2,2,3-trimethylcyclopentenyl moiety from a natural source.

Although many *Laurencia* sesquiterpenes exhibit antibacterial and antifungal bioactivities,[13−15] new compounds **2**−**4** were found to be inactive against the fungus *Cladosporium cucumerinum*. Further studies should be conducted to test other bioactivities, such as cytotoxic and anti-inflammatory properties, of these new compounds, as well as to understand the true biological/ecological role of these metabolites in the life cycle of the algae.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer. NMR spectra were measured on a Bruker DRX-400 spectrometer with the residual $CHCl_3$ (δ_H 7.26 ppm; δ_C 77.01 ppm) as an internal standard. EIMS and HREIMS spectra were recorded on a Finnigan-MAT-95 mass spectrometer. All solvents were of analytical grade (Shanghai Chemical Plant, Qindao, People's Republic of China). Reversed-phase HPLC (Agilent 1100 series liquid chromatography using a VWD G1314A detector at 210 nm and a semipreparative ODS-HG-5 [5 μm particle size, 10 mm (i.d.) $\times$ 25 cm] column) was employed for the purification. Commercial Si gel (Qing Dao Hai Yang Chemical Group Co., 200−300 and 400−600 mesh) was used for column chromatography, and precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC. Sephadex LH-20 (Amersham Biosciences) was also used for column chromatography.

Biological Material. The alga *L. okamurai* was collected by hand along the coast of Nanji Island in the East China Sea, Zhejiang Province, China, at a depth of 0.5−1 m, in June 1999, and the algal material was stored at −20 °C until processed. A voucher specimen (No. MA99-01) was deposited at the Shanghai Institute of Materia Medica, SIBS-CAS for inspection.

Extraction and Isolation. The fresh algal material (dry weight, 500 g) of *L. okamurai* was exhaustively extracted with acetone (3 × 1 L). The acetone extract was concentrated in vacuo to give a residue (36.1 g), which was partitioned between Et_2O and H_2O. The Et_2O-soluble portion (25.8 g) was chromatographed by Si gel CC using light petroleum ether with increasing amounts of Et_2O as eluent to give 12 fractions (I−XII) on the basis of TLC analysis. Fraction II, eluted with pure petroleum ether, was further chromatographed on Si gel (petroleum ether) to afford compounds **4** (3.7 mg) and **6** (850 mg). Fraction IV, eluted with petroleum ether/Et_2O (8:2), was further purified by Si gel CC using a stepped gradient (petroleum ether/Et_2O, 9:1−7.5:2.5) to yield **3** (3.2 mg) and **7** (2.5 g). Fraction VI, eluted with petroleum ether/Et_2O (7.5:2.5), was further separated on Si gel CC (petroleum ether/Et_2O, 8:2, as eluent) and followed by RP-HPLC purification (MeOH/H_2O, 7.5:2.5, as eluent) to afford two pure minor compounds, **1** (2.1 mg) and **2** (3.5 mg).

3β-Hydroxyaplysin (2): colorless oil; $[\alpha]_D^{24}$ −20.0 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 (4.51), 232 (3.55), 299 (3.51) nm; IR ν_{max} (KBr) 3380, 2924, 2852, 1714, 1614, 1579, 1257, 1232, 1090, 1040, 924 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 310.0591 (calc for $C_{15}H_{19}O_2Br$, $[M]^+$ 310.0568).

Laurokomurenene A (3): colorless oil; $[\alpha]_D^{24}$ +13.2 (*c* 0.42, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (5.01), 232 (4.10), 299 (3.55) nm; IR ν_{max} (KBr) 3520, 2958, 2935, 1728, 1610, 1551, 1387, 1230, 1103, 1003, 924 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 294.0614 (calc for $C_{15}H_{19}OBr$, $[M]^+$ 294.0619).

Laurokomurene B (4): colorless oil; $[\alpha]_D^{24}$ +10.0 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (4.85), 249 (4.21) nm; IR ν_{max} (KBr) 3045, 3025, 2965, 2935, 2875, 1610, 1518, 1440, 1377, 1365 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 200.1554 (calc for $C_{15}H_{20}$, $[M]^+$ 200.1565).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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