Terpenes and Polybromoindoles from the Marine Red Alga *Laurencia decumbens* (Rhodomelaceae)

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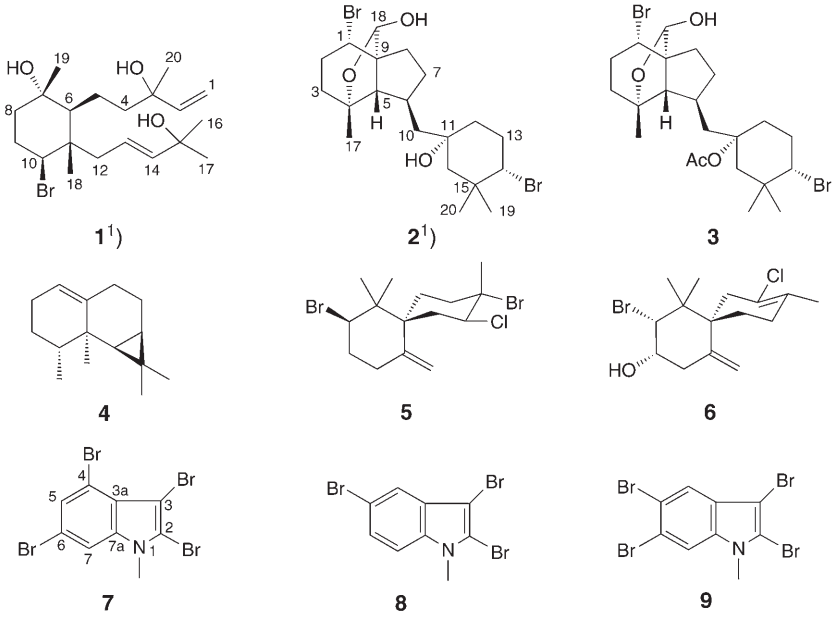
Two new brominated diterpenes, namely, laurendecumtriol (**1**) and 11-*O*-deacetylpinnaterpene C (**2**), one new polybromoindole, 2,3,4,6-tetrabromo-1-methyl-1*H*-indole (**7**), and six known natural products were isolated and identified from the marine red alga *Laurencia decumbens*. Their structures were elucidated on the basis of detailed spectroscopic and mass-spectrometric analysis as well as by comparison with literature data. Based on 2D-NMR experiments, the previously reported NMR data for pinnaterpene C (**3**) were reassigned.

Introduction. – The marine red-algal species of the genus *Laurencia* (order Ceramiales, family Rhodomelaceae) have been the subject of intensive studies due to the occurrence of a structurally diverse range of halogenated secondary metabolites [1]. Recently, the chemical investigation of *Laurencia* species from Chinese-sea waters, such as *L. okamurai*, *L. tristicha*, and *L. similis* were performed, which resulted in the isolation and identification of a variety of new structures [2–6]. As part of our continuing studies directed toward the discovery of novel naturally occurring halogenated metabolites from the genus *Laurencia* [5][6], we examined the chemical constituents of *L. decumbens* that was collected offshore Weizhou Island from the South China-Sea waters. From this species, we isolated and identified three new compounds including two new brominated diterpenes, named laurendecumtriol (**1**) and 11-*O*-deacetylpinnaterpene C[1]) (**2**), and one new polybromoindole, 2,3,4,6-tetrabromo-1-methyl-1*H*-indole (**7**). In addition, six known compounds, pinnaterpene C (**3**) [7], 1(10)-aristolene (**4**) [8], obtusane (**5**) [9], elatol (**6**) [10], 2,3,5-tribromo-1-methyl-1*H*-indole (**8**) [5][11], and 2,3,5,6-tetrabromo-1-methyl-1*H*-indole (**9**) [11], were also isolated and identified. The isolation and structural determination of compounds **1**–**9** are the main subject of this paper. To the best of our knowledge, this is the first report on the chemical investigation of *L. decumbens*.

Results and Discussion. – The dried and powdered alga *L. decumbens* was extracted with $CHCl_3$/MeOH 1:1 (*v*/*v*). After solvent removal, the residue was further extracted with 95% aqueous EtOH. The concentrated extracts were partitioned between H_2O and AcOEt to afford the AcOEt-soluble fraction, which was purified by column chromatography (silica gel, *Sephadex LH-20*), and prep. TLC, to yield compounds **1**–**9**.

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



Compound **1** was obtained as colorless crystals. Structurally, this compound was closely related to luzodiol, a diterpene with a rarely described C-skeleton that was recently identified from *L. luzonensis* [12]. The IR spectrum of **1** indicated the presence of OH groups (3394 cm^{-1}). The ESI-MS (positive mode) exhibited a characteristic quasimolecular-ion cluster at *m/z* 427 and 425 (1:1; $M + Na]^+$), indicating the presence of one Br-atom in **1**. The molecular formula was determined to be $C_{20}H_{35}BrO_3$ by HR-ESI-MS (*m/z* 425.1656 ($[M + Na]^+$, $C_{20}H_{35}{}^{79}BrO_3Na^+$), suggesting three degrees of unsaturation. Based on the ^{1}H- and ^{13}C-NMR (*Table*), HMBC (*Fig.*), and NOESY data, the chemical structure was established for compound **1**, which was named laurendecumtriol[1]). However, the relative configuration at C(3) and C(15) of **1** remain unknown.

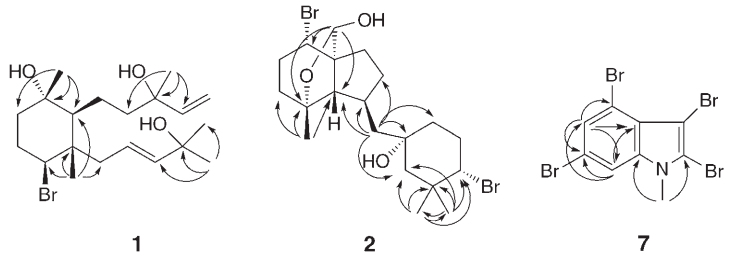


Figure. *Selected HMBC correlations for* **1**, **2**, *and* **7**

Table. *[1]H- and [13]C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) *of Compounds* **1**–**3**[1]). Assignments were corroborated by $^1H,^1H$-COSY, HMQC, and HMBC experiments.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1) or H−C(1)	5.10 (*dd*, *J* = 10.8, 1.1), 5.23 (*dd*, *J* = 17.3, 1.1)	112.1 (*t*)	4.11 (*dd*, *J* = 11.8, 6.1)	58.7 (*d*)	4.09 (*dd*, *J* = 11.7, 6.1)	58.7 (*d*)
H−C(2) or CH_2(2)	5.93 (*dd*, *J* = 17.3, 10.8)	144.9 (*d*)	2.02–2.05, 2.20–2.25 (2*m*)	32.5 (*t*)	2.02–2.08, 2.19–2.25 (2*m*)	32.5 (*t*)
C(3) or CH_2(3)		73.4 (*s*)	1.38–1.42, 1.62–1.66 (2*m*)	40.7 (*t*)	1.37–1.42, 1.60–1.65 (2*m*)	40.7 (*t*)
CH_2(4) or C(4)	1.60–1.67 (*m*)	44.4 (*t*)		83.1 (*s*)		83.0 (*s*)
CH_2(5) or H−C(5)	1.49–1.57 (*m*)	21.0 (*t*)	1.36 (*d*, *J* = 10.7)	63.6 (*d*)	1.34 (*d*, *J* = 10.5)	63.5 (*d*)
H−C(6)	1.10–1.15 (*m*)	49.6 (*d*)	2.44–2.51 (*m*)	35.7 (*d*)	2.38–2.43 (*m*)	36.0 (*d*)
C(7) or CH_2(7)		72.9 (*s*)	1.33–1.37, 2.10–2.13 (2*m*)	33.7 (*t*)	1.30–1.35, 2.02–2.08 (2*m*)	32.5 (*t*)
CH_2(8)	1.44–1.50, 1.60–1.66 (2*m*)	42.0 (*t*)	2.05–2.10, 2.13–2.18 (2*m*)	32.2 (*t*)	1.30–1.35, 1.58–1.62 (2*m*)	31.8 (*t*)
CH_2(9) or C(9)	2.00–2.04, 2.37–2.46 (2*m*)	30.0 (*t*)		62.3 (*s*)		62.2 (*s*)
H−C(10) or CH_2(10)	4.06 (*dd*, *J* = 12.8, 3.9)	63.9 (*d*)	1.26–1.32 (*m*), 1.72 (*d*, *J* = 13.9)	52.3 (*t*)	1.87 (*dd*, *J* = 14.4, 11.3), 2.20–2.26 (*m*)	45.2 (*t*)
C(11)		43.9 (*s*)		72.6 (*s*)		83.4 (*s*)
CH_2(12)	2.17 (*d*, *J* = 7.4)	41.8 (*t*)	1.42–1.46, 1.61–1.65 (2*m*)	39.7 (*t*)	1.33–1.38, 2.38–2.43 (2*m*)	37.0 (*t*)
H−C(13) or CH_2(13)	5.57 (*dt*, *J* = 15.4, 7.4)	121.1 (*d*)	1.99–2.02, 2.32–2.42 (2*m*)	30.5 (*t*)	2.03–2.08, 2.18–2.22 (2*m*)	30.3 (*t*)
H−C(14)	5.81 (*d*, *J* = 15.4)	142.6 (*d*)	3.93 (*dd*, *J* = 12.7, 3.8)	65.8 (*d*)	3.93 (*dd*, *J* = 12.6, 4.0)	64.9 (*d*)
C(15)		70.9 (*s*)		36.6 (*s*)		36.5 (*s*)
Me(16) or CH_2(16)	1.33 (*s*)	30.1 (*q*)	1.32 (*d*, *J* = 14.5), 1.79 (*dd*, *J* = 14.5, 3.4)	50.1 (*t*)	1.20 (*d*, *J* = 14.8), 2.57 (*dd*, *J* = 14.8, 3.6)	46.6 (*t*)
Me(17)	1.33 (*s*)	30.0 (*q*)	1.33 (*s*)	21.9 (*q*)	1.29 (*s*)	21.9 (*q*)
Me(18) or H−C(18)	1.12 (*s*)	18.0 (*q*)	5.33 (br. *s*)	99.1 (*d*)	5.32 (*d*, *J* = 4.2)	99.0 (*d*)
Me(19)	1.17 (*s*)	30.8 (*q*)	1.24 (*s*)	22.6 (*q*)	1.10 (*s*)	21.7 (*q*)
Me(20)	1.30 (*s*)	27.7 (*q*)	1.01 (*s*)	32.6 (*q*)	1.02 (*s*)	32.4 (*q*)
AcO					2.01 (*s*)	22.8 (*q*), 170.8 (*s*)

The ^{13}C-NMR (DEPT) spectrum of **1** indicated the presence of twenty C-atoms including five Me, six CH_2, and five CH groups, and four quaternary C-atoms. The ^{1}H,^{1}H-COSY data were consistent with the four spin systems or structural units CH_2=CH (from CH_2(1) to H–C(2)), CH_2CH_2CH (from CH_2(4) to H–C(6)), CH_2CH_2CH (from CH_2(8) to H–C(10)), and CH_2CH=CH (from CH_2(12) to H–C(14))[1]. The HMBC cross-peaks Me(20)/C(2), C(3), and C(4), Me(19)/C(6), C(7), and C(8), Me(18)/C(6), C(10), C(11), and C(12), Me(17)/C(14), C(15), and C(16), and Me(16)/C(14), C(15), and C(17) established the connectivities of the above four structural units at quaternary C-atoms C(3), C(7), C(11), and C(15) (*Fig.*). The above spectral evidences confirmed the planar structure for **1**, closely related to that of luzodiol [12]. However, the double C=C bond at C(14) in luzodiol was changed to C(13) in **1**, and an additional OH group was present at C(15) of **1**. This was supported by the fact that in the ^{13}C-NMR spectrum, the Me(16) and Me(17) signals, resonating at δ(C) 25.7 and 17.8 in luzodiol, were obviously downfield shifted to δ(C) 30.1 and 30.0 respectively, in **1**. In addition, the appearance of an oxygenated quaternary C-atom signal at δ(C) 70.9 for C(15) further supported this deduction. The relative configuration of **1** was determined upon analysis of the coupling constant and NOESY data. The large J(13,14) (15.4 Hz) indicated the *trans*-configuration for the C(13)=C(14) bond. In the NOESY experiment, both the CH_2(5) and Me(19) exhibited ^{1}H,^{1}H-NOESY correlations with Me(18), indicated their *cis*-orientation. The H–C(10) should be *trans*-oriented with respect to Me(18), since no NOESY cross-peak could be detected between H–C(10) and Me(18).

Compound **2** was obtained as colorless oil. The IR spectrum indicated the presence of OH groups (3405 cm^{-1}) in the molecule. The ESI-MS (positive mode) exhibited a characteristic quasimolecular-ion cluster at *m/z* 505, 503, and 501 (1:2:1; $[M + Na]^+$), indicating the presence of two Br-atoms. The molecular formula was determined to be $C_{20}H_{32}Br_2O_3$ by HR-ESI-MS (*m/z* 503.0593 ($[M+Na]^+$, $C_{20}H_{32}{}^{79}Br^{81}BrO_3Na^+$)), suggesting four degrees of unsaturation. Detailed comparison with pinnaterpene C (**3**) revealed that **2** differed from **3** only at C(11), *i.e.*, instead of the presence of an AcO group, an OH group was attached to C(11) of **2** [7]. The ^{1}H- and ^{13}C-NMR (*Table*) and HMBC (*Fig.*) data further confirmed the chemical structure of compound **2** to be 11-*O*-deacetylpinnaterpene C[1]). Based on the very similar NMR spectral data, the configuration of **2** was assigned to be the same as for pinnaterpene C. However, it should be noted that, according to our 2D-NMR experiments (^{1}H,^{1}H-COSY, HMQC, and HMBC), the reported NMR data of pinnaterpene C (**3**) [7] should be, most likely, reassigned, and these reassigned data of **3** are also shown in the *Table*.

The ^{13}C-NMR (DEPT) spectrum of **2** showed the presence of 20 C-atoms including three Me, eight CH_2, and five CH groups, and four quaternary C-atoms. The lack of signals for an Ac group in the ^{13}C-NMR spectrum suggested that **2** was the deacetylated pinnaterpene C (**3**). This was clearly supported by δ(C) of C(11) which was higher-field shifted to 72.6, as well as by the adjacent lower-field shifted δ(C) of C(10), C(12), and C(16), as compared to the corresponding δ(C) of pinnaterpene C. The HMBC cross-peaks CH_2(10)/C(5), C(6), C(7), C(11), C(12), and C(16), Me(17)/C(3), C(4), and C(5), H–C(18)/C(1), C(4), and C(5), Me(19)/C(14), C(15), C(16), and C(20), and Me(20)/C(14), C(15), C(16), and C(19) (*Fig.*) were consistent with the proposed structure of **2**.

Compound **7** was obtained as a colorless crystal mixture with **8** and **9** (ratio *ca.* 2:10:1, based on NMR-signal intensities). They displayed one spot on TLC, and attempts to separate the three compounds by different column-chromatography steps as well as by prep. TLC with different solvent systems failed. Fortunately, **7**–**9** could be distinguished by the different NMR-signal intensities. Aided by 2D-NMR (^{1}H,^{1}H-COSY, HMQC, and HMBC (*Fig.*)) and MS analysis, the structure of **7** could be established as 2,3,4,6-tetrabromo-1-methyl-1*H*-indole.

Structural confirmation of **8** and **9** was readily achieved by the analysis of their NMR and MS data and by comparison with those reported in [5][11]. The low-resolution EI-MS of **7** exhibited a characteristic quasimolecular ion cluster at *m/z* 451, 449, 447, 445, and 443 (1:4:6:4:1), indicating the presence of 4 Br-atoms. The molecular formula was determined to be $C_9H_5Br_4N$ by HR-FAB-MS (pos.; *m/z* 451.7150 ($[M+H]^+$, $C_9H_6{}^{81}Br_4N^+$)), suggesting six degrees of unsaturation. The ^{13}C-NMR (DEPT) data exhibited the presence of nine C-atoms, including one Me and two CH groups, and six quaternary C-atoms. The ^{1}H-NMR spectrum showed three signals at δ(H) 7.47 (*d*, *J* = 1.5 Hz, H–C(5)), 7.41 (*d*, *J* = 1.5 Hz, H–C(7)), and 3.77 (*s*, Me–N(1)). Detailed comparison with the NMR data for the known compounds **8** [5][11] and **9** [11] suggested that **7** possessed a tetrabromoindole skeleton. The ^{1}H-NMR and ^{1}H,^{1}H-COSY plots exhibited two *m*-coupled aromatic proton signals (H–C(5) and H–C(7)), and the HMBC plot displayed cross-peaks for H–C(5)/C(4), C(6), C(7), and C(3a), H–C(7)/C(5), C(6), and C(3a), and Me–N(1)/C(2) and C(7a) (*Fig.*).

Polybromoindoles have been produced only by few *Laurencia* species, such as *L. similis* and *L. brongniartii* [5][11]. To the best of our knowledge, *L. decumbens* is the third *Laurencia* species producing polybromoindoles, which may be formed by a biosynthetic pathway similar to those present in the above two species. The polybromoindole-type metabolites suggest some affinity between these species. In addition, our results represent an unusual example of the co-occurrence of highly brominated indole alkaloids, halogenated and nonhalogenated sesquiterpenes, and brominated diterpenes in a single *Laurencia* species, *L. decumbens*.

This work was financially supported by the *National Natural Science Foundation of China* (No. 30530080), by the National '*Marine 863 Project*' (No. 2007AA09Z403), and by the *Department of Science & Technology of Shandong Province* (No. 2006GG2205023). The authors are grateful to Prof. *B.-M. Xia*, Institute of Oceanology, Chinese Academy of Sciences, for identifying the algal material. The program *Bairen Jihua* from the Chinese Academy of Sciences (awarded to *B.-G. W.*) is also gratefully acknowledged.

Experimental Part

General. Column chromatography (CC): silica gel (*Qingdao Haiyang Chemical Group Co.*; 200–300 mesh) and *Sephadex LH-20* (*Sigma*). TLC: precoated silica gel plates (*Qingdao Haiyang Chemical Group Co.*; *GF-254*). M.p.: *SGW-X-4* micro melting-point-apparatus; uncorrected. Optical rotation: *Atago-Polax-L* polarimeter. IR Spectra: *Nicolet-NEXUS-470* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. NMR Spectra: *Bruker-Avance-500* spectrometer; at 500 (^{1}H) and 125 MHz (^{13}C); δ in ppm, *J* in Hz. Low- and high-resolution MS: *VG-Autospec-3000* spectrometer; in *m/z* (rel. %).

Algal Material. The red alga *L. decumbens* KÜTZING was collected from South-China-Sea waters offshore Weizhou Island in April 2006, and was identified by Prof. *B.-M. Xia*, Institute of Oceanology, Chinese Academy of Sciences (IOCAS). A voucher specimen (HZ0604a) was deposited at the Key Laboratory of Experimental Marine Biology of the IOCAS.

Extraction and Isolation. The dried and powdered alga *L. decumbens* (500 g) was extracted with $CHCl_3$/MeOH 1:1(*v*/*v*). After solvent removal, the residue was further extracted with 95% aq. EtOH. The concentrated extracts were combined and partitioned between H_2O and AcOEt. The AcOEt-soluble fraction was purified by CC (SiO_2, petroleum ether AcOEt gradient): *Fractions I–VI*. *Fr. I* (eluted with petroleum ether) was further purified by prep. TLC (cyclohexane): **4** (15.1 mg), **5** (3.6 mg), and **7**/**8**/**9** (9.0 mg). *Fr. II* (eluted with petroleum ether/AcOEt 30:1) was also purified by prep. TLC (petroleum ether): **6** (11.8 mg). *Fr. IV* (eluted with petroleum ether/AcOEt 5:1) was further separated by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) and prep. TLC: **3** (13.1 mg). *Fr. V* (eluted with petroleum ether/AcOEt 2:1) was further separated by CC (SiO_2 and *Sephadex LH-20*, $CHCl_3$/MeOH 1:1) and prep. TLC: **1** (5.4 mg) and **2** (4.4 mg).

Laurendecumtriol (= rel-*(1*R,*2*R,*3*R,*6*R*)-3-Bromo-α-ethenyl-6-hydroxy-2-[(2*E*)-4-hydroxy-4-methylpent-2-en-1-yl)-α,2,6-trimethylcyclohexanepropanol*; **1**): Colorless crystals. M.p. 119–121°. $[\alpha]_D^{18} = +2.5$ (*c* = 0.17, $CHCl_3$). IR (KBr): 3394, 2971, 2932, 1640, 1457, 1374, 1148, 900. ^{1}H- and ^{13}C-NMR: *Table*.

ESI-MS: 427, 425 (1:1, $[M + Na]^+$). HR-ESI-MS: 425.1656 ($[M + Na]^+$, $C_{20}H_{35}{}^{79}BrO_3Na^+$; calc. 425.1667).

11-O-Deacetylpinnaterpene C (= rel-*(1R,3aR,4R,7S,7aR,9S)-4-Bromo-1-{[(1R,4R)-4-bromo-1-hydroxy-3,3-dimethylcyclohexyl]methyl}-octahydro-7-methyl-7,3a-(epoxymethano)-3a*H*-inden-9-ol*; **2**): Colorless oil. $[\alpha]_D^{18} = +6.6$ ($c = 0.22$, $CHCl_3$). IR (KBr): 3405, 2963, 2933, 1472, 1375, 1201, 1087. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 505, 503, 501 (1:2:1, $[M + Na]^+$). HR-ESI-MS: 503.0593 ($[M + Na]^+$, $C_{20}H_{32}{}^{79}Br^{81}BrO_3Na^+$; calc. 503.0595).

*2,3,4,6-Tetrabromo-1-methyl-1*H*-indole* (**7**): Colorless crystals. IR (KBr): 1500, 1460, 1417, 781. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.47 (*d*, $J = 1.5$, H–C(5)); 7.41 (*d*, $J = 1.5$, H–C(7)); 3.77 (*s*, Me–N(1)). ^{13}C-NMR (125 MHz, $CDCl_3$): 118.5 (*s*, C(2)); 92.5 (*s*, C(3)); 113.9 (*s*, C(4)); 128.2 (*d*, C(5)); 116.0 (*s*, C(6)); 112.3 (*d*, C(7)); 123.0 (*s*, C(3a)); 137.5 (*s*, C(7a)); 32.9 (*q*, Me–N(1)). EI-MS: 451 (15), 449 (64), 447 (100), 445 (70), 443 (18) (M^+); 436 (3), 434 (12), 432 (18), 430 (13), 428 (4); 370 (4), 368 (12), 366 (13), 364 (5); 289 (14), 287 (27), 285 (15); 208 (15), 206 (15); 127 (22); 112 (26). HR-FAB-MS (pos.): 451.7150 ($[M + H]^+$, $C_9H_6{}^{81}Br_4N^+$; calc. 451.7152).

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Received May 14, 2007