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Note

Algicidal Diterpenes from the Brown Alga *Dictyota dichotoma*

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Bioassay-guided fractionation of an ethanol extract of the brown alga, *Dictyota dichotoma*, led to the isolation of a novel chlorine-containing perhydroazulene diterpene together with two known diterpenes, dictyolactone and sanadaol. The structure of the novel compound, named dictyol J, was elucidated on the basis of spectroscopic information. Dictyolactone showed the highest algicidal activity against red-tide phytoplanktons among the three isolated compounds.

Key words: algicidal compound; *Dictyota dichotoma*; dictyolactone; sanadaol; dictyol J

Seaweeds have been shown to produce allelopathic substances toxic to the microalgae responsible for red tide, and several studies have been aimed at the development of a novel environmentally benign method to control harmful algal blooms (HAB) by utilizing seaweeds.[1)] In our previous study,[2)] we found that the green alga, *Ulva fasciata*, had the highest algicidal activity against several microalgae of the HAB species among thirty-seven species of seaweed collected from the coastal region of Nagasaki prefecture, and determined the algicidal principles of the green alga to be such polyunsaturated fatty acids (PUFAs) as hexadeca-4,7,10,13-tetraenoic acid, octadeca-6,9,12,15-tetraenoic acid and α-linolenic acid. To further investigate the algicidal compounds other than PUFAs, we turned our attention to the metabolites of brown and red algae. We screened again nine seaweeds involving seven Phaeophyta and two Rhodophyta for their algicidal activity against the red-tide phytoplankton, *Heterosigma akashiwo*, and found that the ethanol extract of the brown algae, *Dictyota dichotoma*, had high activity. This brown alga has been identified as a rich source of diterpenes with various carbon frameworks, many of which have interesting biological activity involving cytotoxic[3–6)] and antimicrobial[7,8)] activities. We describe in this paper the isolation and identification of the algicidal principles of *D. dichotoma*.

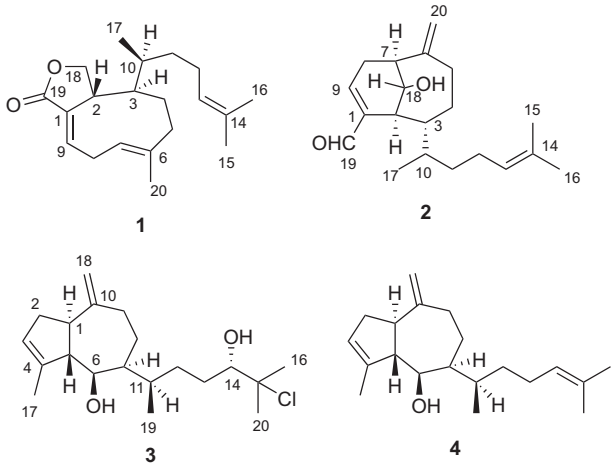

Fig. 1. Diterpenes of *Dictyota dichotoma*.

Chromatographic separation of the ethanol extract of *D. dichotoma* by monitoring the algicidal activity[2)] against *H. akashiwo* resulted in the isolation of three algicidal compounds, **1** (23.0 mg), **2** (3.0 mg) and **3** (4.7 mg). Compounds **1** and **2** were identified as the known diterpenes, dictyolactone[9)] and sanadaol,[10)] that had been previously isolated from the sea hare, *Aplysia depilans*, and the brown alga, *Pachydictyon coriaceum*, respectively, by comparing their spectral data with those reported in the literature.

Novel compound **3**, isolated as a colorless oil, had the molecular formula of $C_{20}H_{33}O_2Cl$ as determined by HREIMS data at 340.2156 (M^+). A strong absorption band at 3419 cm^{-1} in its IR spectrum as well as two D_2O exchangeable signals at δ_H 2.00 and δ_H 2.45 ppm in its ^{1}H-NMR spectrum (Table 1) indicated the presence of two hydroxyl groups. The ^{1}H-NMR spectrum showed two one-proton resonances at δ_H 3.61 and δ_H 4.01 ppm, both of which were shifted downfield to δ_H 5.38 and δ_H 5.64 ppm, respectively, by esterification with 2,4-dini-

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Abbreviations: HAB, harmful algal bloom; PUFA, polyunsaturated fatty acid

Table 1. ^{13}C-NMR,[a] ^{1}H-NMR,[b] HMBC and NOESY Data for Dictyol J (**3**)

C/H	^{13}C[c] (δ)	^{1}H (δ)	Multiplicity, J (Hz)	HMBC (H to C)	NOESY
1	45.8	2.66	*q*, $J = 9.2$	C-2, C-5, C-6, C-10, C-18	H-6, H-7
2	33.8	2.22 (α)	*m*	C-4	
		2.51 (β)	*m*		H-5, H-18
3	123.9	5.33	*br.s*	C-1, C-5	H-17
4	141.5				
5	59.9	2.33	*m*		H-2β
6	74.2	4.01	dd, $J = 8.2, 3.2$	C-4, C-8	H-1, H-12α, H-7
7	48.7	1.45–1.47	*m*	C-8, C-9, C-19	H-1, H-9α, H-12α
8	23.9	1.50–1.58	*m*	C-6, C-7, C-9, C-10, C-11	H-19
9	40.6	2.11 (α)	*m*		H-7
		2.60 (β)	*ddd*, $J = 14.8, 5.3, 2.7$	C-1, C-7, C-8, C-18	H-18
10	152.5				
11	33.0	1.50–1.58	*m*	C-12	
12	29.9	1.87 (α)	*m*	C-13, C-19	H-6, H-7, H-14
		1.27 (β)	*m*	C-11, C-13, C-14, C-19	H-19
13	27.8	1.47–1.64	*m*	C-12	
14	77.4	3.61	*dd*, $J = 10.5, 1.5$		H-6, H-12α, H-16, H-20
15	76.7				
16	27.0	1.55	*s*	C-14, C-15, C-20	H-14, H-20
17	16.0	1.81	*dd*, $J = 2.6, 1.4$	C-3, C-4, C-5	H-3, H-6
18	107.0	4.74	*d*, $J = 1.5$	C-1, C-9, C-10	H-2β
		4.75	*br.s*	C-1, C-9, C-10	H-9β
19	17.7	0.99	*d*, $J = 6.0$	C-7, C-11, C-12	H-8, H-12β
20	29.4	1.61	*s*	C-14, C-15, C-16	H-14, H-16, OH^1, OH^2
OH^1		2.00	*br*		OH^2, H-20
OH^2		2.45	*br*		OH^1, H-20

[a]Recorded at 125 MHz in $CDCl_3$.
[b]Recorded at 500 MHz in $CDCl_3$.
[c]Assignment was made with the aid of HMQC spectral data.

trobenzoyl chloride in pyridine, indicating that the two methines had been hydroxylated. The two oxymethine signals at δ_H 3.61 and δ_H 4.01 ppm were respectively correlated with the carbon signals at δ_c 77.4 and δ_C 74.2 ppm in the HMQC spectrum. The field of resonance of the sp^3 carbon bearing an oxygen or a halogen atom revealed an additional one quaternary carbon signal at δ_C 76.7 ppm, besides the two oxymethine signals. It was consequently proved that the chlorine atom was attached to the quaternary carbon. The other characteristic features in the ^{1}H-NMR spectrum include the presence of an olefinic methine (δ_H 5.33, *t*), an exomethylene (δ_H 4.74, *d* and δ_H 4.75, *br.s*), an olefinic methyl (δ_H 1.81, *dd*), a secondary methyl (δ_H 0.99, *d*) and two tertiary methyls (δ_H 1.55, *s* and δ_H 1.61, *s*).

A detailed analysis of the 1D and 2D NMR data involving DEPT, HMQC and ^{1}H–^{1}H COSY spectra revealed four structural units that are depicted in Fig. 2a, these being partial structures of the perhydroazulene diterpenes frequently found in the Dictyotaceae family. The methine unit was most likely located at the 7-position, the junction point between the perhydroazulenol moiety and the side chain; however, the COSY correlations between H-7/H-8, H-7/H-6 and H-7/H-11 were obscure due to overlapping of the signals of H-7, H-8 and H-11. Determination of the connectivity of the four units as well as verification of their proposed structures was made by the HMBC spectrum. Long-range correlation between C-7/H-8 and C-7/H-19 indicated that the methine at C-7 was connected to C-8 and C-11, and correlation between C-14/H-16, C-14/H-20, C-16/H-14 and C-20/H-14 indicated the connection between C-14 and C-15.

Since all of the perhydroazulene diterpenes of the algae of the Dictyotaceae family so far isolated are of 1*R*,5*S*,6*R*,7*S* configuration as far as we know and since most have positive optical rotation values as exemplified by pachydictiol A (**4**),[11] compound **3**, having the optical rotation value $[\alpha]_D^{24}$ +88.8°, is presumed to have the same stereochemistry in the perhydroazulene moiety. In fact, in the NOESY spectrum of **3**, H-1 displayed correlation with H-6 and H-7, and H-2β displayed correlation with H-5 and H-9β, although no correlation between H-1 and H-5 nor between H-5 and H-7 was apparent. Thus, the relative configuration of the perhydroazulene moiety was determined to be 1*R**,5*S**,6*R**,7*S**. The NOESY spectrum showed strong NOE correlation between the two hydroxyl protons due to hydrogen bonding. The stereochemistry of the side chain was determined by assuming the hydrogen-bonded structure as shown in Fig. 2b. The energy-minimized conformation of the 14S* isomer of **3** calculated by semi-empirical computation with CS MOPAC Pro® showed that H-14, H-12α, H-7 and H-6 were located in the vicinity of the α face of the molecule, whereas, with the 14*R** isomer, H-14 was

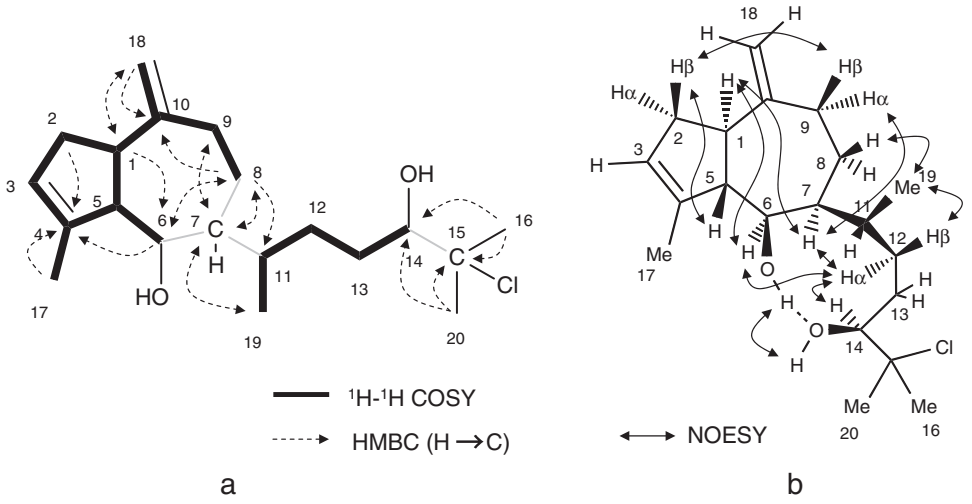

Fig. 2. (a) ^{1}H–^{1}H COSY and Selected HMBC Correlations and (b) Selected NOESY Correlations for Compound **3**.

oriented on the opposite side of the other three protons (β face). In the NOESY spectrum, the methine proton of the stereocenter at C-14 displayed NOE correlation with H-12α, which in turn was correlated with H-7 and H-6. Thus, the relative configuration of the remote stereocenter at C-14 was deduced to be *S**. In addition, the C-19 methyl protons displayed NOE correlation with H-12β and H-8, indicating the relative configuration at C-11 to be *R**. Thus, compound **3** was determined to be a chlorohydrine of pachydictyol A, represented by formula **3**, and named dictyol J.

Isolated compounds **1**–**3** were assayed for their algicidal activity[2] against the three representative HAB species, *H. akashiwo*, *Karenia mikimotoi* and *Alexandrium catenella*. All these compounds showed high (>95%) algicidal activity against *H. akashiwo* and *K. mikimotoi* at a dose of 10–20 µg/ml, dictyolactone (**1**) being the most active. Interestingly, **1** also showed moderate activity ($41.5 \pm 8.2\%$ at 10 µg/ml) against the dinoflagellate, *Alexandrium catenella*, while the other compounds and the known algicidal compound, α-linolenic acid,[2] were totally inactive to this species.

Experimental

Extraction and isolation. Air-dried *D. dichotoma* (78.79 g) was extracted twice with EtOH (450 ml) for 3 days, filtered, and concentrated *in vacuo*. The extract (7.02 g) was chromatographed over Diaion® HP-20 (25-mm i.d. × 300 mm) eluted successively with 25%, 50%, 75% aqueous MeOH, MeOH and then acetone, to yield five fractions (Fr 1 to Fr 5). Part of Fr 4 (1.05 g of 2.71 g) was chromatographed on silica gel 60 (25 mm i.d. × 300 mm) eluted with a hexane–EtOAc gradient with an increasing amount of EtOAc from 10% to 50% to give two active fractions, Fr 4-7 (39.1 mg) and Fr 4-9 (42.0 mg). Fr 4-7 was separated by reversed-phase HPLC (Cosmosil 5C18-MS-II, 20 × 250 mm, 80% CH_3CN) to yield compound **1** (23.0 mg). Fr 4-9 was separated in the same manner to give compounds **2** (3.0 mg) and **3** (4.7 mg).

Compound **1** (dictyolactone). Colorless oil, $[\alpha]_D^{24}$ −166.7° (*c* 1.77, CH_3OH). HRMS *m*/*z* (M^+): calcd. for $C_{20}H_{30}O_2$, 302.2246; found, 302.2227. EIMS *m*/*z*: 302 (M^+), 287, 259, 257, 233, 221, 191, 175, 164, 137, 119, 109, 82 (base), 69, 41. IR ν_{max} (KBr) cm^{-1}: 2922, 1763, 1645, 1456, 1362, 1242, 1191, 1003, 979. NMR δ_H (400 MHz, $CDCl_3$): 0.94 (3H, d, $J = 6.4$ Hz, H-17), 1.20 (2H, m, H-11), 1.44–1.79 (4H, m, H-3, H-4, H-10), 1.58 (3H, s, H-15), 1.67 (3H, s, H-16), 1.72 (3H, s, H-20), 1.86–1.96 (2H, m, H-12), 1.99 (1H, dd, $J = 11.9$, 5.4 Hz, H-5a), 2.24 (1H, br. d, $J = 11.9$ Hz, H-5b), 2.71 (1H, d, $J = 7.1$ Hz, H-2), 2.91 (1H, ddd, $J = 17.1$, 7.7, 4.1 Hz, H-8a), 3.11 (1H, ddt, $J = 17.1$, 11.7, 1.9 Hz, H-8b), 4.03 (1H, dd, $J = 9.5$, 7.1 Hz, H-18a), 4.53 (1H, d, $J = 9.5$ Hz, H-18b), 5.04 (1H, t, $J = 7.0$ Hz, H-13), 5.37 (1H, dd, $J = 11.7$, 3.0 Hz, H-7), 6.95 (1H, dt, $J = 7.4$, 2.0 Hz, H-9). NMR δ_C (125 MHz, $CDCl_3$): 17.5 (C-20), 17.6 (C-17), 17.7 (C-15), 25.7 (C-16), 26.0 (C-12), 28.8 (C-8), 30.6 (C-4), 32.7 (C-10), 37.4 (C-11), 40.2 (C-5), 44.0 (C-2), 47.4 (C-3), 68.2 (C-18), 123.0 (C-7), 124.2 (C-13), 131.7 (C-14), 135.4 (C-1), 136.5 (C-6), 140.4 (C-9), 173.3 (C-19).

Compound **2** (sanadaol). Colorless oil, $[\alpha]_D^{24}$ +45.5° (*c* 0.11, $CHCl_3$). HRMS *m*/*z* (M^+): calcd. for $C_{20}H_{30}O_2$, 302.2246; found, 302.2235. EIMS *m*/*z*: 302 (M^+), 284, 255, 220, 202, 145, 131, 109, 69 (base), 41. NMR δ_H (400 MHz, $CDCl_3$): 0.77 (3H, d, $J = 6.8$ Hz, H-17), 1.00 (1H, m, H-4a), 1.14–1.24 (1H, m, H-11a), 1.46–1.54 (1H, m, H-3), 1.54–1.62 (2H, m, H-10, H-11b), 1.62 (3H, s, H-16), 1.64–1.67 (1H, m, H-4b), 1.69 (3H, s, H-15), 1.90–2.00 (1H, m, H-12a), 2.02–2.09 (1H, m, H-12b), 2.09–2.12 (1H, br, OH), 2.27 (2H, m, H-5), 2.56 (1H, dd, $J = 20.8$, 4.0 Hz, H-8a), 2.76 (1H, m, H-8b), 2.88 (1H, m, H-7), 3.19 (1H, br. d, $J = 4.4$ Hz, H-2), 3.78 (2H, m, H-18), 4.90 (1H, deformed d, $J = 3.1$ Hz, H-20), 5.17 (1H, t, $J = 7.2$ Hz, H-13), 6.80 (1H, t, $J =$

3.6 Hz, H-9), 9.47 (1H, s, H-19). NMR δ_C (125 MHz, $CDCl_3$): 17.6 (C-17), 17.7 (C-16), 24.2 (C-4), 25.4 (C-12), 25.7 (C-15), 29.8 (C-5), 31.8 (C-8), 35.9 (C-11), 36.3 (C-10), 37.6 (C-3), 39.2 (C-2), 46.0 (C-7), 68.9 (C-18), 116.6 (C-20), 125.3 (C-13), 130.9 (C-14), 143.9 (C-1), 146.3 (C-6), 150.6 (C-9), 193.2 (C-19). IR ν_{max} (KBr) cm^{-1}: 3446 (OH), 2918, 1687 (C=O), 1156.

Compound **3** (dictyol J). Colorless oil, $[\alpha]_D^{24}$ +88.8° (*c* 0.24, CH_3OH). HRMS *m/z* (M^+): calcd. for $C_{20}H_{33}O_2Cl$, 340.2169; found, 340.2156. EIMS *m/z* (relative intensity): 342 ($M^+ + 2$, 16.1%), 341 ($M^+ + 1$, 10.6%), 340 (M^+, 46.9%), 322, 286, 246, 245, 227, 187, 159 (100), 145, 120, 107, 81, 80, 69, 55. IR ν_{max} (KBr) cm^{-1}: 3419, 2928, 1635, 1456, 1387, 887. ^1H- and ^{13}C-NMR: see Table 1.

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