Dictyterpenoids A and B, Two Novel Diterpenoids with Feeding-Deterrent Activity from the Brown Alga *Dilophus okamurae*

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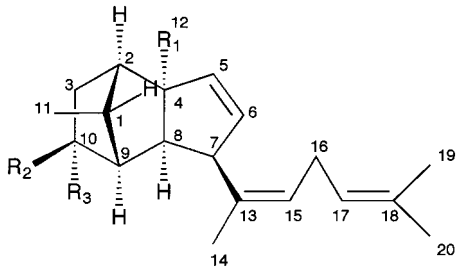
In our continuing efforts to study the presence of biologically active compounds that control seaweed−herbivore interactions, we examined the presence of such chemicals in freshly collected *Dilophus okamurae* Dawson. Our results demonstrated the presence of two diterpenoids, named dictyterpenoid A (**1**) and dictyterpenoid B (**2**), having a novel carbon skeleton, along with 10 known diterpenes, **3**−**12**. The structures of these novel metabolites were elucidated by spectroscopic and chemical methods. The isolated compounds showed feeding-deterrent activity against the young abalone *Haliotis discus hannai*.

Brown algae of the family Dictyotaceae (Dictyotales, Phaeophyceae) are known to produce diverse diterpenoid secondary metabolites with a wide variety of carbon frameworks, e.g., germacrane, selinane, cubebane, bourbonane, and perhydroazulene skeletons, with an additional prenyl unit on the side chain.[1−3] Some of these diterpenoids function as chemical deterrents against marine herbivores such as fishes and sea urchins.[4−8]

In our continuing studies on feeding deterrents from Japanese algae of the family Dictyotaceae,[9−14] we previously reported spatane-type,[9,12] secospatane-type,[11,12] and cubebane-type[10,12] diterpenoids from *Dilophus okamurae* Dawson collected at Karakuwa Peninsula, Miyagi Prefecture, on the coast of the Pacific Ocean. We sampled this alga from Awashima, Niigata Prefecture, on the coast of the Japan Sea. Awashima is a small island (ca. 6 km from south to north and ca. 2 km from east to west), situated about 50 km north-northeast of Niigata city. On the west coast of Awashima, algal communities of the family Dictyotaceae are widespread with *Dilophus okamurae* Dawson and *Dictyota dichotoma* (Hudson) Lamouroux as the dominant species. On the other hand, the east coast is dominated by members of the Sargassaceae family such as *Sargassum hemiphyllum* (Turner) C. Agardh, *S. confusum* C. Agardh, and *S. patens* C. Agardh. Ecological observation revealed the presence of herbivorous gastropods, such as top shells and sea urchins, in greater abundance on the east coast as compared to the west coast. Thus, we harvested *Dilophus okamurae* from the west coast and investigated the feeding-deterrent compounds. Bioassay-guided separation has led to the isolation of two novel diterpenes, named dictyterpenoid A (**1**) and dictyterpenoid B (**2**), together with 10 known diterpenes, **3**−**12**.

In this paper we describe the isolation and structural elucidation of these diterpenoid compounds along with their feeding-deterrent activities against the young abalone *Haliotis discus hannai*.



1: R_1 = CHO, R_2 = R_3 = O
2: R_1 = CH_2OH, R_2 = R_3 = O
13: R_1 = CH_2OH, R_2 = OH, R_3 = H

Results and Discussion

The neutral extract, which showed a moderate feeding-deterrent activity (*Ei* = 0.53) against the young abalone *Haliotis discus hannai*, was chromatographed on an alumina column using a hexane−EtOAc step gradient that gave five active fractions (except the fourth fraction) with potent feeding-deterrent activity. Hence, these active fractions were further subjected to a combination of normal-phase and reversed-phase HPLC to yield two new compounds, dictyterpenoid A (**1**) and dictyterpenoid B (**2**), and 10 known compounds, **3**−**12**.

Known compounds were identified by comparisons of spectral data with those of the authentic specimens or with those reported in the literature. Compounds **3** and **5** were identified as secospatanes previously isolated from this species collected at Karakuwa Peninsula, Miyagi Prefecture.[11,12] Compound **6** was a spatane diterpene also previously isolated from this species.[12] Compounds **4** and **7** were identical with a secospatane-type and a spatane-type diterpenoid, respectively, from *Dilophus marginatus*.[15] Compound **8** was identical with dictyone obtained from *Dictyota dichotoma*.[16] Compounds **9** and **10** were identified as pachydictyol A from *Pachydictyon coriaceum*[17] and isopachydictyol A from *Dictyota dichotoma*,[18,19] respectively. Compounds **11** and **12** were identified as 10-acetoxy-18-hydoxy-2,7-dollabelladiene from the sea hare *Dolabella californica*[20,21] and dictyolactone from the sea hare *Aplysia depilans*,[22] respectively. Feeding-deterrent activities of these compounds were evaluated using the cellulose plate method (see Experimental Section). Compounds **11** and **12**

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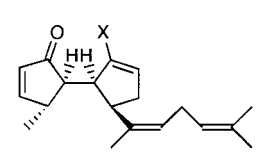
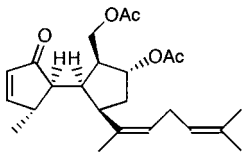


3: X = CO_2CH_3
4: X = CHO



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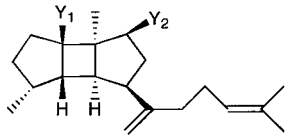
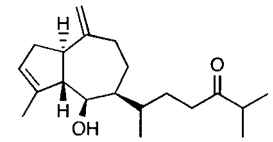


6: Y_1 = OH, Y_2 = H
7: Y_1 = OAc, Y_2 = OH



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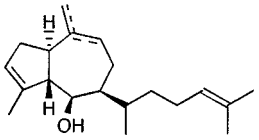
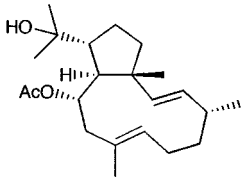


9: exo
10: endo



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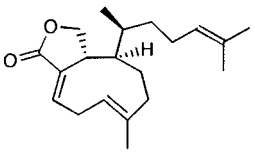

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showed strong feeding-deterrent activities, which are comparable to those of compounds **5** and **7**, thus suggesting that these diterpenoid secondary metabolites may synergistically function as chemical deterrents against marine herbivores as reported for other Dictyotaceae.[4−8]

Dictyterpenoid A (**1**), $[\alpha]^{24}_D$ +58° (*c* 0.23, $CHCl_3$), had the molecular formula $C_{20}H_{26}O_2$ established by LRFIMS and HREIMS. Its IR spectrum showed absorptions ascribable to a saturated five-membered ring ketone group at ν_{max} 1747 cm^{-1} and a formyl group at ν_{max} 1716 cm^{-1}; the latter was evident from a signal at δ_H 9.70 (1H, s) in the ^1H NMR spectrum (Table 1). The five-membered ring ketone was also supported by a signal at δ_C 215.0 in the ^{13}C NMR spectrum (Table 1).[23] Furthermore, the ^1H NMR spectrum (Table 1) showed the presence of a secondary methyl group [δ_H 0.93 (3H, d, J = 6.8 Hz)], three vinyl methyl groups [δ_H 1.62 (3H, br s), 1.70 (3H, br s), and 1.71 (3H, br s)], two trisubstituted double bonds [δ_H 5.08 (1H, br t, J = 6.8 Hz) and 5.37 (1H, br t, J = 6.8 Hz)], and a disubstituted double bond [δ_H 5.60 (1H, dd, J = 5.9, 2.5 Hz) and 6.17 (1H, dd, J = 5.9, 2.0 Hz)].

Dictyterpenoid B (**2**), $[\alpha]^{16}_D$ +27° (*c* 0.58, $CHCl_3$), was analyzed for $C_{20}H_{28}O_2$ and showed in its IR spectrum the presence of a hydroxyl group at ν_{max} 3430 cm^{-1} and a saturated five-membered ring ketone group at ν_{max} 1743 cm^{-1}. The ^1H NMR spectrum (Table 2) showed signals due to a hydroxymethyl group [δ_H 3.67 (1H, br d, J = 10.3 Hz) and 3.78 (1H, br d, J = 10.3 Hz)] as well as those due to a secondary methyl group [δ_H 0.91 (3H, d, J = 6.8 Hz)], three vinyl methyl groups [δ_H 1.61 (3H, br s), 1.68 (3H, br s), and 1.70 (3H, br s)], two trisubstituted double bonds [δ_H 5.08 (1H, br t, J = 6.8 Hz) and 5.32 (1H, br t, J = 6.8 Hz)], and a disubstituted double bond [δ_H 5.67 (1H, dd, J = 5.9, 2.4 Hz) and 6.05 (1H, dd, J = 5.9, 2.0 Hz)], as in the case of **1**.

Table 1. ^{13}C NMR (100 MHz, DEPT), ^1H NMR (400 MHz), and HMBC Data[a] for Dictyterpenoid A (**1**)

C[b,c]	^{13}C δ	^1H δ	J (Hz)	HMB (H→C)
1	39.5	2.58	br q, J = 6.8	C-8, C-10
2	44.0	2.61	br dd, J = 4.4, 2.4	C-9
3	36.1	1.99	dd, J = 19.0, 2.4 (Ha)	C-1, C-2, C-10
		2.22	dd, J = 19.0, 4.4 (Hb)	C-2, C-10
4	77.0			
5	127.8	5.60	dd, J = 5.9, 2.5	C-4, C-6, C-7, C-8
6	141.6	6.17	dd, J = 5.9, 2.0	C-4, C-5, C-7, C-8
7	50.8	4.07	br d, J = 9.3	C-6, C-8, C-13, C-14, C-15
8	44.3	2.88	d, J = 9.3	C-4, C-5, C-6, C-7, C-10, C-12
9	57.9	2.27	br s	C-1, C-2, C-3, C-4, C-8, C-10
10	215.0			
11	12.1	0.93	d, J = 6.8	C-2, C-9
12	200.0	9.70	s	
13	132.0			
14	23.3	1.70	br s	C-7
15	128.5	5.37	br t, J = 6.8	C-7, C-14, C-16, C-17
16	27.5	2.68	m	C-13, C-15, C-17, C-18
17	122.1	5.08	br t, J = 6.8	C-16, C-19, C-20
18	132.0			
19	17.8	1.62	br s	C-17, C-18, C-20
20	25.7	1.71	br s	C-17, C-18, C-19

[a] Measured in chloroform-d_1. [b] Assignment was made with the aid of the HSQC spectrum. [c] The numbering system corresponds to that used for spatane diterpenes.

Table 2. ^{13}C NMR (100 MHz, DEPT) and ^1H NMR (400 MHz) Data[a] for Dictyterpenoid B (**2**)

C[b,c]	^{13}C δ	^1H δ	J (Hz)
1	40.0	2.58	br q, J = 6.8
2	44.4	2.61	m
3	35.7	2.17	m
4	64.3		
5	133.3	5.67	dd, J = 5.9, 2.4
6	138.4	6.05	dd, J = 5.9, 2.0
7	51.0	3.98	br d, J = 9.3
8	47.1	2.14	d, J = 9.3
9	58.4	2.28	br s
10	215.2		
11	12.5	0.91	d, J = 6.8
12	66.1	3.67	br d, J = 10.3
		3.78	br d, J = 10.3
13	132.0		
14	23.5	1.68	br s
15	127.7	5.32	br t, J = 6.8
16	27.4	2.63	br t, J = 6.8
17	122.5	5.08	br t, J = 6.8
18	131.9		
19	17.8	1.61	br s
20	25.7	1.70	br s

[a] Measured in chloroform-d_1. [b] Assignment was made with the aid of the HSQC spectrum. [c] The numbering system corresponds to that used for spatane diterpenes.

The ^1H and ^{13}C NMR spectra (Table 1) of **1** were very similar to those (Table 2) of **2**. Detailed comparisons of the NMR data of both compounds suggested that dictyterpenoid A (**1**) has a formyl group instead of a hydroxymethyl group in dictyterpenoid B (**2**). This was confirmed by the following reaction. Treatment of **1** with sodium borohydride in EtOH for 10 min at room temperature yielded a hydroxy ketone which was identical with dictyterpenoid B (**2**) in all respects. Moreover, treatment of **1** with sodium borohydride in EtOH for 2 h at room temperature produced a dihydroxy compound **13**, $C_{20}H_{30}O_2$, which gave valuable

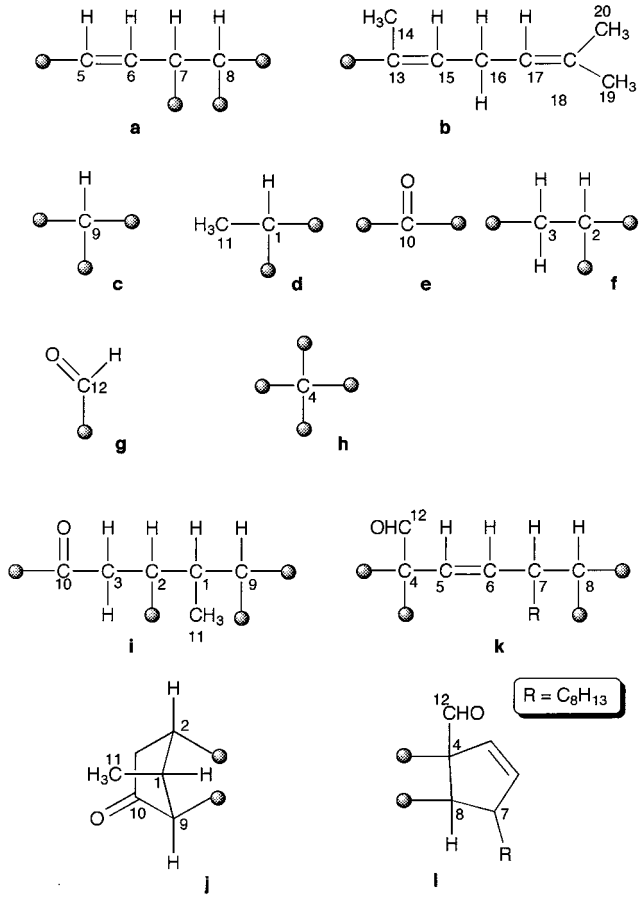

Figure 1. Partial structural units of dictyterpenoid A (**1**).

information pertaining to the position of the ketonic group and the partial structure in its proximity (vide infra).

The ^{1}H and ^{13}C NMR spectra coupled with 2D NMR spectra of ^{1}H−^{1}H COSY and HSQC substantiated the molecular formula of dictyterpenoid A (**1**), which is shown in the partial structural units **a**−**h** (Figure 1).[24] The 1,5-dimethyl-1,4-hexadienyl moiety (unit **b**) is frequently encountered in diterpenoids from the family Dictyotaceae. The chemical shift value (δ_C 23.3)[25] of the C-14 suggested that the double bond between C-13 and C-15 has *Z*-configuration, which was further confirmed by the NOESY spectrum (vide infra). Furthermore, judging from the *J*-value (5.9 Hz) of the pertinent protons, the double bond in **a** must be a portion of cyclopentene ring.[26] The chemical shift value (δ_H 2.22 and 1.99) and the large geminal coupling constant ($^2J_{HH}$ = 19.0 Hz) of one (unit **f**) of the two methylene groups in **1** strongly suggested that this methylene group is adjacent to a carbonyl group (unit **e**). This was confirmed by the ^{1}H−^{1}H COSY spectrum of diol **13**, which indicated the presence of a •−CH(OH)−CH_2−CH−• grouping. The above-mentioned spectral data revealed that there were no other unsaturated bonds apart from those of the ketone, aldehyde, and three double-bond moieties. Therefore, dictyterpenoid A (**1**), which has eight degrees of unsaturation, must be composed of three carbocyclic rings.

Confirmation of the partial structural units and determination of their connectivities were made with the aid of a HMBC spectrum, whose results are summarized in Table 1. A long-range correlation between C-13 (δ_C 132.0) and the methine proton (δ_H 4.07) of C-7 and correlations between C-7 (δ_C 50.8) and both the methyl protons (δ_H 1.70) of C-14 and the vinyl proton (δ_H 5.37) of C-15 confirmed

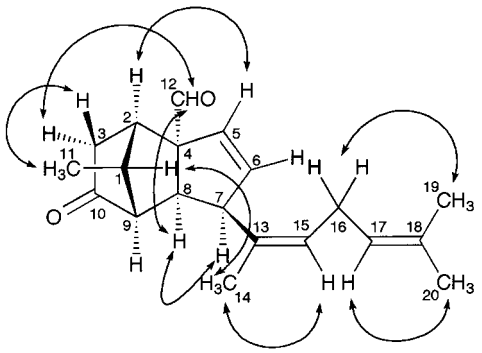

Figure 2. Selected NOEs from the NOESY spectrum of dictyterpenoid A (**1**).

the connection of C-7 in **a** with C-13 in **b**. Furthermore, the isolated secondary methyl group (δ_H 0.93) (unit **d**) showed cross-peaks to both carbons at C-9 (δ_C 57.9) (unit **c**) and C-2 (δ_H 44.0) (unit **f**). The connection of C-10 (unit **e**) with C-3 (unit **f**), which is discussed above, was supported by a cross-peak between signals for C-10 and H_2-3, thus leading to the expanded structure **i**. Since the carbonyl carbon signal of C-10 in **i** showed cross-peaks to H-9 and H-1, the unit **i** was expanded to the unit **j** containing a five-membered ring ketone. In addition, the quaternary carbon at δ_C 77.0 (unit **h**) showed cross-peaks to the vinyl protons, H-5 (δ_H 5.60) and H-6 (δ_H 6.17) (unit **a**), and the aldehyde proton at δ_H 9.70 (unit **g**), leading to the partial structural units **a**, **b**, **g**, and **h**, which could be combined to give the expanded structure **k**. Moreover, the signal for H-8 at δ_H 2.88 showed cross-peaks to the quaternary carbon line for C-4 and the aldehyde carbon (δ_C 200.0), confirming the connection of C-8 with C-4 to lead to a cyclopentene unit, **l**. Finally, the cross-peak between the carbonyl carbon signal at C-10 in **j** and the methine proton at C-8 in **l** indicated the connection of C-9 with C-8 and the connection of C-2 with C-4, thus leading to the planar formula **1** with a tricyclo[5.2.1.0^{2,6}]dec-3-ene skeleton for dictyterpenoid A.

The relative stereochemistry was determined from the NOESY spectrum, whose results are depicted in Figure 2. The NOEs were observed between H-8/H-12 and H-8/H-7, thus indicating that these protons on the cyclopentene ring are oriented in the same direction. In addition, the NOEs between H_α-3/H-12, H-2/H-5, H-1/H_3-14, H_β-3/H_3-11, and H-15/H_3-14 confirmed the relative configurations of dictyterpenoid A, as shown in formula **1**. The stereochemistry of the hydroxyl group at C-10 in compound **13** was deduced as follows. As in $NaBH_4$ reduction of dictyterpenoid A (**1**), the reducing reagent attacks from the less hindered α-side of **1**, the resulting hydroxyl group must have a *β*-oriented configuration. The close proximity of the methyl group (H_3-11) of C-1 and the hydroxyl group at C-10 was evident from the lower chemical shift (δ_H 1.06) of the H_3-11 in **13**.

In consequence, the structures of dictyterpenoid A and B, including the relative configuration, are represented by formulas **1** and **2**, respectively. Dictyterpenoids A and B are presumably derived from a spatane-type precursor via a secospatane skeleton. A possible biogenetic pathway from geranylgeranyl pyrophosphate (GGPP) is shown in Figure 3.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a JASCO A-102 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured in $CDCl_3$ or C_6D_6 solution with TMS as the internal standard by using a JEOL-JNM-EX-400 spectrometer. FIMS were obtained

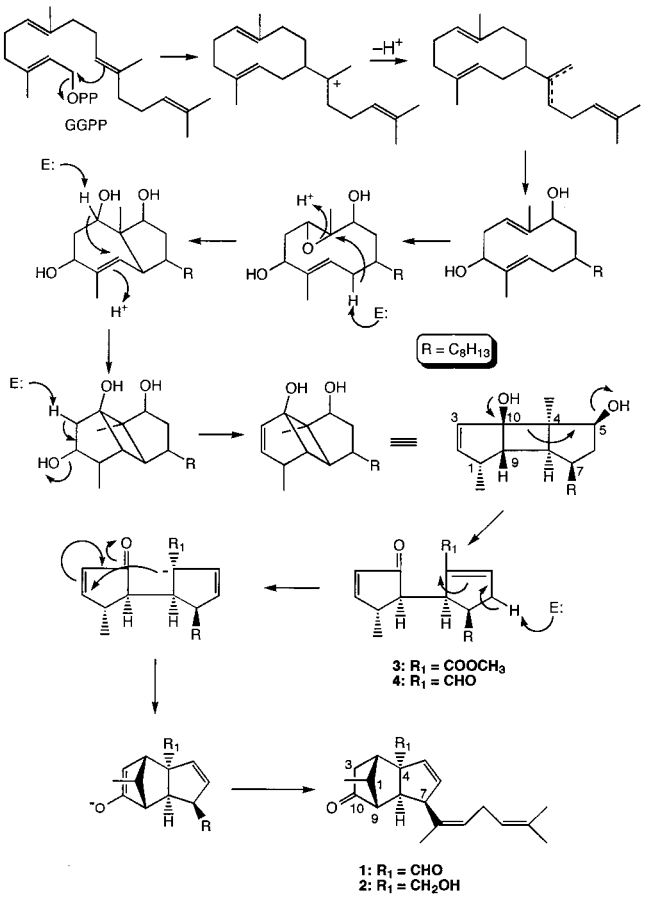

Figure 3. Possible biogenetic pathway for dictyterpenoids A (**1**) and B (**2**).

on a JEOL JMS-01SG-2 spectrometer. EIMS and HREIMS were obtained on a JEOL JMS-DX-300 spectrometer. Optical rotations were measured on a JASCO DIP-140 polarimeter. Aluminum oxide (Merck, Aluminumoxide 90, activity II-III) and Si gel (Merck, Kieselgel 60, 70−230 mesh) were used for column chromatography. A Si gel plate (Merck, Kieselgel 60 F_{254S}) was used for preparative TLC (PTLC). HPLC was carried out using JASCO Megapak SIL-C_{18} (for reversed-phase) and JASCO Megapak SIL-CN (for normal phase) columns. All known compounds were identified by comparisons of the spectral data with those of the authentic specimens or with those reported in the literature.

Plant Material. *Dilophus okamurae* Dawson was collected on May 10, 1992, on the west coast of Awashima, Niigata Prefecture, and identified by Prof. M. Masuda, Division of Biological Sciences, Graduate School of Science, Hokkaido University.

Extraction and Isolation. Partially dried algae (400 g) were extracted with Me_2CO. The Me_2CO extract was concentrated in vacuo and then partitioned between Et_2O and H_2O. The Et_2O layer was successively shaken with 5% KOH and 0.5 M HCl to separate the acidic and basic part, respectively. The Et_2O layer was then washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to leave a neutral, brown oil (13 g), which showed feeding-deterrent activity ($Ei = 0.53$). A portion (2.3 g) of the neutral oil was fractionated by column chromatography on alumina with a step gradient (hexane and EtOAc) to give five fractions (F1−F5), which exhibited feeding-deterrent activity except for the fourth fraction (F4).

The fraction (F2)[27] (188 mg) ($Ei = 0.67$) eluted with hexane/EtOAc (19:1) was further subjected to reversed-phase HPLC with MeOH/H_2O (90:10) to give four fractions. The first fraction was then subjected to normal-phase HPLC with hexane/EtOAc (100:0.5) to give compound **3**[11,12] (0.017% of dry alga) and a mixture which was further separated by successive reversed-phase HPLC with MeOH/H_2O (85:15) and normal-phase HPLC with hexane/*i*-PrOH (100:0.1) to give dictyone (**8**)[16] (0.0029%) and an unidentified compound.[28] The second fraction was then separated by normal-phase HPLC with hexane/*i*-PrOH (100:0.1) followed by reversed-phase HPLC with MeOH/H_2O (85:15) to afford 10-acetoxy-18-hydoxy-2,7-dollabelladiene (**11**)[20,21] (0.0033%) and dictyolactone (**12**)[22] (0.0033%). The third fraction was separated by reversed-phase HPLC with MeOH/H_2O (85:15) followed by normal-phase HPLC with hexane/*i*-PrOH (100:0.5) to give compound **6**[12] (0.0072%). The fourth fraction was further subjected to repeated normal-phase HPLC with hexane/*i*-PrOH (100:0.1) to give pachydictyol A (**9**)[17] (0.0072%) and isopachydictyol A (**10**)[18,19] (0.0016%).

Moreover, the fraction (F3) (728 mg) ($Ei = 0.73$) eluted with hexane/EtOAc (9:1) was further subjected to reversed-phase HPLC with MeOH/H_2O (85:15) followed by a combination of repeated reversed-phase HPLC with MeOH/H_2O (80:20) and normal-phase HPLC with hexane/*i*-PrOH (100:0.5) to give dictyterpenoid A (**1**) (0.057%) and compounds **4**[15] (0.013%), **5**[11,12] (0.0042%), and **7**[15] (0.030%).

The fraction (F5) (241 mg) ($Ei = 0.57$) eluted with EtOAc was further separated by reversed-phase HPLC with MeOH/H_2O (80:20) followed by a combination of reversed-phase HPLC with MeOH/H_2O (70:30) and repeated normal-phase HPLC with hexane/*i*-PrOH (100:5) to give dictyterpenoid B (**2**) (0.014%).

Dictyterpenoid A (1): colorless oil; $[\alpha]^{24}_D$ +58° (*c* 0.23, $CHCl_3$); IR (neat) ν_{max} 1747, 1716, 1156, 1021, 788, 727 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, Table 1; LRFIMS *m/z* (rel int) 298 $[M]^+$ (29), 242 $[M - C_4H_8]^+$ (100); LR-EIMS *m/z* (rel int) 242 $[M - C_4H_8]^+$ (33), 213 (14), 185 (16), 171 (23), 159 (19), 147 (30), 131 (22), 117 (24), 105 (26), 91 (44), 77 (31), 69 (40), 55 (43), 41 (100); HR-EIMS *m/z* 242.1311 (calcd for $C_{16}H_{18}O_2$, 242.1307, $M - C_4H_8$).

Dictyterpenoid B (2): colorless oil; $[\alpha]^{16}_D$ +27° (*c* 0.58, $CHCl_3$); IR (neat) ν_{max} 3430, 1743, 1167, 1045, 796, 732 cm^{-1}; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data, Table 2; ^1H NMR (C_6D_6) δ 0.64 (3H, d, $J = 6.8$ Hz, H_3-11), 1.58 (3H, br s, H_3-19), 1.58 (3H, br s, H_3-14), 1.72 (3H, br s, H_3-20), 1.91 (1H, m, H-2), 1.95 (2H, m, H_2-3), 2.07 (1H, d, $J = 9.3$ Hz, H-8), 2.28 (1H, br s, H-9), 2.40 (1H, br q, $J = 6.8$ Hz, H-1), 2.72 (2H, br t, $J = 6.8$ Hz, H_2-16), 3.27 (1H, br d, $J = 10.8$ Hz, H-12a), 3.34 (1H, d, $J = 10.8$ Hz, H-12b), 3.89 (1H, br d, $J = 9.3$ Hz, H-7), 5.24 (1H, br t, $J = 6.8$ Hz, H-17), 5.34 (1H, br t, $J = 6.8$ Hz, H-15), 5.42 (1H, dd, $J = 5.9$, 2.5 Hz, H-5), 5.70 (1H, dd, $J = 5.9$, 2.0 Hz, H-6); ^{13}C NMR (C_6D_6) CH_3: δ 12.4 (C-11), 17.8 (C-19), 23.5 (C-14), 25.8 (C-20), CH_2: δ 27.8 (C-16), 35.6 (C-3), 66.0 (C-12), CH: δ 39.9 (C-1), 45.5 (C-2), 47.4 (C-8), 51.1 (C-7), 58.4 (C-9), 123.1 (C-17), 128.5 (C-15), 134.4 (C-5), 137.6 (C-6), C: δ 64.3 (C-4), 131.8 (C-18), 133.8 (C-13), 215.2 (C-10); LRFIMS *m/z* (rel int) 300 $[M]^+$ (24), 244 $[M - C_4H_8]^+$ (100); LREIMS *m/z* (rel int) 282 $[M - H_2O]^+$ (2), 244 $[M - C_4H_8]^+$ (100), 229 (6), 213 (9), 199 (7), 185 (32), 171 (41), 157 (21), 143 (37), 131 (69), 119 (28), 105 (42), 91 (49), 77 (30), 69 (48), 55 (48), 41 (87); HREIMS *m/z* 244.1457 (calcd for $C_{16}H_{20}O_2$, 244.1463, $M - C_4H_8$).

Conversion of 1 into 2. To a solution of **1** (10 mg) in EtOH (30 μL) was added $NaBH_4$ (0.3 mg) in EtOH (60 μL). The mixture was stirred for 10 min at room temperature, then the solvent was removed in vacuo, H_2O was added, and the solution was extracted with Et_2O. The Et_2O extract was washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated to yield an oily substance, which was purified by PTLC with hexane/EtOAc (1:1) to give a colorless oil (3.0 mg), whose IR and NMR data as well as the optical rotation were identical with those of dictyterpenoid B (**2**).

Conversion of 1 into 13. To a solution of **1** (12 mg) in EtOH (30 μL) was added $NaBH_4$ (1.5 mg) in EtOH (60 μL). The mixture was stirred for 2 h at room temperature, then the solvent was removed in vacuo, H_2O was added, and the solution was extracted with Et_2O. The Et_2O extract was washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated to yield an oily substance, which was purified by PTLC with hexane/EtOAc (1:1) to give **13** (5.0 mg): colorless oil; $[\alpha]^{24}_D$ +50° (*c* 0.22, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.06 (3H,

d, $J = 7.3$ Hz, H_3-11), 1.63 (3H, br s, H_3-19), 1.63 (1H, br ddd, $J = 14.2$, 3.9, 3.9 Hz, H-3b), 1.70 (3H, br s, H_3-20), 1.71 (3H, br s, H_3-14), 1.71 (1H, d, $J = 9.8$ Hz, H-8), 1.79 (1H, br s, H-9), 1.89 (1H, br d, $J = 3.9$ Hz, H-2), 2.10 (1H, br dd, $J = 14.2$, 7.3 Hz, H-3a), 2.21 (1H, br q, $J = 7.3$ Hz, H-1), 2.67 (2H, m, H_2-16), 3.54 (1H, br d, $J = 10.7$ Hz, H-12a), 3.65 (1H, d, $J = 10.7$ Hz, H-12b), 3.75 (1H, br dd, $J = 7.3$, 3.9 Hz, H-10), 3.90 (1H, br d, $J = 9.8$ Hz, H-7), 5.09 (1H, br t, $J = 7.3$ Hz, H-17), 5.29 (1H, br d, $J = 7.3$ Hz, H-15), 5.51 (1H, dd, $J = 5.9$, 2.5 Hz, H-5), 5.96 (1H, dd, $J = 5.9$, 2.0 Hz, H-6); ^{13}C NMR ($CDCl_3$) CH_3: δ 12.7 (C-11), 17.8 (C-19), 23.6 (C-14), 25.7 (C-20), CH_2: δ 27.4 (C-16), 33.3 (C-3), 66.4 (C-12), CH: δ 40.1 (C-1), 45.8 (C-2), 49.9 (C-8), 50.9 (C-7), 51.6 (C-9), 77.3 (C-10), 122.9 (C-17), 126.7 (C-15), 133.4 (C-5), 137.9 (C-6), C: δ 63.4 (C-4), 131.7 (C-18), 134.0 (C-13); LRFIMS *m/z* (rel int) 302 $[M]^+$ (29), 246 $[M - C_4H_8]^+$ (100); HRFIMS *m/z* 246.1617 (calcd for $C_{16}H_{22}O_2$, 246.1628, $M - C_4H_8$).

Bioassay. The simple and convenient feeding-deterrent assay was performed using cellulose TLC sheets according to a previous protocol.[29] Relative feeding-deterrent activity was defined by the following equation (*Ei*: electivity index, *Pi*: average number of biting traces of the control (PC), *pi*: average number of biting traces of each sample).[30] Significant differences ($p < 0.01$ or 0.05) of feeding-deterrent activity were assessed by the *t*-test.

$$Ei = \frac{Pi - pi}{Pi + pi}$$

The feeding-deterrent activities of the isolated compounds **1**−**12** against the young abalone *Haliotis discus hannai* were as follows: *Ei*: **1** = 0.59, **2** = 0.23, **3** = 0.65, **4** = 0.49, **5** = 0.85, **6** = 0.66, **7** = 0.90, **8** = 0.48, **9** = 0.59, **10** = 0.36, **11** = 0.93, and **12** = 0.74.

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