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SPATANE-TYPE DITERPENES WITH BIOLOGICAL ACTIVITY FROM THE BROWN ALGA *DILOPHUS OKAMURAI*

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Key Word Index—*Dilophus okamurai*, Dictyotaceae, brown alga; structure; biological activity, spatane-type diterpenoids.

Abstract—The neutral methanol extract of the brown alga *Dilophus okamurai* was found to inhibit the settlement and the metamorphosis of the swimming larvae (veliger) of the abalone *Haliotis discus hannai* Ino. The active components were identified as spatane-type diterpenes.

INTRODUCTION

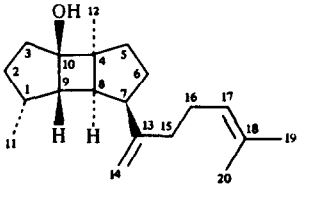
Among Japanese gastropods, the abalone *Haliotis discus hannai* Ino is known to feed preferentially on the brown algae of the family Laminariaceae, such as Eisenia bicyclis (Kjellman) Setchell, *Ecklonia cava* Kjellman, *Undaria pinnantifida* (Harvey) Suringar and *Laminaria* species [1, 2]. The feeding attractants [3–5] and the feeding stimulants [6–8] for young abalone have been studied. By contrast, these herbivorous abalones can scarcely be seen in the community of the brown alga *Dilophus okamurai* Dawson which grow in a depth of water very similar to that of *Eisenia bicyclis*, thus suggesting that *Dilophus okamurai* contains anti-feeding substance against the abalone.

The neutral methanol extract of *D. okamurai* was found to inhibit the settlement and the metamorphosis of the swimming larvae (veliger) of the abalone *Haliotis discus hannai* Ino. Screening investigations of the extract have led to the isolation of two active diterpenes, each with a spatane skeleton. In this paper, we report the isolation and the structures and biological activity of these metabolites as well as the structure of an inactive spatane diterpene.

RESULTS AND DISCUSSION

The brown alga *D. okamurai* was collected in December 1984 at Karakuwa Peninsula, Miyagi Prefecture. Partly-dried alga was extracted with methanol, and the concentrated methanol extracts were partitioned between ether and water. The ethereal layer was successively shaken with 5% KOH and 1 M HCl to separate the acidic and the basic component, respectively. The neutral fraction strongly inhibited the settlement and the metamorphosis of the swimming larvae (veliger) of the abalone *Haliotis discus hannai* Ino. The neutral fraction was fractionated, as outlined in the Experimental, by a combination of open column chromatography and HPLC to yield two active diterpenoids, **1** and **2**

The first of the active metabolites (**1**) had the molecular formula $C_{20}H_{32}O$ (HR-EIMS: *m/z* 288.2447, calcd for $C_{20}H_{32}O$, 288 2453). Its IR spectrum showed an absorption maximum at ν_{max} 3400 cm^{-1} characteristic of a hydroxyl group, the tertiary nature of which was evident from a signal due to a quaternary carbon atom at δ 93.7 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum of **1** revealed the presence of a secondary methyl group at δ 0.92, a tertiary methyl group at δ 1.20 and two vinyl



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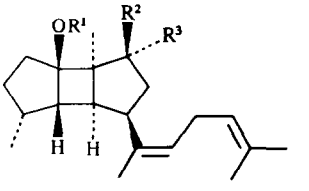


	R¹	R²	R³
2	H	H	H
3	H	OH	H
4	Ac	OAc	H
6	Ac	OH	H
7	H	OAc	H

methyl groups at δ 1 60 and 1 67 In addition, the remaining signals at δ 4 82 (1H, *br s*), 4 94 (1H, *br s*) and 5 08 (1H, *br t*, $J=7$ Hz) indicated the presence of an exomethylene group and a trisubstituted double bond, which was substantiated by the ^{13}C NMR spectrum The low-field region in the ^{13}C NMR spectrum showed exomethylene signals at δ 148 4 (*s*) and 109 8 (*t*) and trisubstituted double bond signals at δ 131 4 (*s*) and 124 4 (*d*) This, along with the molecular formula, required that compound **1** had a tricyclic carbon framework

The ^{1}H–^{1}H 2D-COSY spectrum of **1** gave the limited information that compound **1** contained a 1-methylene-5-methyl-4-hexenyl side chain. On the other hand, the 2D-COSY ^{1}H NMR spectra recorded in the presence of various amounts of the shift reagent Eu(dpm)$_3$ provided stereochemical and connectivity relationships for compound **1**, leading to partial formula **A** (Fig 1) A combination of the partial formula **A** with the partial formula **B** led to 10 possible planar formulae for compound **1**. Of these 10 formulae, formula **5** with a spatane carbon skeleton agreed best with the experimental data. At this stage we were aware that a spatane derivative with the same planar structure as that of our compound, had been isolated from the Australian brown alga *Dilophus marginatus* [9]. However, the spectral properties of our compound, although very similar, were not identical with those [9] reported

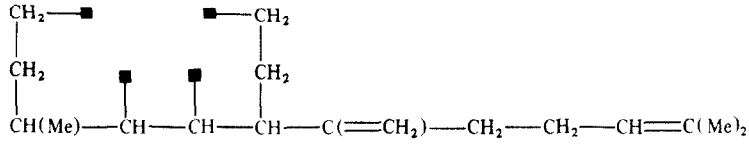
The stereochemistry was deduced with the aid of the chemical shifts (Fig 2) in the ^{1}H NMR spectrum of **1** measured in the presence of 0.60 mol equivalent of Eu (dpm)$_3$ The X-ray diffraction study of the *p*-bromobenzoate derivative of spatol [10], the first example of a spatane diterpenoid, showed that the cyclobutane ring has both cyclopentane rings joined in a *cis*-anti-*cis* fashion in a manner identical with that in bourbonene Similarly, a Dreiding model study also indicated that the most stable tricyclic spatane skeleton seems to adopt a *cis*-anti-*cis* configuration which was supported by the chemical shifts in the LIS ^{1}H NMR spectrum of **1** As already described, the hydroxyl group is *trans* to the tertiary methyl group, reflecting a slight down-field shift of the tertiary methyl signal Furthermore, the secondary methyl group is also *trans to* the hydroxyl group since the signal of the secondary methyl group was slightly shifted, while that of the methine proton at C-1 showed a large shift In addition, the stereochemistry of the side chain at C-7 was assigned the same β-configuration as that shown in the hitherto known spatane diterpenes, since the signal of the methine proton at C-7 was observed slightly down-field in the LIS spectra If the side chain at C-7 had α-configuration, the signal of H-7 would be expected to be observed considerably down-field because H-7 would be situated *cis* to the hydroxyl group at C-10 Furthermore, the reason why the signal of H-9, *cis* to the hydroxyl group at C-10, was observed considerably up-field appears to be explicable by the shielding effect of the C-13 (14) double bond which is situated close to H-9

Consequently, the structure of compound **1** is best represented by formula **1**, though this is not fully conclusive

Another active metabolite, compound **2**, exhibited spectral properties almost identical with those [9] reported for one of the other spatane derivatives isolated from *D marginatus*. Although a direct comparison could not be achieved because of the lack of spectral data, the metabolites seem to be identical

Attempts to obtain further information for the structure were not possible due to decomposition of the sample during the measurement of its ^{13}C NMR spectrum Therefore, without further evidence, formula **2** was tentatively assigned for compound **2** on the basis of the comparison of its spectral data with those of the diacetate **4** derived from the inactive spatane diterpene **3**, which was also isolated from the same alga, coupled with the co-existence of **1**–**3** in the same alga

The inactive metabolite **3** was isolated as the diacetate **4** The spectral properties of **4** were very similar to those [9] reported for another spatane derivative (**6**) also isolated *D marginatus* The structure of **4** was deduced in a manner similar to that used for **1**, i e. ^{1}H–^{1}H 2D-COSY spectrum of **4** together with partial decoupling experiments The *cis* relationship of both hydroxyl groups in **3** could be inferred from the fact that the tertiary hydroxyl group at C-10 was readily acetylated by acetic anhydride and pyridine at room temperature, whereas the tertiary hydroxyl group at C-10 in **1** was resistant to acetylation with acetic anhydride and pyridine This unusual acetylation is due to acetyl migration from the acetoxyl group at C-5 in the course of the aceteylation reaction in a manner very similar to the acyl migration of *N*-acyl-ψ-nortropine [11] If the geometry of both hydroxyl groups at C-5 and C-10 in **3** is *cis*, the cyclic intermediate formed from the monoacetate **7** is a stable six-membered ring and the acetyl migration seems to occur from C-5 to C-10 followed by further acetylation of the free hydroxyl group at C-5 to afford the diacetate **4** The configuration at C-5 was also supported by the observation of a NOE between H-5 and H$_3$-12 in the NOE difference spectra Moreover,



A

■—OH ■—Me ■ x 2

B

Fig 1 Partial formulae (■ quaternary carbon)

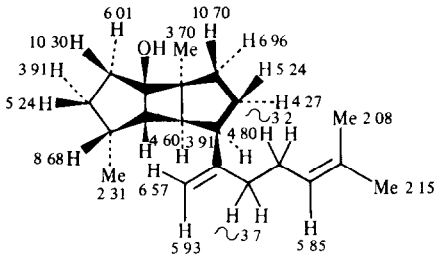


Fig 2 Stereochemistry and chemical shifts in the ^{1}H NMR spectrum of **1** in the presence of 0 60 mol equiv of Eu(dpm)$_3$.

as depicted in Fig 3, the NOE difference spectra provided the stereochemical relationships in compound **4**, leading to formula **4** for the diacetate **4**

The active metabolites, **1** and **2**, inhibited the settlement and the metamorphosis of the swimming larvae (veliger) of the abalone *Haliotis discus hannai* Ino at a concentration of 5 ng/ml [ED$_{50}$]. Details of the biological evaluation will be presented elsewhere. It remains to be proved whether or not these two spatane diterpenes are true anti-feedants.

EXPERIMENTAL

^{1}H NMR· 270 MHz and ^{13}C NMR · 67.8 MHz, $CDCl_3$, TMS as int standard, LR-MS. 70 ev; CC Al_2O_3 (Merck, Al_2O_3 90, activity II-III), packed CC ODS (Fuji, packed column RQ-2); HPLC· Megapak SIL-C$_{18}$ (JASCO), Megapak SIL-CN (JASCO) and Finepak SIL-CN (JASCO)

Isolation. Dilophus okamurai Dawson was collected by hand using scuba equipment (−3 to −5 m) at Karakuwa Peninsula, Miyagi Prefecture, in Dec. 1984. Half- dried alga was extracted (× 2) with MeOH, and the concd MeOH extracts were partitioned between Et_2O and H_2O The ethereal layer was successively shaken with KOH (5%) and HCL (1 M) to separate the acidic and the basic components, respectively. Of the neutral, the acidic, the basic and the water-soluble fractions, the neutral fraction displayed a strong inhibitory activity against the settlement and the metamorphosis of the swimming larvae (veliger) of the abalone *Haliotis discus hannai* Ino The neutral extract (20 g) was fractionated by CC over Al_2O_3 The C_6H_6 fraction showed inhibitory activity and was subjected to packed CC on ODS The active fraction eluted with MeOH was then chromatographed on reverse-phase HPLC (MeOH–H_2O, 19 1) The active fraction was further repeatedly chromatographed on HPLC (hexane–*iso*–PrOH) to yield **1** (9 mg) and **2** (6 mg) The inactive spatane derivative **3** was obtained as the diacetate **4** in 0 15% yield

Compound **1** Oil, $[\alpha]_D^{20}$ −43 3° (*c* 0 962, $CHCl_3$); IR ν_{max}^{neat} cm^{-1} 3400, 3080, 1643, 1277, 1249, 1218, 1145, 1105, 1070, 1027, 948 and 891, ^{1}H NMR· δ0 92 (3H, *d*, *J* = 7 Hz), 1 20 (3H, *s*), 1 3–1 6 (5H, *m*), 1 60 (3H, *br s*), 1 7–2 0 (5H, *m*), 2 0–2.2 (3H,*m*), 2 30 (2H, *m*), 2 50 (2H, *m*), 4 82 (1H,*br s*), 4 94 (1H, *br s*) and 5 08 (1H, *br t*, *J* = 7 Hz), ^{13}C NMR (INEPT) Me, δ17 5, 17 7, 20 7 and 25.7, CH_2, 15 1, 26 4, 27 2, 31 2, 33 5, 36 5 and 109 8, CH, δ34 6,43 7, 44 4, 54 8 and 124 4, C, δ47 9, 93 7, 131 4 and 148 4, MS *m/z* (rel int): 288 2 [M]$^+$ (2), 273 [M−Me]$^+$(1), 270 [M−H_2O]$^+$ (1), 260 (40), 177 (20), 147 (19), 121 (27), 109 (56), 107 (28), 105 (20), 95 (21), 93 (26), 91 (26), 81 (29), 79 (26), 69 (92), 67 (25), 55 (29), 43 (36) and 41 (100), HRMS *m/z* 288 2447 Calc for $C_{20}H_{32}O$, 288 2453

Compound **2** Oil, $[\alpha]_D^{25}$ +146° (*c* 0 843, $CHCl_3$), IR ν_{max}^{neat} cm^{-1}. 3340, 3020, 1647, 1298, 1240, 1220, 1192, 1180, 1143, 1110, 1090, 1038, 1022, 981 and 965, ^{1}H NMR· δ 0.80 (3H, *d*, *J* = 7 Hz), 1.03 (3H, *s*), 1 63 (3H, *br s*), 1 69 (3H, *br s*), 1 73 (3H, *br s*), 1 1–1 8 (6H, *m*), 1 8–2 2 (6H, *m*), 2 67 (2H, *m*), 2 85 (1H, *ddd*, *J* = 12, 6, 6 Hz), 5 09 (1H, *br t*,*J* = 7 Hz) and 5 16 (1H, *br t*, *J* = 7 Hz), ^{13}C NMR (INEPT). Me, δ 14 0, 17 7, 20 3, 23 9 and 25 7, CH_2, δ 26 8, 29 5, 33 2, 34.6 and 36.9, CH, δ 35 6, 42 4, 43 9, 50 0, 123 6 and 125 4, C; δ 47 9, 82 2, 131.0 and 134.8, MS *m/z* (rel int) 288 [M]$^+$ (2), 270 [M−H_2O]$^+$ (3), 203 (6), 191 (6), 175 (6), 159 (5), 147 (10), 135 (25), 134 (100), 121 (24), 119 (21), 109 (16), 107 (12), 105 (16), 95 (7), 93 (23), 91 (14), 83 (17), 81 (22), 79 (12), 77 (9), 69 (14), 67 (10), 55 (17), 43 (12) and 41 (31), HRMS *m/z* 288.2445 Calc for $C_{20}H_{32}O$, 288 2453

Compound **4** Oil, $[\alpha]_D^{25}$ +60 4° (*c* 1 05, $CHCl_3$), IR ν_{max}^{neat} cm^{-1}· 1740, 1250, 1193, 1110, 1102, 1028, 980 and 890, ^{1}H NMR. δ0.81 (3H, *d*, *J* = 7 Hz, H_3-11), 1 08 (3H, *s*, H_3-12), ~1 30 (1H, *m*, H-2β), ~1 60 (1H, *m*, H-3β, overlapped), 1 64 (3H, *br s*, H_3-20), 1 69 (3H, *d*, *J* = 1 Hz, H_3-19), 1 75 (3H, *d*,*J* = 1 Hz, H_3-14), ~1 81 (1H, *m*, H-2 , overlapped), 1 84 (1H, *dd*, *J* = 14, 6 Hz, H-6α), ~1 87 (1H, *dd*, *J* = 6, 6 Hz, H-8, overlapped), 2 03 (3H, *s*, Ac), 2.04 (3H, *s*, Ac), ~2 20 (1H, *m*, H-1), 2 30 (1H, *ddd*, *J* = 14, 13, 4 5 Hz, H-6β), 2.43 (1H, *dd*, *J* = 6, 5 Hz, H-9), 2 47 (1H, *dd*, *J* = 13, 6 Hz, H-3α), 2 70 (2H, *m*, H_2-16), 3 18 (1H, *ddd*, *J* = 13, 6, 6 Hz, H-7), 5 10 (1H, *d*, *J* = 4 5 Hz, H-5), 5 10 (1H, *br t*, *J* = 7 Hz, H-17) and 5 22 (1H, *br t*, *J* = 7 Hz, H-15), ^{13}C NMR (INEPT). Me, δ 13.7, 13 8, 17 7, 21 2, 21 3, 23 7 and 25 7, CH_2, δ 26.9, 33.2, 34 6 and 36.1, CH, δ 34 6, 41 3, 43 1, 46 4, 77 9, 123 3 and 126 6, C, δ 51 2, 88 4, 131 3, 133.3, 170 1 and 170 2, MS *m/z* (rel int): 328 [M−HOAc]$^+$(7), 268 [M−2HOAc]$^+$(22), 212 (17), 199 (18), 188 (26), 173 (22), 159 (17), 145 (60), 133 (39), 132 (95), 119 (26), 109 (48), 107 (28), 105 (25), 98 (55), 95 (17), 93 (35), 91 (22), 83 (33), 81 (26), 79 (18), 69 (43), 67 (21), 55 (33), 43 (100) and 41 (47), HRMS *m/z* 328.2422 and 268 2195 Calc for $C_{22}H_{32}O_2$, 328.2401, M−HOAc, and $C_{20}H_{28}$, 268.2190, M−2 HOAc

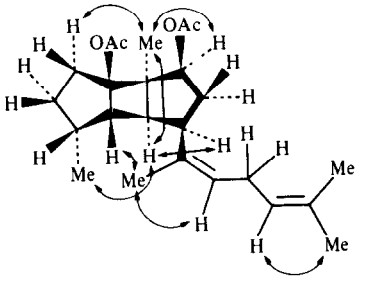


Fig 3. NOE (↔).

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