CHEMISTRY LETTERS, pp. 1629-1632, 1988.

A New Feeding-Deterrent Diterpenoid from the Brown Alga *Dilophus okamurai* Dawson

Kazuya KURATA,* Kazunari SHIRAISHI,† Tohru TAKATO,† Kazuya TANIGUCHI,††
and Minoru SUZUKI*†††

Department of Industrial Chemistry, Hakodate Technical College, Hakodate 042

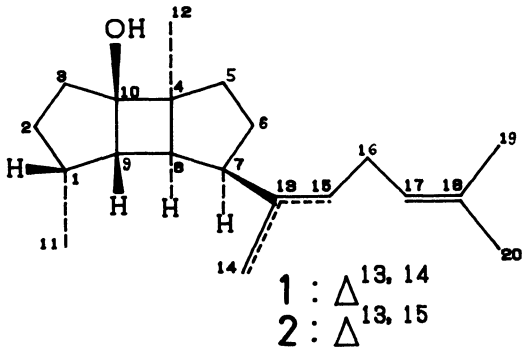
†Miyagi Prefectural Fisheries Experimental Station, Ishinomaki, Miyagi 986-21

††Tohoku Regional Fisheries Research Laboratory, Shiogama 985

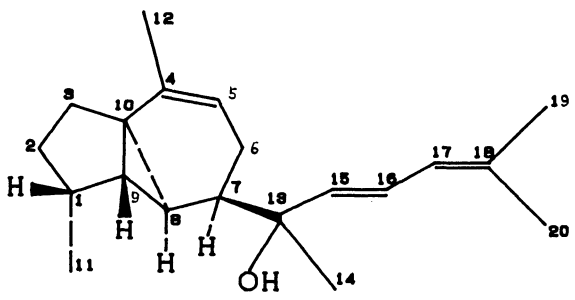
†††Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060

A new unusual metabolite with feeding-deterrent activity, closely related to spatane-type diterpenoids, has been isolated from the title alga, and its structure was determined by the spectroscopic method.

In connection with our current interest on the biologically active metabolites from marine sources, we recently reported[1] the structures of two spatane diterpenes **1** and **2** which have been isolated from the brown alga *Dilophus okamurai* Dawson. The compounds **1** and **2** strongly inhibited the settlement and the metamorphosis of the swimming larvae (veliger) of the abalone *Haliotis discus hannai* Ino. In addition, the above diterpenes **1** and **2** were also found to be the strongly active feeding deterrents for the young abalone.[2] Further examination of the weekly active fractions led to the isolation of an active compound **3**, the structure of which is described in this paper.



1 : $\Delta^{13,14}$
2 : $\Delta^{13,15}$



3

Table 1. ^{13}C and ^{1}H NMR data for compound **3**

Carbon No.[a]	^{13}C δ[b]	^{1}H δ[c]	Multiplicity, J/Hz
1	34.4(d)	2.27	m
2	29.7(t)	2.04 Hβ	ddd, J=12.0,12.0,8.4
		0.85 Hα	m
3	29.7(t)	1.7 Hα	m
		1.6 Hβ	m
4	136.3(s)		
5	117.0(d)	5.22	br d, J=7.0
6	22.4(t)	1.95 Hβ	m
		1.6 Hα	m
7	40.8(d)	1.8	m
8	20.1(d)	1.13	br dd, J=4.8,4.4
9	34.1(d)	1.48	dd, J=4.4,4.4
10	30.5(s)		
11	18.3(q)	1.02	d, J=6.6
12	21.3(q)	1.81	br d, J=1.5
13	75.6(s)		
14	26.1(q)	1.32	s
15	137.8(d)	5.72	d, J=15.4
16	123.7(d)	6.48	dd, J=15.4,11.0
17	124.6(d)	5.84	br d, J=11.0
18	134.5(s)		
19	18.2(q)	1.78	br s
20	26.0(q)	1.76	br s

a) The numbering system for **3** corresponds to those used for spatane diterpenes.
b) Measured at 67.9 MHz ($CDCl_3$, TMS=0).
c) Measured at 270 MHz ($CDCl_3$, TMS=0).

Repeated high performance liquid chromatography (JASCO, Megapak SIL-C_{18} or SIL-CN) of the active fractions yielded compound **3** (2.0% of the extract).

Compound **3**, oil, $[\alpha]_D^{20}$ -8.13° (c 1.15; $CHCl_3$), had a molecular formula $C_{20}H_{30}O$ (m/z 286; M^+).[3] The IR spectrum[4] revealed a strong band at ν_{max} 3458 cm^{-1} while ^{13}C NMR spectrum showed a quaternary carbon at δ 75.6, both indicative of a tertiary hydroxyl group. The presence of a conjugated diene moiety was evident from the UV spectrum, λ_{max} (EtOH) 238 nm (ε 33000). The ^{1}H NMR spectrum (Table 1) showed signals due to one secondary, one tertiary, and three olefinic methyl groups and due to four vinylic protons. Furthermore, the ^{13}C NMR spectrum (Table 1) indicated the presence of five CH_3 groups, three CH_2 groups, eight CH groups (four olefinic), and four nonprotonated carbons (two olefinic). Above-mentioned data, along with the molecular formula, required that compound **3** had a tricyclic carbon skeleton.

The ^{1}H-^{1}H and ^{1}H-^{13}C shift-correlated 2D-NMR spectra, the latter of which was very informative to distinguish signals overlapping as complex multiplets in

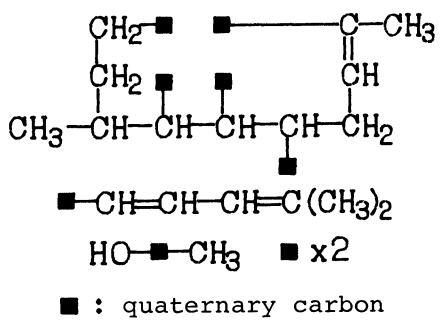


Fig. 1. Partial structures.

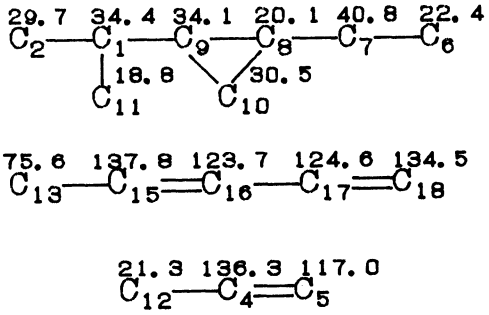


Fig. 2. Partial sequences.

the ^{1}H NMR spectrum, indicated the presence of partial structures in **3** as shown in Fig. 1. The geometry of the 1,2-disubstituted double bond was assigned as E-configuration by a large coupling constant, $J_{15,16}$=15.4 Hz. The presence of a 1-hydroxy-1,5-dimethylhexa-2,4-dienyl grouping was suggested by a base peak at m/z 125 [$C_8H_{13}O^+$, HR-MS; 125.0964 (Δmms -0.2)] in the MS spectrum.[4] The INADEQUATE spectrum[5-7] indicated the correlation peaks of the ^{13}C-^{13}C pairs, giving rise to the partial sequences of carbon atoms. As shown in Fig. 2, the presence of a cyclopropane ring moiety and a C_8-unit discussed above was unambiguously confirmed. In view of the above data in conjunction with the biogenetical standpoint (co-occurrence[8] of **3** with several spatane-type diterpenes in the same alga), the planar formula **3** with an unusual carbon skeleton containing a cyclopropane ring[9] has been assigned for compound **3**.

The relative stereochemistry for **3** except for that of C-13 was confirmed by difference NOE spectral studies. Irradiation of the cyclopropyl methine proton at δ 1.13 (C_8-H) induced positive NOE enhancement on the signals at δ 0.85 due to C_2-Hα, δ 1.7 due to C_3-Hα, δ 1.8 due to C_7-H, δ 5.72 due to C_{15}-H, and δ 6.48 due to C_{16}-H. When another cyclopropyl proton at δ 1.48 (C_9-H) was irradiated, positive NOEs were seen for the signals due to C_6-Hβ (δ 1.95), C_1-H (δ 2.27), C_{15}-H, and C_{16}-H. Furthermore, irradiation of the methyl group on C-13 (δ 1.32) induced positive NOEs on the signals due to C_7-H, C_8-H, C_{15}-H, C_{16}-H, and C_6-H_2. Above-mentioned NOE results together with the NOESY spectrum are consistent with the relative stereochemistry, excluding the configuration at C-13, depicted in the structural formula **3**.

Accordingly, the structure of **3** must be represented by formula **3**, which has a cubebene carbon skeleton with a further prenylated side chain. Compound **3** showed

weak feeding-deterrent activity which has been evaluated by Avicel plate method.[2]

We are grateful to Prof. Kanzo Sakata, Faculty of Agriculture, Shizuoka University, for valuable suggestion. This research is a result of financial support from the Marine Ranching Plan of Agriculture, Forestry, and Fisheries Agency, Japan, under Contribution No. MRP 88-IV-1-(1)-9.

References

1) K. Kurata, M. Suzuki, K. Shiraishi, and K. Taniguchi, Phytochemistry, 27, 1321 (1988).
2) The Avicel plate method[10] was used for the bioassay. The ethanol solutions (25 μml) of a standard phosphatidylcholine (PC) (10 μg) and the samples, which were prepaired by mixing 100 μg of each of fractions and pure compounds with PC (10 μg), were applied with a microsyringe on to the sample zone (25 mm in diameter) on an Avicel plate. Feeding-deterrent activity of each sample was evaluated by comparing the number of biting traces left on the plates with that of the standard PC. Details of the biological tests will be reported elsewhere.
3) HR-MS; **3**: m/z 286.2278 (calcd for $C_{20}H_{30}O$, 286.2296).
4) **3**: UV (EtOH), λ_{max} 238 nm (ε 33000) and λ_{inf} 232 (ε 31000) and 247 (ε 23000) nm; IR (neat), ν_{max} 3584, 3458, 1656, 1197, 1156, 1120, 1084, 1064, 1038, 1027, 989, 964, 873, 826, 796, and 781 cm^{-1}; LR-MS (70 eV), m/z (rel. intensity) 286 (7; M^+), 268 (8; M^+-H_2O), 215 (12), 162 (11), 161 (18), 159 (15), 145 (11), 135 (12), 125 (100), 119 (19), 107 (23), 105 (65), 93 (12), 91 (18), 83 (17), 81 (13), 69 (10), 55 (13), 43 (61), and 41 (21).
5) A. Bax, R. Freeman, and T. A. Frenkiel, J. Am. Chem. Soc., 103, 2102 (1981).
6) A. Bax, R. Freeman, T. A. Frenkiel, and M. H. Levitt, J. Magn. Reson., 43, 478 (1981).
7) The 2D-INADEQUATE spectrum of **3** (ca. 130 mg) was taken on a JEOL FX-500 spectrometer, using the propagation time (τ=1/4Jcc) of 25 ms optimized for Jcc=10 Hz). We are grateful to Dr. Mitsuhiko Ikura, High-Resolution NMR Laboratory, Faculty of Science, Hokkaido University, for measurement of the INADEQUATE spectrum.
8) K. Kurata and M. Suzuki, unpublished data.
9) Tricyclic diterpenes with the same carbon framework as **3** have been obtained from the brown alga Dilophus marginatus.[11]
10) K. Sakata, T. Itoh, and K. Ina, Agric. Biol. Chem., 48, 425 (1984).
11) B. N. Ravi and R. J. Wells, Aust. J. Chem., 35, 129 (1982).

(Received July 1, 1988)