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CYCLOZONARONE, A SESQUITERPENE-SUBSTITUTED BENZOQUINONE DERIVATIVE FROM THE BROWN ALGA *DICTYOPTERIS UNDULATA*

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Key Word Index—*Dictyopteris undulata*; Dictyotaceae; brown alga; sesquiterpene-substituted benzoquinone derivative; feeding-deterrent.

Abstract—A new sesquiterpene-substituted benzoquinone derivative, which we have named cyclozonarone, has been isolated from the brown alga *Dictyopteris undulata* collected in 'Isoyake areas'. Its structure was determined on the basis of spectroscopic evidence. Cyclozonarone showed potent feeding-deterrent activity toward young abalones.

INTRODUCTION

Crustose coralline red algae dominate extensive areas of the sublittoral rocky surfaces, which are characteristically depauperate in foliose macroalgae and are referred to as 'sea-urchin barren ground', 'coralline flats' or 'Isoyake areas' (in Japanese). Sea urchins and other benthic invertebrates are generally associated with such crustose coralline algal communities.

In Isoyake areas at Tobi, Kaneura, Akita prefecture, *Dictyopteris undulata* and *Dictyota dichotoma* grow sympatrically with the abalone *Haliotis discus*, suggesting that these algae have a defence mechanism against marine herbivorous animals through producing some feeding-deterring substance. We used a feeding-deterrent bioassay (cellulose plate method against the young abalone *Haliotis discus hannai*) to isolate the deterrent compounds zonarol (**1**), isozonarol (**2**), chromazonarol (**3**), zonarone (**4**) and isozonarone (**5**) from *D. undulata* [1]. Further investigation led to the isolation of a new feeding-deterrent compound (**6**), named cyclozonarone, whose structure is reported in this paper.

RESULTS AND DISCUSSION

The neutral and acidic fractions obtained from the methanol extract of dried *D. undulata* showed potent feeding-deterrent activity. The neutral fraction was fractionated by column chromatography over alumina. The fraction eluted with ethyl acetate was further subjected to repeated reverse-phase HPLC (MeOH–H_2O, 9:1) followed by preparative TLC to yield cyclozonarone (**6**) (0.08% based on the neutral extract) along with zonarol (**1**) [2], isozonarol (**2**) [2], chromazonarol (**3**) [3, 4], zonarone (**4**) [2] and isozonarone (**5**) [2].

Cyclozonarone (**6**), $[\alpha]_D^{19}$ $-89.1°$ ($CHCl_3$), was analysed for $C_{21}H_{24}O_2$ by HR-EIMS (*m/z* 308.1777 [M]); the UV spectrum showed absorptions at λ_{max} 257 nm (ε 22 800), $\lambda_{inf.}$ 265 nm (ε 19 200) and λ_{max} 348 nm (ε 3200) indicative of the 1,4-naphthoquinone moiety. The presence of a 6,7-disubstituted 1,4-naphthoquinone grouping in **6** was strongly supported by signals in the ^{1}H NMR spectrum (Table 1) at δ_H 7.98 (1H, *s*), 7.74 (1H, *s*), 6.90 (1H, *d*, J = 10.3 Hz) and 6.89 (1H, *d*, J = 10.3 Hz), the last two signals of which consisted of a typical AB-type quartet. Furthermore, the ^{1}H NMR spectrum indicated the presence of three tertiary methyl groups. The ^{1}H–^{1}H COSY spectrum gave limited information, from which the presence of ■–CH_2–CH_2–CH_2–■ and ■–CH–CH_2–CH_2–■ (■ quaternary carbon atom) groups were defined. The above results, and the ^{13}C NMR data, indicated the presence of the partial structural units A, B, C, D and E in the molecule (Fig. 1).

Detailed analysis of the ^{1}H-detected heteronuclear multiple-bond ^{1}H–^{13}C correlation spectrum (HMBC) together with biogenetic considerations led to the gross structure for cyclozonarone. In the HMBC spectrum (summarized in Table 1), one (H-15) of the two isolated protons on the 1,4-naphthoquinone ring showed cross-peaks with five quaternary naphthoquinone carbons and a methylene carbon (C-7) at δ_C 30.7 due to the methylene group in the partial unit B. The other isolated proton (H-14) on the 1,4-naphthoquinone ring showed cross-peaks with five quaternary naphthoquinone carbons and a quaternary carbon (C-10) at δ_C 38.7, the last of which in

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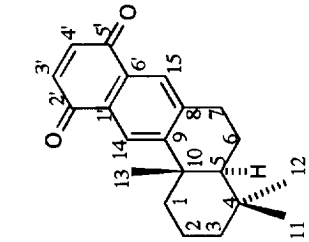
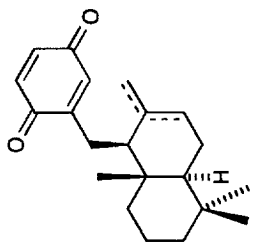
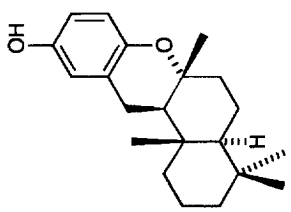


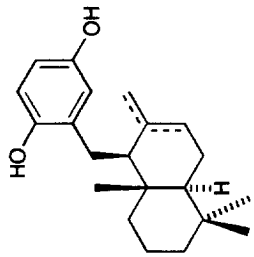
6 Cyclozonarone



4 Zonarone (exo)
5 Isozonarone (endo)



3 Chromazonarol



1 Zonarol (exo)
2 Isozonarol (endo)

turn showed correlations with a tertiary methyl (H_3-13) at δ_H1.21, a methine proton (H-5) at δ_H1.31 due to the methine group in the unit B and methylene protons H_2-1 and H_2-2 due to two methylene groups in the unit A. Furthermore, the remaining two tertiary methyl groups revealed correlations with the same carbons, e.g. the quaternary carbon (C-4) at δ_C33.6, the methine carbon (C-5) at δ_C49.8 due to the methine group in the unit B and a methylene carbon (C-3) at δ_C19.1 due to another methylene group in the unit A, thus indicating that these methyl groups must constitute a *gem*-dimethyl group.

As cyclozonarone (**6**) possesses 10 degrees of unsaturation, **6** must consist of four carbocyclic skeleton including the 1,4-naphthoquinone ring. Thus the planar structure of cyclozonarone must be represented by formula **6**. The coexistence of cyclozonarone (**6**) with zonarol (**1**) and other related metabolites **2**–**5** suggested that **6** possesses the same *trans* ring junction. This was further supported by the *J*-value (J = 12.7 Hz) of H-5 resulting from vicinal axial–axial coupling.

A possible biogenetic pathway is outlined in Scheme 1. Farnesyl hydroquinone derived from farnesyl pyrophosphate and hydroquinone may give zonarol (**1**) and isozonarol (**2**) by acid-catalysed cyclization. Chromazonarol (**3**) may result from **1** or **2** also by acid-catalysed cyclization. On *in vitro* transformation with *p*-toluene sulphonic acid in benzene, zonarol (**1**) gave chromazonarol (**3**) [3]. Oxidation of **1** and **2** should yield zonarone (**4**) and isozonarone (**5**), respectively. However, oxidation of **1** and **2** with Jones reagent yielded **4** and **5**, respectively [2]. Cyclozonarone (**6**) appears to be biosynthesized via a diene precursor derived from zonarone (**4**).

The feeding-deterrent activity of compounds **1**–**6** was tested at a concentration of 75 μg of each sample by the cellulose plate method [5]. Compounds **1**–**6** exhibited potent feeding-deterrent activity (electivity index (Ei): **1** = 0.85, **2** = 0.78, **3** = 0.80, **4** = 0.92, **5** = 0.85 and **6** = 0.93) against the young abalone *Haliotis discus hannai*. It appeared that in Isoyake areas *D. undulata* grew sympatrically with marine herbivores as a result of producing these compounds, especially zonarol (**1**), isozonarol (**2**) and chromazonarol (**3**), which occur in large quantities in this alga.

EXPERIMENTAL

^{1}H NMR: 400 MHz and ^{13}C NMR: 100 MHz, $CDCl_3$, TMS as int. standard; LR-MS and HR-MS; 70 eV; CC: Al_2O_3 (Merck, Al_2O_3, activity II–III); prep. TLC (PTLC): silica gel (Merck, Kieselgel 60 F_{254S}); HPLC: Megapak SIL-C_{18} (JASCO). All known compounds were identified by comparison of the spectral data with those reported.

Bioassay. The bioassay was carried out by the cellulose plate method using cellulose TLC aluminium sheets (Merck, No. 5552) [5]. Feeding-deterrent activity was evaluated by comparing the number of biting traces left on the sheets with that of the standard phosphatidylcholine (PC). Relative activity (electivity index: Ei) was

Table 1. ^{1}H NMR, ^{13}C NMR and HMBC data for cyclozonarone (**1**)

H/C	δ_H, *J* (Hz)	δ_C	Long range correlations
1	1.43 *m* 2.44 *br d*, 11.7	38.6	C2, C3, C5, C9, C10, C13
2	1.67 *m* 1.76 *m*	41.5	C1, C4, C10
3	1.22 *m* 1.52 *m*	19.1	C1, C2, C4, C5, C11, C12
4		33.6	
5	1.31 *dd*, 12.7, 2.4	49.8	C1, C3, C4, C6, C7, C9, C10, C11, C12
6	1.78 *m* 1.95 *br dd*, 13.7, 7.8	16.6	C5, C6, C10
7	2.97 *ddd*, 18.0, 10.7, 7.8 3.10 *dd*, 18.0, 6.9	30.7	C5, C6, C8, C9, C15
8		142.9	
9		157.0	
10		38.7	
11	0.97† *s*	21.7*	C3, C4, C5, C12
12	0.99† *s*	33.2*	C3, C4, C5, C11
13	1.21 *s*	24.5	C1, C5, C9, C10
14	7.98 *s*	127.4	C8, C9, C10, C1′, C2′, C6′
15	7.74 *s*	123.1	C7, C8, C9, C1′, C5′, C6′
1′		129.1	
2′		185.3	
3′	6.90 *d*, 10.3	138.6	C1′, C2′, C4′, C5′
4′	6.89 *d*, 10.3	138.9	C2′, C3′, C5′, C6′
5′		185.3	
6′		129.8	

*†Assignments may be interchanged.

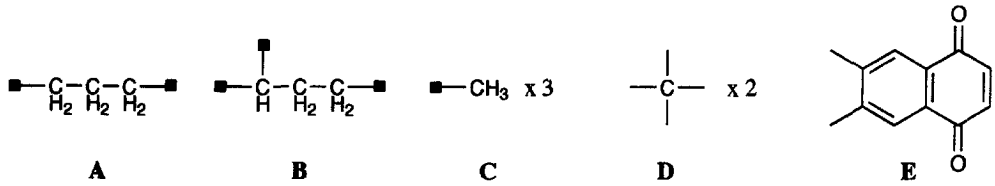


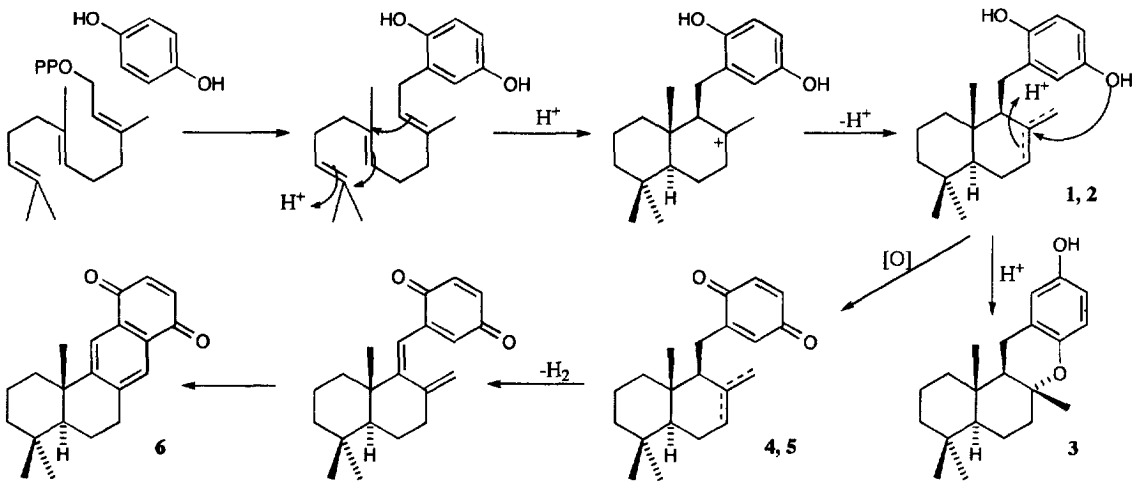
Fig. 1. Partial structural units for cyclozonarone (**6**) (■: quaternary carbon atom).

defined by the following equation (Pi: average number of biting traces of the control (PC), pi: average number of biting traces of each sample) [6]. Significant differences ($P < 0.01$ or 0.05) of feeding-deterrent activity were assessed by a *t*-test.

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

Isolation. As described in a previous paper [1], dried *Dictyopteris undulata* (0.95 kg), collected at Tobi, Kaneura, Akita prefecture, on July 1991, was extracted with MeOH. The neutral and acidic fractions (34 g and 14 g, respectively) obtained in the usual manner showed potent feeding-deterrent activity. The neutral fraction was fractionated by CC over Al_2O_3. The fraction eluted with EtOAc was further subjected to repeated reverse-phase HPLC (MeOH–H_2O, 9:1) followed by prep. TLC to yield cyclozonarone (**6**) (0.08% based on the neutral extract) along with zonarol (**1**) [2] (17%), isozonarol (**2**) [2] (10%), chromazonarol (**3**) [3, 4] (2%), zonarone (**4**) [2] (7%) and isozonarone (**5**) [2] (3.3%). Zonarol (**1**), isozonarol (**2**) and chromazonarol (**3**) were also obtained from the acidic fraction in 14.6, 8.1 and 10.8% yield, respectively.

Cyclozonarone (**6**). Oil; $[\alpha]_D^{19}$ $-89.1°$ ($CHCl_3$; *c* 0.330); UV λ_{max} (MeOH) nm: 257 (ε 22 800), $265_{inf.}$ (ε 19 200) and 348 (ε 3200); IR, ν_{max} (film) cm^{-1}: 1668, 1651, 1599, 1378, 1367, 1334, 1295, 1230 and 1119; ^{1}H NMR: Table 1; ^{13}C NMR: Table 1; LR-EIMS *m/z* (rel. int.): 308 (44) $[M]^+$, 293 (77), 237 (19), 225 (67), 223 (38), 212 (21), 211 (100) $[M - C_7H_{13}]^+$, 197 (35), 69 (58),



Scheme 1. Possible biogenesis for cyclozonarone (**6**) and related metabolites (**1–5**).

55 (23) and 41 (34); HR-EIMS *m/z*; 308.1796 and 211.0747. Calc. for $C_{21}H_{24}O_2$, 308.1777 [M] and $C_{14}H_{11}O_2$, 211.0759 [M − C_7H_{13}].

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