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FEEDING-DETERRENT BROMOPHENOLS FROM *ODONTHALIA CORYMBIFERA*

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Key Word Index—*Odonthalia corymbifera*; Rhodomelaceae; red alga; bromophenol; feeding-deterrent activity.

Abstract—A new diarylmethane-type bromophenol with potent feeding-deterrent activity has been isolated from the red alga *Odonthalia corymbifera*, and its structure was determined as 2,2′,3-tribromo-4,5,5′,6′-tetrahydroxy-3′-methoxymethyldiphenylmethane on the basis of spectroscopic evidence. ©1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The red alga *Odonthalia corymbifera* (Gmelin) Greville (Rhodomelaceae, Ceramiales) is common in the shallow waters of the Pacific coast of Hokkaido. The algal communities in the depth of −3 to −6 m on the Hiroo coast, eastern Hokkaido, mainly consisted of *O. corymbifera* and the brown algae, such as *Laminaria angustata* Kjellman and *Costaria costata* (C. Agardh) Saunders. In order to increase the standing stock of the sea urchin *Strongylocentrotus intermedius* which is a commercially important animal in Japan, laboratory-reared sea urchins with test length of 5–10 mm in diameter were released at 800 animals/m^2 in the algal communities at Hiroo coast in May 1988. Only *O. corymbifera* was not grazed by the young sea urchins and grew extensively six months later. These results suggested that *O. corymbifera* has a chemical defence mechanism against marine herbivorous animals through producing some feeding-deterrents. Thus, we examined for feeding-deterrents by using the bioassay (cellulose plate method) against the young abalone *Haliotis discus hannai* and were able to isolate six bromophenols with feeding-deterrent activity.

RESULTS AND DISCUSSION

The fresh alga of *O. corymbifera* was collected at Hiroo coast, eastern Hokkaido, in July 1991, and extracted with methanol. The methanolic 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 fraction to leave the neutral fraction. Of these fractions including the water-soluble fraction, the acidic fraction was found to display potent feeding-deterrent activity. The active acidic fraction was then subjected to a combination of reversed-phase HPLC and preparative TLC to yield six compounds (**1**–**6**) which showed potent feeding-deterrent activity against the young abalone *Haliotis discus hannai*. These compounds were shown to be bromophenols by spectral analyses. Compound **1** was identified as lanosol previously isolated from *Polysiphonia lanosa*, *Odonthalia corymbifera*, *Rhodomela larix*, *R. subfusca* and other species [1–7]. Compounds **2** and **3** were methyl [2–8] and ethyl [3] ether derivatives of lanosol, respectively, which are probably artifacts [3, 4, 6]. Compounds **4** and **5** were also known metabolites which were previously isolated from *Rhodomela larix* [6, 7]. Moreover, dipotassium 2,3-dibromo-5-hydroxybenzyl-1′,4-disulfate (**7**) [1, 2, 4, 5] was also obtained from the aqueous layer.

A new minor bromophenol **6**, had the molecular formula of $C_{15}H_{23}Br_3O_5$, which was isomeric with **5**. The spectral data of **6** were very similar to those of **5**. Treatment of **6** with acetic anhydride and pyridine gave the tetraacetate **8**, $C_{23}H_{31}Br_3O_9$, whose acetyl groups were defined to be phenolic by IR (ν_{max} 1778 cm^{-1}) and ^{1}H NMR spectrum (δ 2.18, 2.20, 2.26 and 2.32 (each 3H, *s*)). Detailed comparison of the spectral

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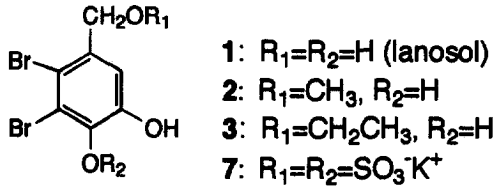

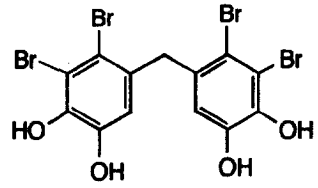

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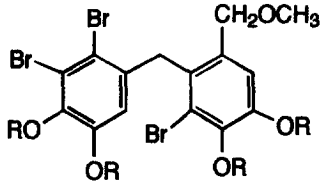
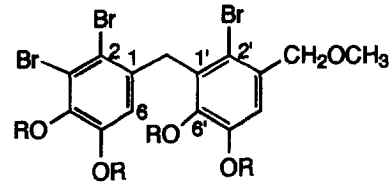


5: R=H
9: R=Ac



6: R=H
8: R=Ac

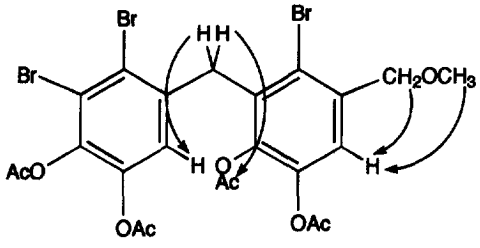
data of **6** and **8** with those of **5** and its acetate **9** [7] together with biogenetic viewpoint allowed us to assign structure **6** for this new bromophenol. Confirmation of the structure was obtained by the measurements of NOE difference spectra. As depicted in Fig. 1, in the acetate **9** the methylene group flanked by two aryl groups revealed NOEs between the methoxyl Me along with the aromatic proton at *ortho*-position. However, in the acetate **8** no NOE was observed between the pertinent metylene group and the methoxyl Me, but instead NOE was observed between the pertinent methylene and the acetoxyl Me at the *ortho*- position.

Compounds **1**–**6** displayed potent feeding-deterrent activity (electivity index *Ei*: **1** = 0.90, **2** = 0.87, **3** = 0.78, **4** = 0.91, **5** = 0.91 and **6** = 0.92) against the young abalone *Haliotis discus hannai*, while only two diarylmethane derivatives **4** and **5** displayed potent feeding-deterrent activity (*Ei*: **4** = 0.90 and **5** = 0.75) against the young sea urchin *Strongylocentrotus intermedius*. Although **6** was not tested because of the small quantity, it also seems to have potent feeding-deterrent activity against the young sea urchin. It is interesting to note that dipotassium 2,3-dibromo-5-hydroxybenzyl-1′,4-disulfate (**7**) did not show any feeding-deterrent activity against both the young sea urchin and the young abalone. It seemed that *O. corymbifera* exists sympatrically with the sea urchin *S. intermedius* as a result of producing these brominated phenolic compounds.

EXPERIMENTAL

General. ^{1}H NMR: 400 MHz, TMS as int. standard. LR-MS and HR-MS; 70 eV. Prep TLC: silica gel 60 F_{254S} (Merck). HPLC: Megapak SIL-C_{18} (JASCO). All known bromophenols were identified by comparison of the spectral data with those of the authentic specimens.

Bioassay. The bioassay was carried out by the cellulose plate method using the cellulose TLC aluminum sheets (Merck, no. 5552) [9]. Feeding-deterrent activities against the young abalone *Haliotis discus hannai* and the young sea urchin *Strongylocentrotus intermedius* were 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 defined by the following equation (*Pi*:



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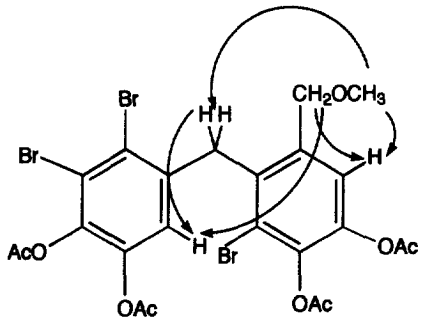

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Fig. 1. NOEs from NOE difference spectra of **8** and **9**.

average number of biting traces of the control (PC), *pi*: average number of biting traces of each sample) [10]. 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. *Odonthalia corymbifera* (Gmelin) Greveille was collected at Hiroo coast, eastern Hokkaido, in July 1991. The fresh alga (2.1 kg) was soaked in methanol, and the methanol solution was concentrated *in vacuo* to leave the residues which were then partitioned between ether and water. The ethereal layer was successively shaken with 5% KOH and 1 M HCl to separate the acidic (12.2 g), the basic (trace) and the neutral fr. (0.13 g). The acidic fr. which showed potent feeding-deterrent activity ($Ei = 0.83$) against the young abalone, was subjected to reversed-phase HPLC [MeOH–H_2O (70:30)] followed by repeated prep. TLC (C_6H_6–MeOH–AcOH (8:1:1)) to give lanosol (**1**) (2.4 g) [1–7], lanosol methyl ether (**2**) (0.48 g) [2–8], lanosol ethyl ether (1.6 g) (**3**) [3], **4** (0.85 g) [6], **5** (2.3 g) [6, 7] and **6** (0.010 g). Dipotassium 2,3-dibromo-5-hydroxybenzyl-1′,4-disulfate (**7**) (26.4 g) [1, 2, 4, 5] was also obtained from the aq. layer.

6. Colourless gum; IR, ν_{max} (film) cm^{-1}. 3382, 1599, 1424, 1277, 1169, 1081, 939 and 859; ^1H NMR (CD_3OD): δ 3.35 (3H, *s*; OCH_3), 4.12 (2H, *br s*; Ar-CH_2-Ar), 4.44 (2H, *s*; Ar-CH_2-O), 6.15 (1H, *s*; H-6) and 6.92 (1H, *s*; H-4′).

Acetylation of **6**. Acetylation of **6** (4 mg) was carried out with acetic anhydride (150 μl) and pyridine (150 μl) at room temp by the usual method to give the acetate, which was purified by prep. TLC to afford **8** (5 mg); colourless crystals; mp 195–196° (MeOH); IR, ν_{max} ($CHCl_3$) cm^{-1}. 1778, 1370, 1197, 1180, 1143 and 1015; ^1H NMR ($CDCl_3$): δ 2.18, 2.20, 2.26, 2.32 (each 3H, *s*; Ac), 3.50 (3H, *s*; OCH_3), 4.25 (2H, *s*; Ar-CH_2-Ar), 4.51 (2H, *br s*; Ar-CH_2-O), 6.52 (1H, *s*; H-6) and 7.43 (1H, *s*; H-4′); LR-EIMS *m/z* (rel. int.): 684, 682, 680, 678 (0.3:0.7:0.7:0.3) $[M]^+$, 642, 640, 638, 636 (1.0:2.4:2.4:1.0) $[M - CH_2 = C = O]^+$, 600, 598, 596, 594 (2.0:2.4:2.4:0.7) $[M - CH_2 = C = O \times 2]^+$, 445, 443, 441 (5.1:9.4:5.0), 403, 401, 399 (5.1:9.3:5.5) and 43 (100); HR-EIMS *m/z* 677.8740. Calcd for $C_{23}H_{21}{}^{79}Br_3O_9$, 677.8735 [M].

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