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Chemical ecology of the Antarctic nudibranch *Bathydoris hodgsoni* Eliot, 1907: defensive role and origin of its natural products

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Abstract

A variety of Antarctic marine invertebrates contains secondary metabolites that may provide defense against potential predators. However, only in a few cases have tissues, extracts or isolated compounds of these invertebrates been tested against sympatric predators. The Antarctic nudibranch *Bathydoris hodgsoni* Eliot, 1907 contains hodgsonal, a compound only present in the external body (mantle tissues), which may protect the slugs from predators. To test this defensive hypothesis for hodgsonal, we carried out a series of experiments using the sympatric omnivorous seastar *Odontaster validus* Koehler, 1906 as a potential predator. Our experiments revealed that natural concentrations of hodgsonal elicit significant feeding deterrent responses in *O. validus*. Furthermore, hodgsonal is probably biosynthesized *de novo* by the nudibranch, since it was not detected in the viscera (as it should be in the case of a dietary compound), its concentration in the mantle (0.05–0.15% dry mass) is quite constant in individuals from different localities and depths, and its sequestration from a particular dietary source is unlikely because *B. hodgsoni* is an omnivorous feeder.

Keywords: Antarctica; Chemical ecology; Defense; Feeding deterrence; Nudibranch molluscs

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1. Introduction

The marine benthos of Antarctica has been subject of many taxonomical, zoogeographical and ecological studies (e.g. Dayton et al., 1974; Voss, 1988; Galéron et al., 1992; Arntz et al., 1997). However, chemical ecology studies have only recently begun (reviewed by McClintock and Baker, 1997a) and little is known about the chemistry, biochemistry, and especially the ecological function of secondary metabolites from Antarctic marine invertebrates. As known from temperate and especially tropical regions, secondary metabolites can play important roles in mediating intra- and interspecific relationships among marine organisms, thus regulating the structure of marine communities (e.g. Scheuer, 1990; Paul, 1992; Pawlik, 1992; Hay, 1996). Benthic communities in Antarctica, below the zone of ice scour and anchor ice, are considered to be controlled mainly by biological factors, such as predation and competition (Dayton et al., 1974). Selective pressures may have been sufficient over geological history to drive the evolution of defensive metabolites in Antarctic marine invertebrates (McClintock, 1987; McClintock et al., 1990; Slattery and McClintock, 1997; McClintock and Baker, 1997a; Amsler et al., 1999). Chemical defense in Antarctic marine invertebrates may also be related to the slow growth rates and long lifespans that characterize this fauna (Clarke, 1983; Pearse et al., 1991; Arntz et al., 1994). Chemical defenses have been reported in a variety of phyla of Antarctic marine invertebrates, including bryozoans (Winston and Bernheimer, 1986), porifera (McClintock, 1987; Molinski and Faulkner, 1987; McClintock and Gauthier, 1992), echinoderms (McClintock, 1989; Blunt et al., 1990; McClintock and Vernon, 1990; McClintock et al., 1994b), cnidarians (Slattery et al., 1990), nemerteans (Heine et al., 1991), tunicates (McClintock et al., 1991, 1992a), brachiopods (McClintock et al., 1993) and molluscs (McClintock and Janssen, 1990; McClintock et al., 1992b, 1994b). However, relatively few natural products have been fully described in marine Antarctic invertebrates from a chemical point of view (see review by McClintock and Baker, 1997a), and these have been mainly on sponges (e.g. Eggersdorfer et al., 1982; Seldes et al., 1986; Molinski and Faulkner, 1987, 1988; Blunt et al., 1990; Baker et al., 1993, 1995; Puliti et al., 1993; Perry et al., 1994; Trimurtulu et al., 1994; Shin et al., 1995; Fontana et al., 1996, 1997, 1999a) and occasionally on other marine invertebrates such as echinoderms (e.g Kong et al., 1992; De Marino et al., 1997), soft corals (e.g Slattery et al., 1997) and molluscs (e.g. McClintock et al., 1994a).

Opisthobranch molluscs are known from temperate and tropical regions to exhibit a great variety of secondary metabolites; some of these compounds have a defensive function, and, in particular, natural products from nudibranch molluscs show a wide range of structural characteristics, activities and origin (Avila, 1995; Faulkner, 1999 and references therein). However, only few studies have been dedicated to the investigation of the ecological role of the natural compounds of Antarctic opisthobranchs (McClintock et al., 1992b,1994c; Bryan et al., 1998). The chemical ecology of three opisthobranch species has been studied to date. The pteropod *Clione antarctica* Smith, 1902 (McClintock and Janssen, 1990; Bryan et al., 1995), and two nudibranchs: the dorid *Austrodoris kerguelenensis* (Bergh, 1884) (Davies-Coleman and Faulkner, 1991; McClintock et al., 1992b; Gavagnin et al., 1995,1999) and the dendronotid *Tritoniella*

belli Eliot, 1907 (McClintock et al., 1992b,1994c; McClintock and Baker, 1997b; Bryan et al., 1998). Among the nudibranchs, *Austrodoris kerguelenensis* presents several diterpenoic acid glycerides (Davies-Coleman and Faulkner, 1991; Gavagnin et al., 1995, 1999), although the ecological significance of these compounds still remains unclear. *Tritoniella belli* possesses a chimyl alcohol which has feeding deterrent effects against a sympatric predator, and some additional defensive chemicals which have yet to be chemically identified (McClintock et al., 1992b,1994c; Bryan et al., 1998). Recently, we described the main secondary metabolite present in the external mantle tissues of the high Antarctic nudibranch *Bathydoris hodgsoni*, a new drimane sesquiterpene named hodgsonal (Iken et al., 1998). In the present study we examine the role of hodgsonal as a defensive metabolite using an ecologically relevant predator, the Antarctic seastar *Odontaster validus*. We also analyze whether hodgsonal is derived from the diet or produced by de novo biosynthesis.

2. Material and methods

2.1. Collections

Specimens of *Bathydoris hodgsoni* were collected by trawling the sea floor during two cruises aboard the R/V Polarstern to the Eastern Weddell Sea (ANT XIII/3 (EASIZ I), 1996; ANT XV/3 (EASIZ II), 1998) (Table 1; Fig. 1). During the 1996 cruise, individuals were collected at five different stations in the West Cape area (Fig. 1), at depths ranging from 246 to 620 m. In 1998, specimens were collected at nine different stations in the West Cape, Drescher Inlet, Norway Cape and Halley Bay areas (Fig. 1), at depths ranging between 246 and 928 m (Table 1) (Avila et al., 1999). Nudibranchs were directly frozen at -30°C for further chemical analysis and experiments.

Prior to chemical or biological analysis, individuals were identified to species level. *B. hodgsoni* (Fig. 2) is a high Antarctic, circumpolar nudibranch easily identified by several distinct morphological traits, including the body shape and size, and the morphology of the gills (Wägele, 1987). To distinguish this species from other Antarctic *Bathydoris* species, the radular formula and morphology, absence of eyes, and number of gills, as well as some internal anatomical characters were employed as the main taxonomic criteria (Kaiser, 1980; Wägele, 1987, 1989a,b). The radula was dissected from several specimens, preserved in 70% ethanol, and studied later by SEM to allow confirmation of taxonomic classification.

2.2. Stomach content analysis

Subsamples of the stomach contents of five individuals (Table 2) were preserved in 70% ethanol and further examined under a dissecting microscope to identify prey items. The relative percentage by volume of each prey type in the stomach contents was determined for qualitative comparisons (Table 3).

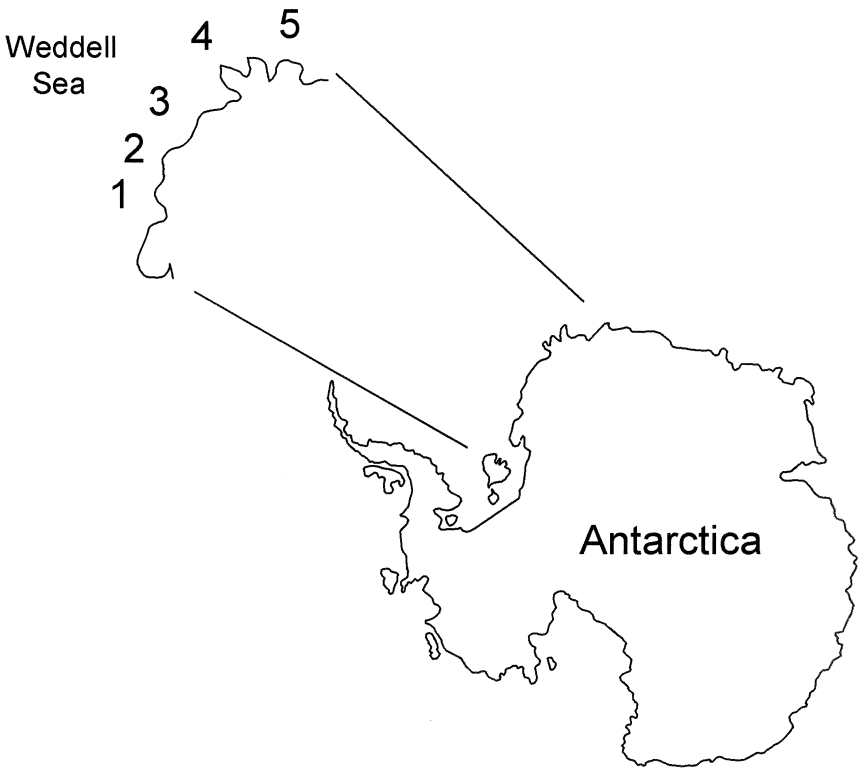

Fig. 1. Schematic view of the Eastern Weddell Sea, indicating the different areas sampled. 1=Halley Bay, 2=West Cape, 3=Drescher inlet, 4=Norway Cape and 5=Atka Bay (Neumayer Station).

2.3. Chemical analysis and isolation of hodgsonal

Chemical analysis consisted of extraction with organic solvents, isolation, purification and structure elucidation of the main compounds, including the main secondary metabolite hodgsonal (Fig. 3) as reported by Iken et al. (1998). Briefly, frozen animals were carefully dissected into mantle and viscera. These body sections were extracted with acetone and further divided into a diethyl ether extract, a butanolic extract and an aqueous residue (Table 4). A complete TLC screening in different conditions was carried out to detect the presence of interesting metabolites in the different fractions. Some interesting minor compounds detected still remain to be chemically elucidated.

From a pool of four individuals, hodgsonal was isolated from the diethyl ether fraction of the mantle by a silica-gel column using a rising diethyl ether–petroleum ether gradient as eluent. The fraction containing the main compound was then methylated with diazomethane in diethyl ether to remove fatty acids, and the reaction mixture was further purified by silica gel column chromatography, yielding 10 mg of pure hodgsonal (Table 5). This was used to test bioactivity of hodgsonal at different concentrations. Similarly,

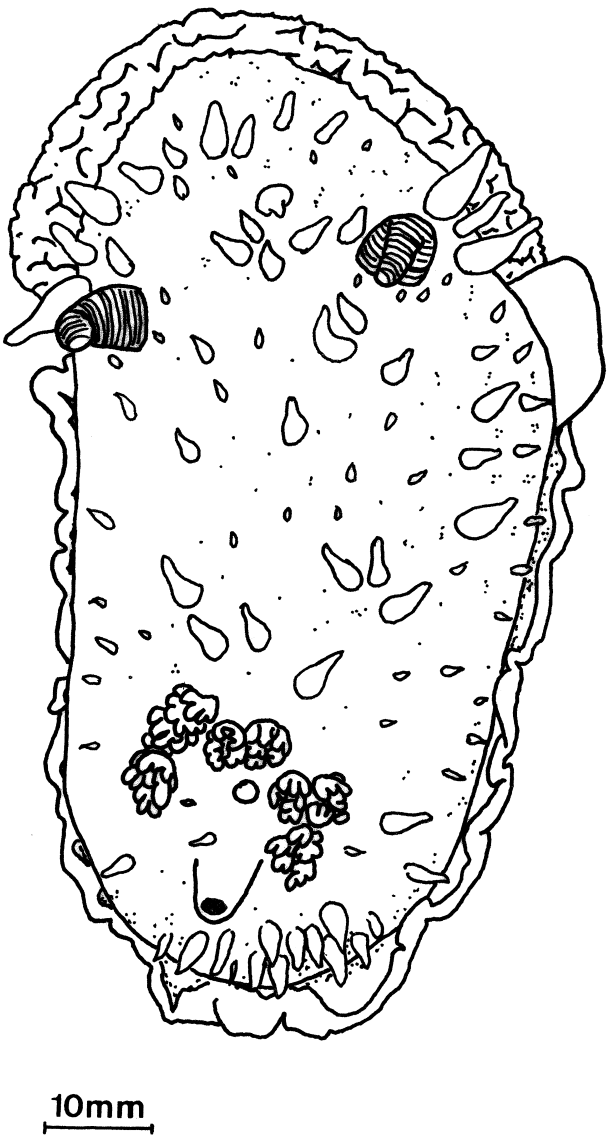

Fig. 2. Schematic dorsal view of a specimen of *Bathydoris hodgsoni* (modified from Wägele, 1989a).

several specimens were studied separately to obtain the percentages of hodgsonal per dry mass (Tables 4 and 5).

One individual was further dissected into gills, foot and dorsal mantle. The extracts of these different external components were examined by TLC to detect differences in the amount of hodgsonal in each body component. Similarly, the dorsal papillae of one specimen were extracted separately in order to compare by quantitative TLC the relative

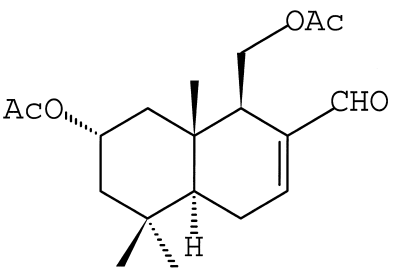

Fig. 3. Chemical structure of hodgsonal as described in our previous study (Iken et al., 1998).

concentration of hodgsonal in the papillae to that in the dorsal mantle skin (without papillae). The different crude extracts, fractions, and pure compound obtained from silica gel chromatography were then tested for feeding deterrence using the sympatric omnivorous seastar *Odontaster validus* (see below).

2.4. Feeding deterrence tests

In the Antarctic marine environment, seastars have replaced fish as the dominant predators of sessile and slow-moving invertebrates (e.g. Dayton et al., 1974; McClintock, 1994). Therefore, the asteroid *Odontaster validus* was chosen from potential predators collected in the benthic trawls for carrying out our tests. Seastars were maintained in fresh seawater aquaria aboard ship and received a maintenance diet of frozen krill (*Euphausia superba* Dana, 1850). In feeding deterrence tests, krill pieces and opisthobranch mantle pieces (size ~ 0.5 cm^2) were offered as food and the reaction of *O. validus* observed. Mantle extracts, fractions or isolated hodgsonal were applied to krill pieces by slowly pipetting them, and then allowing the solvent to evaporate. Control krill pieces were treated with solvent only. In order to calculate the concentration of extract or compound used per dry weight krill, the relationship between wet weight (WW) and dry weight (DW, 8 h at 60°C) was determined for 22 krill pieces. The mean WW and DW were 61.2 ± 3.3 mg and 14.3 ± 0.7 mg, respectively. Based on concentrations of extracts, fractions or compound obtained from the nudibranch, they were loaded on to krill pieces and tested at natural concentrations (Figs. 4–6). When sufficient material was available we tested the extracts, fractions or compound at higher concentrations (several fold) in order to confirm negative results and compensate for material loss while coating krill pieces.

Twenty seastars were starved for 24 h prior to a feeding experiment. They were transferred from large maintenance aquaria to individual 3-l containers with fresh seawater at semidark conditions and at 1 ± 0.5°C. Then, randomly, an experimental or a control krill piece ($n = 10$ each) was placed in the center of the bottom of the experimental container and a seastar carefully placed over the krill piece, so that the mouth of the seastar made contact with the krill. When fresh mantle tissue pieces were tested, we took care to ensure that the external side of the mantle touched the mouth of *O. validus*.

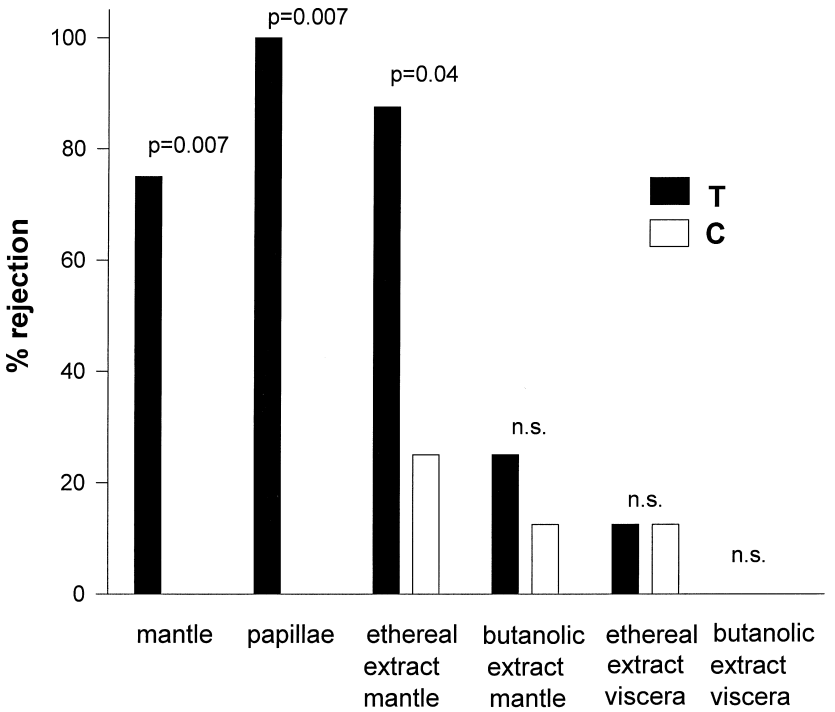

Fig. 4. Feeding deterrence tests of mantle pieces, papillae, and crude extracts (at natural concentrations) of *B. hodgsoni*, using the seastar *O. validus* as predator. Results from the Fisher's exact test are indicated with *P* values; n.s., not significant; T, treatment; C, control.

This experimental design was similar to that used by McClintock and Baker (1997b) who placed food pellets in the ambulacral groove (half way between the mouth and the tip of the arm) and considered movement of the pellet to the mouth and extrusion of the cardiac stomach a positive feeding response. While these investigators followed the pellets for about 20 min, we carried out our tests for 24 h, since we observed that seastars could sometimes ingest or reject food after a few hours. We considered the food item rejected when physical contact between the seastar and the food item ceased. Seastars were observed during the first 45 min, and then checked every 2–3 h up to 24 h to observe whether food items were completely eaten, were still in contact with the seastars, or rejected. As in McClintock and Baker (1997b), we analyzed the data by using a Fisher's exact test (Sokal and Rohlf, 1981) to determine significant differences between control and experimental treatments.

3. Results

A total of 40 specimens of *Bathydoris hodgsoni* were collected in different areas of the Weddell Sea at different depths (Fig. 1, Table 1). Length and wet weight were

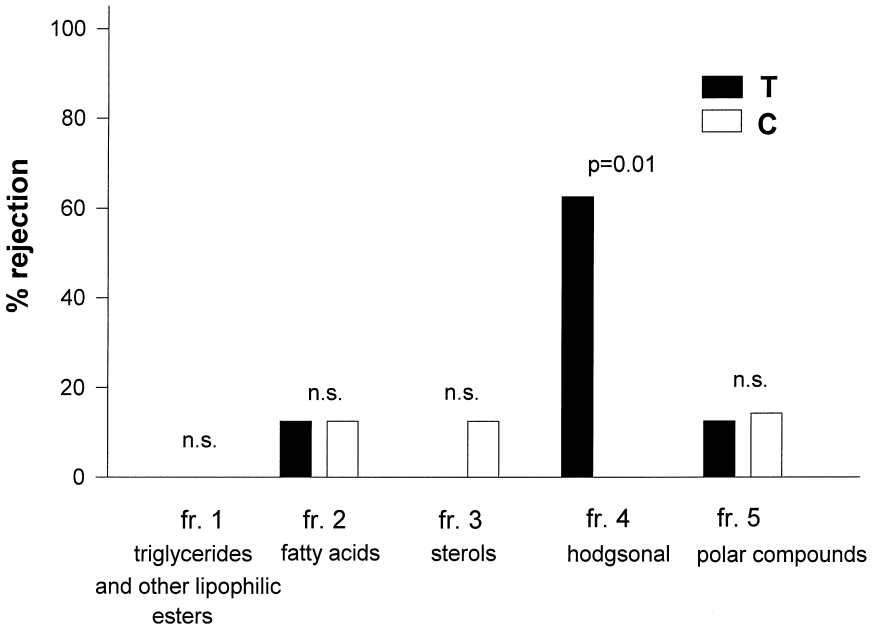

Fig. 5. Feeding deterrence tests of the different fractions obtained from the ethereal extract of the mantle of *B. hodgsoni* (at natural concentrations), using the seastar *O. validus* as predator. Below fraction number the main components of the fractions are indicated. Results from the Fisher's exact test are indicated with *P* values; n.s., not significant; T, treatment; C, control.

recorded for several specimens (Table 2); the use of specimens is also indicated there (chemical analysis, diet or feeding tests). Qualitative stomach content analysis indicated that the individuals examined had been feeding mainly on crinoids, sponges, alcyonarians and gorgonarians (Table 3).

3.1. Location of hodgsonal

As previously reported, *B. hodgsoni* possesses the sesquiterpene hodgsonal (Fig. 3; Iken et al., 1998). Hodgsonal is located only in the external body tissues of individuals from different areas (Fig. 1), and no differences in its concentration were detected by TLC among the different external body sections (gills, foot and dorsal mantle). Similarly, no differences in concentration of hodgsonal between the dorsal papillae and the rest of mantle tissue were detected by calibrated (quantitative) TLC. Several specimens studied separately yielded similar percentages of hodgsonal (Tables 4 and 5), ranging from 0.05 to 0.15% mantle dry mass (Table 5).

3.2. Feeding deterrence tests

Mantle pieces, mantle ethereal extract and papillae caused significant feeding deterrence in *Odontaster validus* ($P < 0.05$, Fig. 4). However, the ethereal extract of the

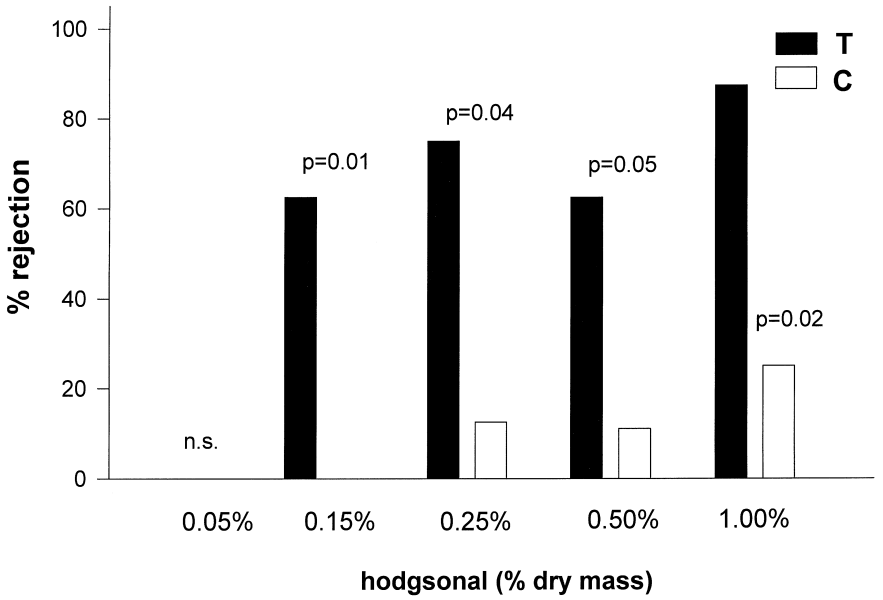

Fig. 6. Feeding deterrence tests for hodgsonal at different concentrations (0.05–1%), using the seastar *O. validus* as predator. Results from the Fisher's exact test are indicated with *P* values; n.s., not significant; T, treatment; C, control.

viscera as well as the butanolic extracts of mantle and viscera were not deterrent against *O. validus* ($P > 0.05$ in the three experiments) at natural or higher (two-fold) concentrations (Fig. 4).

The ethereal extract of the mantle of a single individual was further fractionated by column chromatography, and the different fractions obtained were tested for feeding deterrence against *O. validus*. The fractions containing high R_f compounds (fr. 1; 1.5 mg), fatty acids (fr. 2; 4.6 mg), sterols (fr. 3; 11.6 mg), and low R_f compounds (fr. 5; 5.9 mg) were not deterrent in *O. validus* tests ($P > 0.05$ for all tests) at natural or higher concentrations (Fig. 5).

The crude fraction containing hodgsonal (fr. 4) yielded 5.4 mg. This was further purified to yield 1 mg of pure hodgsonal. Feeding deterrence against *O. validus* was tested by preparing krill with hodgsonal at five concentrations ranging from 0.05 to 1% of dry mass. All except the lowest concentration (0.05%) caused significant feeding deterrence in *O. validus* (Fig. 6).

4. Discussion

Bakus (1974) and Bakus and Green (1974) suggested an inverse correlation between the incidence of chemical defense and latitude, with the highest occurrence of chemical defense in tropical sessile or slow moving marine invertebrates. The decrease in chemical defense in temperate invertebrates was related to the decrease in predation

Table 1
Coordinates for each specimen of *Bathydoris hodgsoni* collected, date, depth and capturing gear for the two expeditions: ANT XIII/3 during 1996 and ANT XV/3 during 1998

Specimen code	No. of specimens	Station	Date	Coordinates	Depth (m)	Gear[a]
ANT XIII/3						
1–4	4	011	13/II	S 73 22.6 W 21 10.6	338	BT
5	1	013	14/II	S 73 36.3 W 22 19.0	620	BT
6–8	3	015	15/II	S 73 42.0 W 22 30.5	446	BT
9–10	2	016	15/II	S 73 53.4 W 22 26.9	246	BT
11	1	017	16/II	S 73 18.0 W 21 09.9	468	BT
ANT XV/3						
12–13	2	078	3/II	S 72 50.5 W 19.24.2	439	BT
14	1	082	3/II	S 72 50.5 W 19 28.0	463	BT
15	1	084	3/II	S 7250.5 W 19 23.0	433	BT
16–18	3	095	5/II	S 73 33.4 W 22 03.5	920	BT
19–20	2	097	5/II	S 73 36.6 W 22 24.7	736	BT
21–26	6	100	5/II	S 73 34.3 W 22 07.0	616	BT
27–28	2	103	5/II	S 73 34.9 W 22 04.5	600	AT
29	1	120	7/II	S 73 35.5 W 22 05.5	928	BT
30–32	3	123	7/II	S 73 36.5 W 22 23.8	748	BT
33–36	4	150	11/II	S 74 35.8 W 26 55.0	789	BT
37	1	189	15/II	S 71 40.3 W 12 43.5	248	AT
38	1	197	16/II	S 71 17.0 W 12 36.2	416	AT
39–40	2	264	25/II	S 72 49.8 W 19 26.2	473	AT

[a] BT, bottom trawl; AT, Agassiz trawl.

pressure by browsing fish. Extending this latitudinal gradient to polar waters where predation by browsing fish is also considered low, chemical defense in Antarctic marine invertebrates would be rare. In Antarctic marine communities, however, considerable

Table 2
Quantitative data and use of *Bathydoris hodgsoni* specimens studied

Specimen code[a]	Length (cm)	WW[b] (g)	DM[b] (g)	Analysis
3	10.5	113.0	28.1	Chemistry/testing/diet
4	20.0	472.0	53.9	Chemistry/testing
5	13.0	148.0	20.3	Chemistry/testing/diet
7	10.5	106.0	16.4	Chemistry/testing/diet
9	12.5	240.0	49.4	Chemistry/testing/diet
10	10.0	83.0	N.a.	Chemistry/testing
11	9.5	96.0	14.1	Chemistry/testing/diet
12	8.0	46.9	N.a.	Testing
16,18,20,21	N.a.	N.a.	N.a.	Testing
22	12.4	180.6	N.a.	Testing
26,32	N.a.	N.a.	N.a.	Testing
39	9.5	72.0	N.a.	Testing

[a] Codes as reported in Table 1.
[b] WW, wet weight; DM, total dry mass; N.a., not available.

Table 3
Relative percentage by volume of the prey items of five specimens of *Bathydoris hodgsoni*

Stomach contents	Relative percentage by volume				
Specimen code	3	5	7	9	11
Sponge spicules and/or tissue	90				30
Crinoid ossicles	5	30	40	10	20
Gorgonarians	2	30	10		
Alcyonarians tissue and/or spicules			10	80	
Bryozoans[a]					30
Seastars[b]					10
Bivalves[c]		10			
Mixed items[d]	3	30	40	10	10

[a] *Cellaria diversa*.
[b] Probably *Epidontaster pentagonalis*.
[c] *Lissarca notocardensis*.
[d] Mixed items include some of the following: polychaete soft tubes, crustacean pieces, sediment, rests of gorgonarians, bryozoans or sponges, unidentified animal soft tissue, and/or calcareous rests.

Table 4
Quantitative data and extract yields of different specimens of *Bathydoris hodgsoni*

Specimen code	Mantle[a] DM[b] (g)	Viscera DM[b] (g)	Mantle ethereal fraction (mg)	Viscera ethereal fraction (mg)	Mantle BuOH[b] fraction (mg)	Viscera BuOH[b] fraction (mg)
3	8.1	17.5	325.0	1500.0	269.0	305.0
4	10.2	37.1	737.1	5981.0	–	–
5	3.8	14.4	184.1	1948.0	–	–
7	3.3	12.1	108.2	965.0	–	–
9	5.0	37.1	201.0	6500.0	170.0	464.0
11	3.4	9.9	101.2	673.0	–	–
39	1.3	N.a.[b]	82.8	N.a.	–	–

[a] Mantle and all external parts of the body.
[b] DM, dry mass; BuOH, butanolic fraction; n.a.: not available.

predation does occur by invertebrates, especially seastars (Dayton et al., 1974), this being a selective pressure for the development of chemical defense in Antarctic invertebrates (McClintock and Baker, 1997a). In recent years, the number of bioactive

Table 5
Percentages of the main secondary metabolite of *Bathydoris hodgsoni*, hodgsonal, in the different specimens analyzed

Specimen code	Amount of hodgsonal (mg)	Hodgsonal in mantle DM (%)
4	6	0.05
9	8	0.15
Pool of 4 specimens[a]	10	0.05
39	1	0.08

[a] Pooled extracts of specimens 3, 5, 7 and 11.

compounds found in Antarctic organisms has increased considerably due to enhanced studies in chemical ecology, proving that chemical defense is not at all restricted to tropical and temperate regions (McClintock and Baker, 1997a). However, most chemical ecology studies of Antarctic organisms have been carried out on organisms from shallow areas (~40 m, accessible by diving) in the Ross Sea and along the Antarctic Peninsula (McClintock and Baker, 1997a). To the best of our knowledge, no chemical ecology studies are known from the Weddell Sea region, nor from deeper waters in the previously mentioned areas. Hence, investigating species from deep waters of the Weddell Sea is a new contribution to our knowledge on the importance of chemical interactions in the Antarctic marine environment.

In our study, the chemical ecology of the nudibranch *Bathydoris hodgsoni* from the Weddell Sea was investigated. The genus *Bathydoris* includes seven known species which are strictly stenotherm cold water species, inhabiting exclusively polar, sub-polar, and deep-sea regions (Wägele, 1989a). *B. hodgsoni* has been found repeatedly in the Weddell Sea, a single specimen is reported from the Ross Sea and two specimens from the Davis Sea, and therefore, it is confined to the high Antarctic shelf areas (Wägele, 1987, 1989a). The specimens of *B. hodgsoni* examined in the present study were morphologically identical to those described by Wägele (1989a), both with respect to external and internal characteristics, as well as radular features. However, we should add that papillae on the mantle of some specimens were more abundant than described previously. This may have been missed in the past because the papillae are easily detached by physical stress associated with trawling. Wägele (1989a) found specimens ranging in length between 1.3 and 16.0 cm, however some of our individuals were up to 20.0 cm in length (Table 2). Our data on the geographic and bathymetric distribution of *B. hodgsoni* also agree with previous data (Wägele, 1987), however, our findings extend the depth range of this species down to 928 m (Table 1).

Feeding assays to test chemical defense in *B. hodgsoni* were conducted with the sympatric seastar *Odontaster validus*. *O. validus* lives in Antarctic waters at depths ranging from shore to 941 m, and it has a circumpolar distribution (Fell and Dawsey, 1969). Furthermore, *O. validus* is an omnivore and opportunistic species which displays a variety of feeding behaviors depending on available prey and circumstances (Dearborn, 1977; McClintock, 1994). *O. validus* may feed on sponges, gastropods, bivalves, nauplii, ostracods, detritus, hydroids, shrimp and different crustacea (Dearborn, 1977). Other possible predators of *B. hodgsoni* living within the same community could be near bottom octopods (*Pareledone spp*), fish (*Trematomus spp*, *Artedidraco spp*), actinia, and asteroids (*Notasterias armata*, *Acodontaster conspicuus*, *Diplasterias brucei*) [Iken and Avila, personal observations from trawls]. Feeding experiments in aquaria, however, were successfully conducted only with *O. validus*, and to a minor extent with fish and actinia (see below).

In the past, tests to demonstrate the existence of chemical defense mechanisms in Antarctic marine invertebrates have included several methods depending on both the prey and the predator. Some of them consisted of testing tube-foot retractions in seastars (e.g. McClintock et al., 1992b, 1993, 1994b; Slattery and McClintock, 1995, 1997; Slattery et al., 1997), feeding fish, cnidarians and amphipods with pellets made of agar (McClintock et al., 1991, 1992b, 1993; Slattery and McClintock, 1995; McClintock and

Baker, 1997b), placing treated shrimp paper disks in the mouth of sea urchins, or pellets or tissues in the ambulacral grooves of the arms of seastars and observing movement to the mouth (McClintock et al., 1994a,c; McClintock and Baker, 1997b; Amsler et al., 1999), or, more recently, coating mucous secretions onto krill pieces (Bryan et al., 1998). Our feeding deterrence experiments consisted in coating the items to test onto the krill at known concentrations, thus allowing quantitative assays, and waiting for 24 h to ensure ingestion or rejection of the food item by the seastar. Although we do not report it here, we also carried out some tests using artificial food cubes, which have been proven very useful for testing feeding deterrence in tropical systems (e.g. Van Alstyne et al., 1992). However, these cubes were not eaten by *O. validus*, and perhaps factors related to the consistency of the cubes may account for this fact.

B. hodgsoni specimens from different locations (Fig. 1) showed identical pattern with respect to the allocation of hodgsonal in their body components. Hodgsonal (Fig. 3) was only present in the external part of the body, supporting its role as a defensive agent, and being uniformly distributed in the different external components (gills, foot, papillae and dorsal skin). Hodgsonal is deterrent at concentrations equal or higher than 0.15% DM (Fig. 6). Natural concentrations range from 0.05 to 0.15% DM (Table 5), and therefore, hodgsonal is deterrent at natural concentrations. The fact that the lowest concentration was not deterrent could be due to the loss of material when preparing the coated krill pieces. Though extracts and compounds were always applied slowly and carefully onto the krill pieces and the solvent allowed to evaporate, some extract or compound is lost to the glass surrounding the krill piece. Hence, concentrations tested were conservative, being lower than the minimum amounts detected in the animals. Furthermore, none of the fractions obtained from the ethereal extract of the mantle were deterrent except the fraction containing hodgsonal. Similarly, no other extract from the mantle or viscera caused a rejection response in *O. validus*, thus confirming that feeding deterrence of *B. hodgsoni* against the seastar *O. validus* is due exclusively to the presence of hodgsonal.

Although we do not show detailed results here due to the low number of replicates, we carried out some feeding tests using additional potential predators: the Antarctic fish *Artedidraco orianae* Regan, 1914, and a yet unidentified white actinia. Both predators were collected at the same localities where the slugs were found. In these experiments, the predators were offered mantle pieces or krill coated with ethereal extract of the mantle of *B. hodgsoni* versus a krill control. The fish, *A. orianae,* rejected mantle pieces as well as krill pieces coated with ethereal extract of the mantle, but they ate the krill used as control [Avila and Iken, unpublished data]. For the actinia, the mantle tissue and the papillae of *B. hodgsoni* were not eaten, although they ate the krill pieces coated with mantle ethereal extract and the controls [Avila and Iken, unpublished data]. These experiments extend the range of predators that could be deterred by the secondary metabolite present in the external parts of the body of *B. hodgsoni*, thus increasing the ecological significance of this chemical defense mechanism.

As mentioned above, hodgsonal is present in the dorsal mantle tissue including the papillae. In *B. hodgsoni,* these papillae measure up to ~1 cm [Avila and Iken, personal observation], and are very constricted at their bases, which could explain their easy detachment. The detachment of the papillae might also be part of the defensive strategy of *B. hodgsoni*: in the event of a predator attack, when it comes into physical contact

with the slug the papillae would detach and the nudibranch could escape while the predator tries to feed on the papillae (which also contain hodgsonal). This would suggest a combined morphological–chemical defense strategy in *B. hodgsoni*, as described in other opisthobranchs, such as sacoglossans (reviewed by Cimino and Ghiselin, 1998) and nudibranchs (Marín et al., 1991).

Percentages of hodgsonal are quite similar in individuals from different localities (0.05–0.15% DM, Table 5). This strongly supports the hypothesis that hodgsonal is de novo biosynthesized by the nudibranch, and not dietarily derived. Moreover, hodgsonal is found only in the external part, being present in all external parts (gills, dorsal mantle, papillae and foot), while being absent in the viscera, also indicating that this compound is not dietarily obtained. Furthermore, *B. hodgsoni* is an omnivorous and opportunistic predator, which has been described to contain in its stomach items such as foraminifera, small porifera, hydrozoa, alcyonaria, bryozoa, ophiuroidea, parts of crustacea and crinoidea, and even stones (Wägele, 1989c). Our analysis of the stomach contents of *B. hodgsoni* are in agreement with the previous study (Wägele, 1989c), and the only remarkable fact would be the presence in our specimens of some bivalves among the dietary items. In the event a defensive metabolite is sequestered from the diet, it is crucial that an individual encounters and feeds regularly on the dietary item containing the defensive metabolite (or a precursor in the case of a biotransformation). This is not the case in an opportunistic feeder such as *B. hodgsoni*.

Hodgsonal is structurally similar to sesquiterpenoids isolated from *Dendrodoris* and *Doriopsilla* species (Cimino et al., 1981; Avila et al., 1991; Spinella et al., 1994), and *Dendrodoris* species are known to produce de novo defensive compounds (Cimino et al., 1983; Fontana et al., 1999b). In fact, Bathydoridoidea is considered the sister taxon of Doridacea (Wägele, 1989a,d, 1997), and this may suggest that the ability of nudibranchs to biosynthesize their own defensive molecules originated very early in the evolution of nudibranch molluscs.

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