Pergamon

Tetrahedron 56 (2000) 9057–9062

TETRAHEDRON

# Structure and Bioactivity of Erebusinone, a Pigment from the Antarctic Sponge *Isodictya erinacea*

Byoungho Moon,[a] Young Chul Park,[a] James B. McClintock[b] and Bill J. Baker[a,*]

[a]*Department of Chemistry, Florida Institute of Technology, Melbourne, FL 32901, USA*
[b]*Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA*

Received 5 June 2000; accepted 31 July 2000

**Abstract**—We have investigated the Antarctic sponge *Isodictya erinacea* as part of our ongoing study of Antarctic chemical ecology. *I. erinacea* was found to produce a tryptophan catabolite as its yellow pigment. The pigment, erebusinone, causes significantly reduced molting and proportionally increased mortality at ecologically relevant concentrations when fed to sympatric predatory amphipods. This appears to be the first example of molt inhibition as a mechanism of chemical defense. 

## Introduction

The marine biota of Antarctica has recently been the subject of a number of chemical investigations.[1] Interest in this biota stems in part to the continuing effort to identify novel chemical diversity for exploitation in drug discovery programs[2–4] and partly from fundamental questions concerning forces that drive the evolution of secondary metabolism.[5] Both of these objectives derive their impetus from the unique ecology of the Antarctic benthos. The physical environment on the Antarctic benthos has been stable for more than 20 million years, a period sufficient for the establishment of a 'biologically accommodated' ecosystem whereby predation and competition are the dominant forces determining species distributions and proportions.[6,7] The continent has been isolated from its lower latitude neighbors even longer. Factors such as physical stability and isolation are important criteria for genetic divergence and this has resulted in high levels of endemism in Antarctica.[8] While too little data are currently available to ascertain the extent to which these factors will lead to high levels of novel and/or diverse secondary metabolic pathways, it is clear that unique, chemically mediated biological interactions have evolved.[5] We describe herein the isolation and characterization of a secondary metabolite from the Antarctic sponge *Isodictya erinacea* which we believe to be responsible for an unusual chemical defense mechanism.

The Antarctic benthos in the vicinity of McMurdo Sound supports a thriving community dominated by sponges.[6] The sponge *I. erinacea* (Topsent, 1916) (Family Esperiopsidae) is a conspicuous member of the community due to its bright yellow coloration, which contrasts sharply with the more common colorless species. *I. erinacea* is an attractive target for chemical investigation because it is free of predation despite its lack of structural protection elements such as spicules.[6] We have previously reported that *I. erinacea* derives protection against a major Antarctic sponge predator, the sea star *Perknaster fuscus*, from its secondary metabolite repertoire.[9] The yellow pigment of this sponge proved intractable in our earlier studies due to its instability at ambient temperature. Recollection of the sponge with attention paid to handling has enabled the isolation of this previously unreported pigment, erebusinone (**1**), which appears to be a tryptophan catabolite.

## Results

*I. erinacea* was collected by scuba from several locations in Erebus Bay on the western coast of Ross Island, Antarctica, at −20 to −30 m under the seasonal ice sheet in the austral summer of 1996. A 105 g (wet) specimen of *I. erinacea*, frozen in liquid nitrogen immediately after collection to prevent degradation of the pigment, was blended and exhaustively extracted in methanol. The methanol extract was concentrated to a largely aqueous slurry and applied to a bed of reversed phase silica gel. The extract was eluted sequentially with water, methanol and ethyl acetate. The deep yellow methanol fraction was concentrated, then applied to LH-20 in methanol, and fractions were collected for each column volume of eluent. Erebusinone (**1**) eluted from LH-20 in the fourth column volume of methanol and was purified by repeated HPLC, yielding 2 mg ($2\times10^{-3}$%) of a yellow solid. The HREIMS spectrum provided a molecular formula for erebusinone of $C_{11}H_{14}N_2O_3$ (Δmmu 0.8). The $^1$H NMR spectrum displayed six signals, including

*Keywords*: marine natural products; Antarctic chemical ecology; sponge; alkaloid; pigment; chemical defense.
* Corresponding author. Tel.: +1-321-674-7376; fax: +1-321-674-8951; e-mail: bbaker@fit.edu

0040–4020/00/$ - see front matter 
PII: S0040-4020(00)00760-2

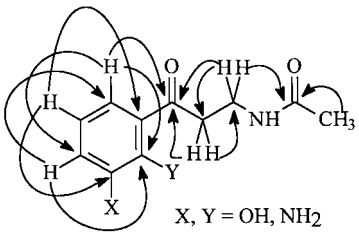



**Figure 1.** Key HMBC correlations establishing the molecular structure of erebusinone (**1**).

a three spin aromatic system (H-6, $\delta$ 7.68, dd; H-4, $\delta$ 7.53, dd; H-5, $\delta$ 6.52, t), two mutually coupled mid-field methylene triplets ($H_2$-9, $\delta$ 3.45 and $H_2$-8, $\delta$ 3.12), and an acetyl singlet ($\delta$ 1.87), accounting for 10 protons. The $^1$H NMR spectrum in DMSO-$d_6$ displayed the remaining four protons as broad signals, and the $H_2$-9 signal appeared in DMSO-$d_6$ as a quartet due to a new coupling to an amide proton. An HMQC spectrum allowed assignment of all protonated carbons observed in the $^{13}$C NMR spectrum. The aromatic ring and the mutually coupled methylene signals could be assembled around the ketone carbonyl at $\delta$ 201.9 based on HMBC correlations observed from H-6, $H_2$-8 and $H_2$-9 to C-7 (Fig. 1). The HMBC spectrum also revealed correlations from $H_2$-9 to the amide carbonyl, which also displayed a correlation to the acetyl methyl group. Two valances on the aromatic ring (X and Y, Fig. 1) could not be accounted for by 2D NMR spectroscopy and an amine and phenolic function remained to be assigned (Fig. 2).

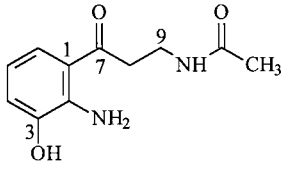
Based on biosynthetic grounds, it was compelling to place the amine group of erebusinone (**1**) in the position *ortho* (C-2) to the acyl group and the phenol in the respective *meta* position (C-3), yielding a derivative of 3-hydroxykynurenine (**2**), a known marine invertebrate-derived product.[10] However, the downfield shift of the *ortho* position (C-2, $\delta$ 145.5) relative to *meta* (C-3, $\delta$ 141.7) suggested C-2 may be substituted by the more electronegative hydroxyl group. Comparison of chemical shifts of erebusinone to isomeric analogues was thus undertaken; methyl 3-hydroxyanthranilate[11] (**3**) and **4**[12] were prepared and their shifts (see Table 1) supported an assignment where the carbon bearing the less electronegative atom resonates

**Table 1.** $^{13}$C NMR shift comparison of erebusinone **1**, hydroxyanthranilate **3** and **4** ($CD_3OD$, 360 MHz)

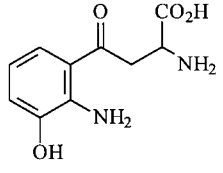
| Carbon # | 1 | 3 | 4 |
|---|---|---|---|
| C-1 | 120.2 | 111.8 | 120.1 |
| C-2 | 145.5 | 146.2 | 151.7 |
| C-3 | 141.7 | 142.2 | 138.4 |
| C-4 | 127.6 | 118.1 | 120.3 |
| C-5 | 115.3 | 116.2 | 120.1 |
| C-6 | 128.9 | 122.7 | 121.8 |
| C-7 | 201.9 | 170.3 | 207.0 |
| C-8 | 39.7 | | 38.9 |
| C-9 | 36.5 | | 36.0 |
| Methyl | 22.7 | 51.9 | 22.6 |
| Amide C=O | 173.2 | | 173.6 |

downfield apparently due to the electron withdrawing effects of the carbonyl substituent. Erebusinone therefore has the same aromatic substitution pattern as 3-hydroxykynurenine, suggesting a biogenesis from the tryptophan catabolic pathway.

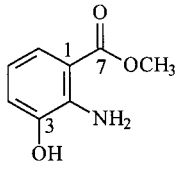
Due to structural similarities between erebusinone (**1**) and 3-hydroxykynurenine (**2**) (see Discussion, below), erebusinone was evaluated for bioactivity against the predatory amphipod *Orchomene plebs*. A freshly collected colony of *I. erinacea* was extracted and the extract chromatographed to yield a bright yellow LH-20 fraction bearing erebusinone; on return to our home institution, this fraction was demonstrated to be chromatographically homogeneous (see Experimental). This erebusinone-bearing fraction was reduced in volume, then divided into twelve aliquots. Over the course of the experiment, agar blocks were prepared from individual aliquots of the erebusinone sample using agar enriched with krill (feeding stimulant) and sand (to weight the agar down). Amphipods were fed ad libitum either erebusinone-enriched disks removed from the agar blocks or control disks prepared the same way as the experimental disks except with an equal volume of methanol rather than the erebusinone sample. Feeding was vigorous in both groups of amphipods; over the course of the first four weeks, erebusinone-enriched diet was consumed at a significantly higher rate than controls. Molt and mortality were monitored twice daily (Fig. 3) for 33 days. At the conclusion of the experiment, nearly twice as many amphipods



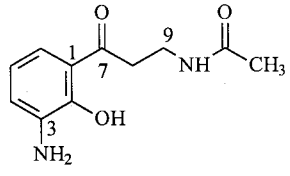

Erebusinone (**1**)




3-Hydroxykynurenine (**2**)




Methyl 3-hydroxyanthranilate (**3**)




(**4**)

**Figure 2.**

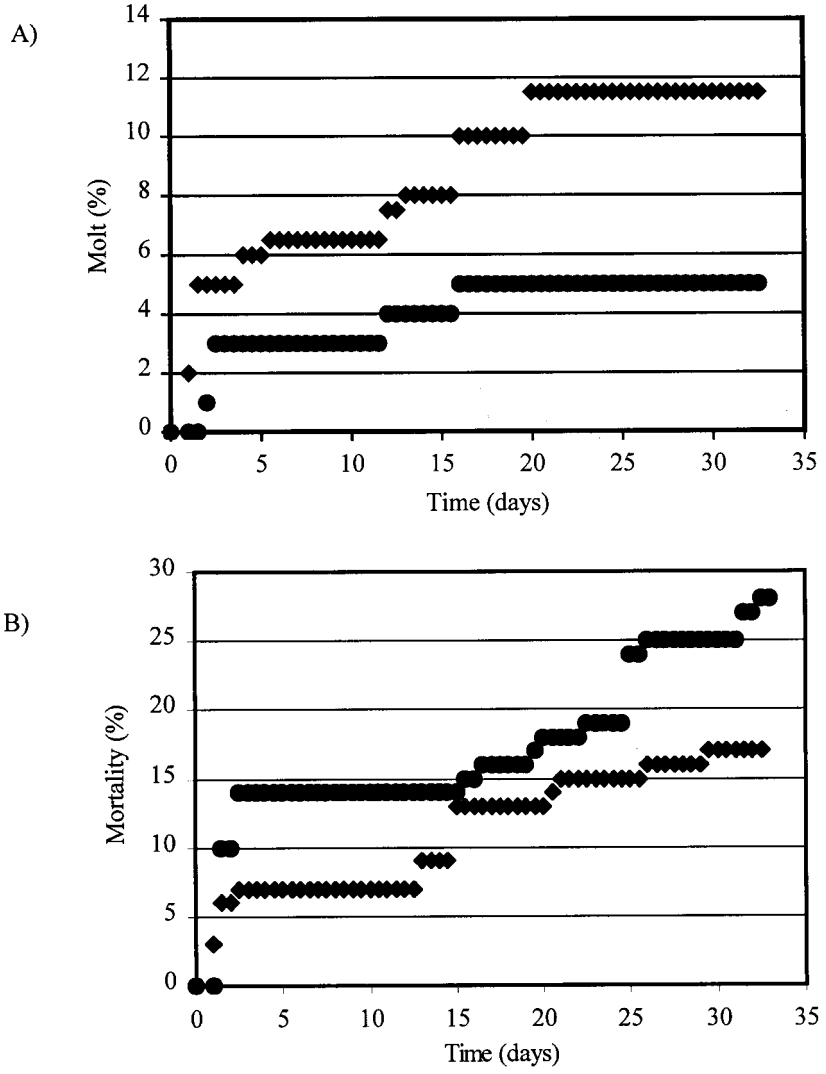



**Figure 3.** (A) Molt events, as measured by shed *O. plebs* carapaces fed control (diamonds) and erebusinone-enriched (circles) diets, as a percentage of total number of amphipods. (B) Mortality in *O. plebs*, as measured by deceased amphipods, fed experimental (circles) and control (diamonds) diets as a percentage of total number of amphipods. Both variables were monitored twice daily.

receiving control diet had molted (Fig. 3A) whereas nearly twice as many amphipods receiving experimental diet had suffered mortality (Fig. 3B). Both results show statistical difference (ANOVA using arcsine transformed percentage data, $P < 0.05$) between experimental and control data sets, suggesting that erebusinone interferes with molting in *O. plebs* leading to premature mortality.

## Discussion

Primitive plants evolved a defense against arthropod predation which takes advantage of the requirement of insects to molt as part of their development.[13] Molting, which is regulated by a complex and still poorly understood series of events, can be induced physiologically by ecdysone-derived hormones. As a chemical defense mechanism, a number of plant species produce derivatives of ecdysone, termed phytoecdysones, some of which are more potent molting inducers in insect predators than the natural hormones. Phytoecdysones produce major abnormalities in insect predators, often leading to premature death of the predator.[13]

In the marine realm, crustacean arthropods molt by a mechanism similar to that in insects.[10] Ecdysone is metabolized to 20-hydroxyecdysone, a crustacean molt-inducing hormone. The molt regulatory pathway involves the catabolism of tryptophan, via 5-hydroxytryptophan and 3-hydroxykynurenine (**2**), to xanthurenic acid, which inhibits the cytochrome P450 oxidase responsible for hydroxylation of ecdysone at C-20. Xanthurenic acid, therefore, is a naturally occurring molt inhibitor in crustaceans.

The structural similarity between erebusinone (**1**) and that of 3-hydroxykynurenine (**2**) led us to study the potential role of erebusinone in mediating molting in an Antarctic predatory crustacean. The Antarctic amphipod *O. plebs* is a general benthic scavenger on the Antarctic benthos and can be a voracious sponge predator; we have found the amphipod boring into or otherwise associated with many McMurdo Sound sponges. *O. plebs* feeding on a diet enriched at the

concentration found naturally in *I. erinacea* suffered significant molt inhibition (Fig. 3A), relative to controls, leading to a proportional increase in mortality (Fig. 3B). Toxic effects of erebusinone can be discounted as causing the reduction in molting since such effects would be expected to randomly kill amphipods, not just amphipods preparing to molt. Lack of predation by *O. plebs* on *I. erinacea* may well result from the detrimental consequences of doing so, in which case this would appear to be the first example in the marine realm of a chemical defense mechanism involving mediation of molting and an unusual example of molt inhibition, rather than induction, as a means of chemical defense.

Because erebusinone (**1**) is a structural analog of a precursor to the natural inhibitor rather than an analog to the inhibitor itself, the mechanism of action of molt inhibition is of interest. Erebusinone may function by being metabolized into a xanthurenic acid analog which acts to swamp or irreversibly bind the cytochrome P450 responsible for molt hormone biosynthesis. Alternately, erebusinone may act elsewhere, such as direct inhibition of the cytochrome P450 oxidase or one of the many other enzymes involved in molt regulation.[10] Further evaluation of the biological activities of erebusinone is underway.

## Experimental

### General procedures

NMR spectral analyses were performed on an 8.46-T NMR instrument operating at 360 MHz for $^{1}H$ and 90 MHz for $^{13}C$. One bond heteronuclear $^{1}H$–$^{13}C$ connectivities were determined by HMQC; two and three bond $^{1}H$–$^{13}C$ connectivities were determined by HMBC optimized for 7 Hz couplings; chemical shifts are reported in ppm with the chemical shift of residual solvent nuclides used as internal standards. The IR spectra were recorded on a Nicolet Magna-IR 550 spectrometer. The UV spectra were recorded on a Hewlett–Packard 8452A diode-array spectrometer. Electron impact mass spectra were recorded on a VG 70SE at the University of Florida. A Waters 510 HPLC pump system with a Waters 490E UV detector and YMC ODS-Aq (10×250 mm, 5 μm) column were used for HPLC purification. All chromatographic solvents were distilled from glass. Unless otherwise noted, chemicals were commercially available and used without further purification.

### Animal material

*I. erinacea* was collected at a depth of 20–30 m from several locations on the coast of Ross Island in McMurdo Sound, Antarctica in the Austral summer of 1996. A voucher specimen (SC 1620) is on hand at Florida Tech. *O. plebs* was collected in McMurdo Sound by placing tethered fish tissue on the benthos overnight; upon retrieval, the amphipods were removed from the fish tissue and transferred to the Crary Laboratory aquarium in bottles (2 L) of ice cold sea water. The animals were maintained in these bottles, kept at ambient temperature (−1°C) on a diet of 4% krill in agar.

### Isolation of erebusinone

Freshly collected sponge (105.5 g wet) was extracted by blending with methanol (3×400 mL). The extract was filtered and the solvent removed under reduced pressure. The residual dark brown oil was subjected to reverse phase vacuum chromatography (18 cm×4 cm) with methanol, water, methanol, then ethyl acetate. The combined methanol fractions (22.5 g after concentration) were chromatographed on Sephadex® LH-20 with methanol as eluent. Four fractions were collected, two column volumes of eluent per fraction. The fourth LH-20 fraction (2.7 g after concentration) was subjected to purification by reversed phase HPLC with 0.01 M ammonium formate/methanol (80:20), $t_R$ 13 min. Further purification by reversed phase HPLC with 0.05% TFA/methanol (80:20) provided erebusinone (2 mg, 0.002% of wet weight, $t_R$ 25 min).

**Erebusinone (1).** yellow solid; IR (KBr) $\nu_{max}$ 3419, 1641, 1221, 1061, 998 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ ($\epsilon$) 372 (176), 312 (372), 238 (1,238) nm; HREIMS found 222.0996 ($M^+$ Δmmu 0.8 for $C_{11}H_{14}N_2O_3$); $^{1}H$ NMR ($CD_3OD$): $\delta$ 7.68 (dd, $J$=6.8, 1.4 Hz, H-6), 7.53 (dd, $J$=6.5, 1.4 Hz, H-4), 6.52 (bt, $J$=7.9 Hz, H-5), 3.45 (t, $J$=6.5 Hz, $H_2$-9), 3.12 (t, $J$=6.5 Hz, $H_2$-8), 1.87 (s, NCOC$H_3$); $^{13}C$ NMR ($CD_3OD$): See Table 1.

**Preparation of methyl 3hydroxyanthranilate (3).** Powdered $K_2CO_3$ (200 mg, 1.44 mmol, 1.1 equiv.) was added to DMSO (3 mL) then, after stirring for 5 min, 3-hydroxyanthranilic acid (200 mg, 1.3 mmol, 1 equiv.) was added, followed immediately by methyl iodide (222 mg, 1.57 mmol, 1.2 equiv.). Stirring was continued for 30 min after which the mixture was poured into water (20 mL) and then extracted with methylene chloride (3×20 mL). The combined organic extracts were washed with water and brine, dried with anhydrous $MgSO_4$ and concentrated. The crude product was purified by column chromatography (5% EtOAc in *n*-hexane) to give 99 mg (45%) of methyl 3-hydroxyanthranilate as a yellow solid: IR (KBr) $\nu_{max}$ 3414, 3328, 1709, 1301, 1281 $cm^{-1}$; EIMS *m/z* 167 (M+); $^{1}H$ NMR ($CD_3OD$): $\delta$ 7.28 (d, 1H), 6.76 (d, 1H), 6.41 (t, 1H), 3.78 (s, 3H); $^{13}C$ NMR: See Table 1.

### Preparation of 4

**A.** Lithium diisopropylamide (LDA) (10 mL, 2.0 M in hexane) was added dropwise to a stirred solution of 2-hydroxy-3-nitroacetophenone (1.63 g, 9 mmol) in anhydrous THF (100 mL) under $N_2$ at −78°C (dry-ice/acetone). The reaction mixture was stirred at −78°C for 30 min. Formaldehyde vapor, obtained by heating paraformaldehyde (7.00 g) at 180°C, was introduced into the reaction vessel with vigorous stirring under nitrogen at −78°C. The reaction mixture was then poured into saturated aqueous $NH_4Cl$ (50 mL) and acidified to pH 3 with concentrated HCl. After extraction with ethyl ether (3×100 mL), and EtOAc (100 mL), the organic layers were combined and dried over anhydrous $MgSO_4$ prior to concentration in vacuo. Purification of the residue by column chromatography on silica gel (EtOAc/hexane, 1:1) yielded 855 mg (45%) of 2′-hydroxy-3′-nitro-3-hydroxypropiophenone as a pale yellow solid: mp 70°C; IR (KBr) $\nu_{max}$ 3427, 3098,

1670, 1584, 1354,1288 $cm^{-1}$; UV ($CH_2Cl_2$) $\lambda_{max}$ ($\epsilon$) 344 (6701); HREIMS found 211.0476 ($M^+$ Δmmu 0.5 for $C_9H_9NO_5$); $^1H$ NMR ($CDCl_3$): $\delta$ 12.72 (s, 1H, –OH), 8.35 (dd, $J$=6.6, 1.6 Hz, 1H), 8.11 (dd, $J$=6.2, 1.6 Hz, 1H), 7.08 (t, $J$=8.0 Hz, 1H), 4.08 (t, $J$=5.3 Hz, 2H), 3.38 (t, $J$=5.8 Hz, 2H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 204.18 (s), 156.01 (s), 137.56 (s), 136.54 (d), 131.58 (d), 125.55 (s), 118.94 (d), 57.75 (q), 42.99 (q).

**B.** A mixture of 2′-hydroxy-3′-nitro-3-hydroxypropiophenone (438 mg, 2.08 mmol), anhydrous $K_2CO_3$ (286 mg, 2.08 mmol) and benzylbromide (246 μL, 2.08 mmol) in dimethoxyethane (10 mL) and DMF (3 mL) was stirred for 24 h at room temperature. The reaction mixture was filtered and then concentrated in vacuo. The residue was diluted with water (50 mL), then extracted with $CH_2Cl_2$ (3×100 mL). The organic layer was dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Column chromatography on silica gel (EtOAc/hexane, 4:6) yielded 380 mg (61%) of 2′-benzyloxy-3′-nitro-3-hydroxypropiophenone as a pale brown oil: mp 57°C; IR (KBr) $\nu_{max}$: 3600–3300 (b), 3085, 2953, 1703, 1611, 1538, 1354, 1242; UV ($CH_2Cl_2$) $\lambda_{max}$ ($\epsilon$): 302 (3,076); HRFABMS found 302.1036 ($(M+H)^+$ Δmmu 0.8 for $C_{16}H_{16}NO_5$; $^1H$ NMR ($CDCl_3$): $\delta$ 7.94 (dd, $J$=6.6, 1.6 Hz, 1H), 7.74 (dd, $J$=6.1, 1.6 Hz, 1H), 7.37 (m, 5H), 7.29 (t, $J$=8.0 Hz, 1H), 3.83 (t, $J$=5.4, 3H), 3.10 (t, $J$=5.4 Hz, 3H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 201.85 (s), 150.25 (s), 144.78 (s), 136.74 (s), 134.98 (s), 133.71 (d), 129.26 (d), 129.14 (d, 2C), 128.99 (d, 2 C), 128.49 (d), 125.08 (d), 79.70 (t), 58.14 (t), 45.54 (t).

**C.** To a stirred solution of 2′-benzyloxy-3′-nitro-3-hydroxypropiophenone (350 mg, 1.16 mmol) in 5 mL of anhydrous $CH_2Cl_2$ at −78°C under $N_2$ was added methanesulfonyl chloride (135 μL, 1.74 mmol), followed by triethylamine (243 μL, 1.74 mmol). After the mixture was stirred for 1 h at −78°C, the solution was poured into water (50 mL), separated, and the aqueous phase was extracted with $CH_2Cl_2$ (2×50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated to yield the mesylate (427 mg, 97%) that was used without further purification. The mesylate (427 mg, 1.13 mmol) in anhydrous DMF (5 mL) was treated with $NaN_3$ (73 mg, 1.13 mmol) with stirring under $N_2$ for 3 h at room temperature. The reaction mixture was then diluted with ethyl ether (50 mL). A white precipitate was formed which was removed by filtration. The filtrate was evaporated under reduced pressure to leave an oil. Purification of the oil by column chromatography on silica gel (hexane/EtOAc/$CH_2Cl_2$, 7:1.5:1.5) yielded 147 mg (40%) of 2′-benzyloxy-3′-nitro-3-azidopropiophenone as pale yellow oil: IR (NaCl) $\nu_{max}$: 3078, 2927, 2117 (–$N_3$), 1709, 1611, 1249; UV ($CH_2Cl_2$) $\lambda_{max}$ ($\epsilon$): 302 (4909); $^1H$ NMR ($CDCl_3$): $\delta$ 7.99 (dd, $J$=6.4, 1.7 Hz, 1H), 7.77 (dd, $J$=6.1, 1.6 Hz, 1H), 7.39 (s, 5H), 7.32 (t, $J$=7.9 Hz, 1H), 5.05 (s, 2H), 3.57 (t, $J$=6.2 Hz, 2H), 3.13 (t, $J$=6.3 Hz, 2H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 199.07 (s), 150.56 (s), 144.80 (s), 136.53 (s), 135.15 (s), 133.98 (d), 129.27 (d), 129.06 (d, 2 C), 128.98 (d, 2 C), 128.67 (d), 79.70 (t), 46.09 (t), 42.26 (t).

**D.** Under a nitrogen atmosphere in a 1 mL round-bottom flask equipped with a rubber septum was placed 2′-benzyloxy-3′-nitro-3-azidopropiophenone (110 mg, 0.34 mmol). To the system was added thiolacetic acid (97 μL, 1.35 mmol). The reaction mixture was stirred at room temperature for 4 h and concentrated in vacuo. The resulting oil was subjected to flash column chromatography on silica gel (EtOAc/hexane, 1:1) to obtain 109 mg (90%) of the 2′-benzyloxy-3′-nitro-3-acetamidopropiophenone as a pale yellow solid: mp 75°C; IR (KBr) $\nu_{max}$: 3295, 3078, 2933, 1709, 1657, 1532; UV ($CH_2Cl_2$) $\lambda_{max}$ ($\epsilon$): 302 (1662); HREIMS found 342.1208 ($M^+$ Δmmu 0.8 for $C_{18}H_{18}N_2O_5$); $^1H$ NMR ($CDCl_3$): $\delta$ 7.95 (dd, $J$=6.5, 1.6 Hz, 1H), 7.73 (dd, $J$=6.1, 1.7 Hz, 1H), 7.36 (s, 5H), 7.31 (t, $J$=7.9 Hz, 1H), 6.08 (bs, $1H_{ex}$), 3.47 (q, $J$=5.9 Hz, 2H), 3.09 (t, $J$=5.8 Hz, 2H), 1.88 (s, 3H); $^{13}C$ NMR ($CDCl_3$): $\delta$ 201.08 (s), 170.19 (s), 150.37 (s), 144.90 (s), 136.64 (s), 135.06 (s), 133.60(d), 129.17 (d), 128.99 (d, 2 C), 128.93 (d, 2 C), 128.48 (d), 79.51 (t), 43.01 (t), 34.50 (t), 23.29 (q).

**E.** A mixture of 2′-benzyloxy-3′-nitro-3-acetamidopropiophenone (68 mg, 0.19 mmol), and 10% Pd/C (30 mg) in ethanol (15 mL) was shaken with hydrogen administered via balloon for 1 h at room temperature (21 mL of hydrogen was consumed). The reaction mixture was filtered to remove the catalyst, and the filtrate was concentrated. Purification of the residue by column chromatography on silica gel (methanol/$CH_2Cl_2$, 5:95) yielded 34 mg (81%) of **4** as a yellow solid: yellow solid: IR (KBr) $\nu_{max}$: 3427, 3322, 2940, 2479, 1663, 1637, 1545, 1453, 1249; UV (MeOH) $\lambda_{max}$ ($\epsilon$) 372 (219), 278 (679), 238 (1323); HREIMS found 222.1052 ($M^+$ Δmmu 4.8 for $C_{11}H_{14}N_2O_3$), 163.0637 ($M^+-CH_3CONH_2$, Δmmu 0.4 for $C_9H_9NO_2$); $^1H$ NMR ($CD_3OD$): $\delta$ 7.19 (dd, $J$=6.8, 1.3 Hz, 1H), 6.93 (dd, $J$=6.3, 1.4 Hz, 1H), 6.70 (t, $J$=7.9 Hz, 1H), 3.51 (t, $J$=6.4 Hz, 2H), 3.21 (t, $J$=6.4 Hz, 2H), 1.87 (s, 3H); $^{13}C$ NMR ($CD_3OD$): See Table 1.

**Bioassay.** Agar blocks for amphipod bioassay were prepared from freshly collected *I. erinacea* (160 g dry/650 mL), which was extracted and chromatographed by reversed phase and LH-20 methods, as described above. The bright yellow LH-20 band bearing erebusinone was reduced in volume then divided into twelve 2.0 mL aliquots. Agar blocks were prepared as needed from individual aliquots of the erebusinone sample by adding the 2.0 mL solution (in methanol) to 52 mL (=1/12 the volume of the original sponge such that the concentration of erebusinone in agar is equivalent to that of the sponge on a volume basis) of 3% agar enriched with 4% krill (feeding stimulant) and 1.5% sand (to weight the agar down). Control blocks were similarly prepared using 2.0 mL of pure methanol. HPLC analysis of one aliquot of the erebusinone sample was carried out on return to our home institution; erebusinone (**1**), identified by $^1H$ NMR spectroscopy, was the sole absorption in the HPLC chromatogram at 254 nm.

*O. plebs* were collected using fish-baited traps in McMurdo Sound, Antarctica. Amphipods selected for experiments were of similar size (mean length=9 mm). Six 250 mL Erlenmeyer flasks containing 30 amphipods each were maintained at ambient temperature (−1°C) in the Crary Laboratory (McMurdo Station, Antarctica) with daily water changes. Experimental animals in three of the flasks were fed an ad libitum diet of disks removed from agar

blocks of 4% finely ground dry krill in 3% agar containing 1.5% sand (to weight the food to the bottom of the flasks) and a concentration of the erebusinone ecologically equivalent to that found in the sponge. Control animals in the other three flasks received the same diet ad libitum but containing an equivalent amount of solvent (methanol) used to solubilize erebusinone in the preparation of the experimental diet. All agar disks were replaced daily with fresh disks. Both experimental and control amphipod flasks were observed twice daily for molt (discarded carapace) and mortality for a 33-day-period. The percent incidence of molting and mortality over the entire experimental period was compared between experimental and control treatments (following arcsine transformation of percentage data) employing an analysis of variance test.

## Acknowledgements

Field and laboratory assistance from Jim Mastro, Chuck Amsler, Thomas Klinger, Thomas Barlow, Jill Baker and Gamini Jayatilake are greatly appreciated. This research was supported by the National Science Foundation (OPP-9530735 to JBM and OPP-9526610 and -9901076 to BJB). We gratefully acknowledge the services of Dr David Powell, Director of Spectroscopic Services at the University of Florida for the mass spectral data. Purchase of the NMR spectrometer was assisted by a grant from NSF. The Antarctic Support Associates Inc., the Antarctic Support Services of the National Science Foundation, and the US Naval Antarctic Support Force provided invaluable logistical support for this project. This manuscript is submitted in honor of Paul J. Scheuer, whose inspiration and guidance has contributed in many ways to make this work possible.

## References

1. Amsler, C. D.; Iken, K. B.; McClintock, J. B.; Baker, B. J. *Marine Chemical Ecology*; McClintock, J. B., Baker, B. J., Eds.; CRC Press: Boca Raton, FL, in press.
2. (a) Blunt J. W.; Munro, M. H. G.; Battershill, C. N.; Copp, B. R.; McCombs, J. D.; Perry, N. B.; Prinsep, M.; Thompson, A. M. *New Journal of Chemistry* **1990**, *40*, 761–775. (b) Perry, N. B.; Ettouati, L.; Litaudon, M.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron* **1994**, *50*, 3987–3992.
3. Jayatilake, G. S.; Baker, B. J.; McClintock, J. B. *Tetrahedron Letters* **1997**, *38*, 7507–7510.
4. Fontana, A.; Ciavatta, M. L.; Amodeo, P.; Cimino, G. *Tetrahedron* **1999**, *55*, 1143–1152.
5. (a) McClintock, J. B.; Baker, B. J. *American Scientist* **1998**, *86*, 254–263. (b) McClintock, J. B.; Baker, B. J. *American Zoologist* **1997**, *37*, 329–342.
6. Dayton, P. K.; Robilliard, G. A.; Paine, R. T.; Dayton, L. B. *Ecological Monographs* **1974**, *44*, 105–128.
7. Dayton, P. K.; Mordida, B. J.; Bacon, F. *American Zoologist* **1994**, *34*, 90–99.
8. Arntz, W. E.; Brey, T.; Gallardo, V. A. *Oceanography and Marine Biology an Annual Review* **1994**, *32*, 241.
9. Moon, B. H.; Baker, B. J.; McClintock, J. B. *Journal of Natural Products* **1998**, *61*, 116–118.
10. Nakanishi, K. In *Biomedical Importance of Marine Organisms*; Fauntin, D. G., Ed.; California Academy of Science Press: San Francisco, 1988; pp 59–67.
11. Kelly, T. R.; Martinez, C.; Mears, R. J. *Heterocycles* **1997**, *45*, 87–93.
12. Full details of the synthesis of **1** and **4** will be reported elsewhere.
13. Williams, C. M. In *Chemical Ecology*; Sondheimer, E., Simeone, J. B., Eds.; Academic Press: New York, 1972; pp 103–132.