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TYRIAN PURPLE PRECURSORS IN THE EGG MASSES OF THE AUSTRALIAN MURICID, *Dicathais orbita*: A POSSIBLE DEFENSIVE ROLE

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Abstract—We report a putative defensive role for the precursors of Tyrian purple in the egg masses of the Australian muricid, *Dicathais orbita*. The fresh egg masses contain a high proportion of tyrindoleninone, which reacts to form tyriverdin and subsequently Tyrian purple and 6-bromoisatin as the eggs develop and the larvae hatch. Antimicrobial testing revealed that tyrindoleninone is toxic to both marine and human pathogens at a concentration of 1 mg/ml. Tyriverdin inhibits the growth of two marine pathogens, as well as the yeast *Candida albicans* at 0.001 mg/ml and was effectively bacteriostatic at 0.0005 mg/ml against three human pathogenic bacteria. Tyriverdin did not appear to significantly lyse the microbial cells. 6-Bromoisatin has mild antimicrobial properties, whereas Tyrian purple exhibited no significant activity. The antimicrobial properties of these compounds and changes in their presence during egg development correlates with ripening in the egg masses of *D. orbita*. This is the first report of the chemical ripening of eggs in a marine environment.

Key Words—Tyrian purple, tyriverdin, tyrindoleninone, 6-bromoisatin, *Dicathais orbita*, egg mass, antimicrobial, marine natural products.

INTRODUCTION

Tyrian purple is the trivial name for a purple dye that has been known since pre-Roman times (Baker, 1974). The chemical constitution of the dye was first

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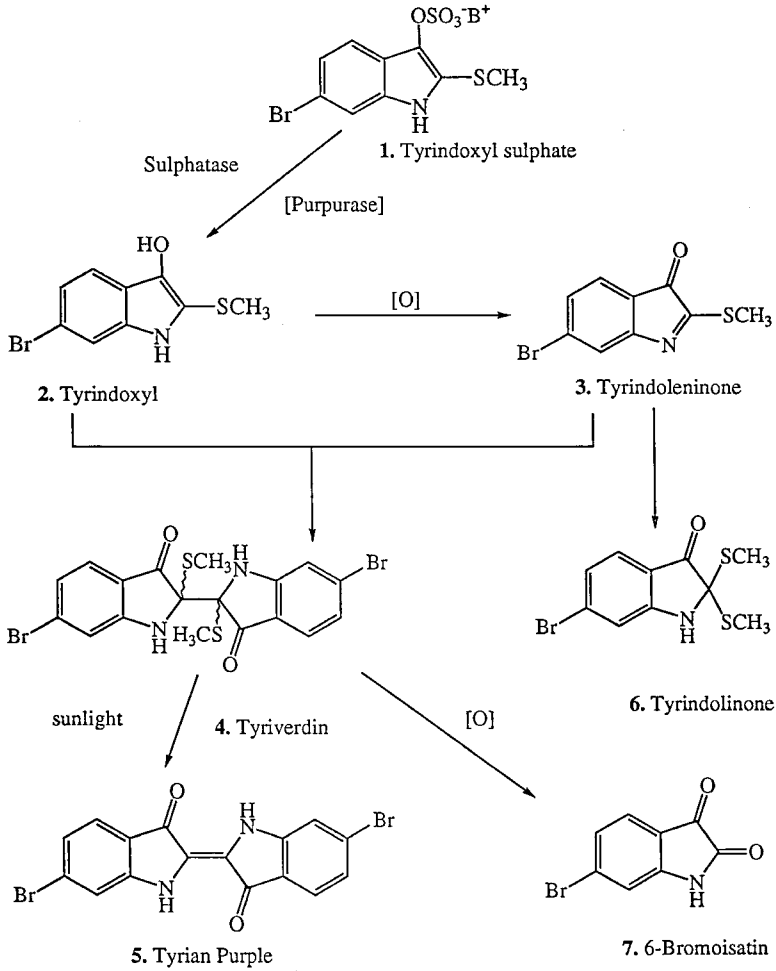

SCHEME 1. The formation of Tyrian purple from the ultimate precursor tyrindoxyl sulfate produced in the hypobranchial glands of the murex mollusk *Dicathais orbita*.

identified by Friedländer (1909) as 6,6′-dibromoindigotin (**5**, Scheme 1). The importance of this dye in several Mediterranean civilizations has triggered much interest in the chemistry of its biosynthesis. Mollusks of the families Muricidae and Thaisidae are the principal sources of the dye, but no purple compound as such is present within the dye-producing hypobranchial glands of these mollusks. The original white fluid from the hypobranchial glands turns yellow first, followed by various shades of green and blue to its final purple coloration fol-

lowing exposure to sunlight (Cole, 1685). The color change is concomitant with the production of an unpleasant sulfurous smell (Prota, 1980).

The ultimate precursor to the dye was isolated from an ethanol extract of the hypobranchial gland of the Australian murex *Dicathais orbita* (Gmelin, 1791) by Baker and Sutherland (1968). These researchers also isolated an enzyme from the glands that initiated the conversion of the salt precursor, tyrindoxyl sulfate (**1** Scheme 1), to the purple product. Subsequently, the intermediate precursors were isolated from diethyl ether extracts of the glands and identified as tyrindoxyl (**2**), which is oxidized to tyrindoleninone (**3**) (Baker and Duke, 1973). These two compounds combine to form tyriverdin (**4**), the immediate precursor of Tyrian purple (Christophersen et al., 1978). Tyrindolinone (**6**), a methanethiol adduct of tyrindoleninone, and 6-bromoisatin (**7**) also have been isolated in small amounts (Baker and Duke, 1973; Baker, 1974). 6-Bromoisatin is a by-product formed by the decomposition of tyriverdin and the amount produced increases relative to 6,6′-dibromoindigotin in the presence of oxygen (Baker, 1974). It has been suggested also that 6-bromoisatin may arise via the enzymatic oxidation of tyrindoxyl sulfate (**1**), the oxidation of tyrindoxyl (**2**), and the hydrolysis of tyrindolinone (**6**) (Clark and Cooksey, 1997).

The role of the Tyrian purple precursors in the hypobranchial glands of muricid mollusks has remained a mystery (Prota, 1980; Max, 1989; Hoffmann, 1990). They may represent excretory products from the breakdown of tryptophan (Fox, 1974; Ziderman, 1990). Several marine indoles, however, exhibit significant pharmacological activity (Christophersen, 1983) and, in particular, isatin is a natural fungicide that protects the embryos of the shrimp, *Lagenidium callinectes* (Gil-Turnes et al., 1989).

There have been no previous investigations into the possible presence of Tyrian purple and its precursors in the egg masses of muricid mollusks. Nevertheless, the egg masses of many species are yellow when freshly laid and turn a distinctive purple color just prior to hatching (personal observations). Disruption of the egg capsules results in the liberation of an offensive sulfurous smell. Cole (1685) suggested that the products of the hypobranchial glands could be reproductive material, but he conducted no experiments to test this idea.

Aristotle indicated the potential importance of the egg masses by suggesting that the best time to collect the murex for their purple dye was before they have produced their "honeycomb-like exudate" (Peck, 1970). Pliny the Elder reinforced this, stating that "this fact, although of utmost importance, is not recognized in the dye factories" (Bailey, 1929; Cap. XXXVIII. Sect. 62. 133). Neither of these workers correctly identified this exudate as the egg masses. However, Bailey (1929) makes a note in his translation of Pliny's *Historia Naturalis* that most mollusks enveloped their eggs with a clear substance that is not unlike a honeycomb in appearance and, indeed, the egg capsules of *D. orbita* bear a striking resemblance (Figure 1).

FIG. 1. The Australian muricid, *Dicathais orbita*, with its egg masses.

In this study, the egg masses of the abundant southern Australian muricid *D. orbita* were examined for the presence of Tyrian purple and its precursors. The antimicrobial activity of the crude egg extracts and isolated compounds was assessed to determine a potential ecological role for these metabolites.

METHODS AND MATERIALS

Collection and Extraction of Egg Masses. The egg masses of *D. (Thais) orbita* are common in the lower intertidal area from late winter through spring (authors' personal observation). The eggs were collected from two sites, Towradgi and Flagstaff Hill (on the Illawarra Coast, NSW, Australia), on several occasions throughout the breeding season (August to October) in 1995, 1996, and 1997. The eggs were collected from vertical rock faces by wading at Towradgi and by snorkelling at Flagstaff Hill. A total of 307.15 g of fresh eggs and 64.75 g of hatching eggs was collected. A voucher specimen (M-em-001) is lodged in the Department of Biological Sciences, University of Wollongong, New South Wales, Australia.

The egg capsules were cut in half and extracted fresh by soaking in solvent for several hours, followed by decanting and replacing the solvent. This was repeated twice with the final soak being overnight. The extracts were then combined and evaporated to dryness in a rotary evaporator with water pump vacuum. The main solvent used was chloroform–methanol (1 : 1, v/v), but additional extracts were made with diethyl ether or ethanol. All solvents were AR grade. A small volume of distilled water was added to the chloroform–methanol extracts to facilitate a chloroform–water/methanol separation before the solvent was evaporated.

Organisms and Culture. Stock cultures of *Candida albicans* ACM4581, *Escherichia coli* ACM845, *Staphylococcus aureus* ACM844, and *Pseudomonas aeruginosa* ACM846 were obtained from the Culture Collection at the University of Queensland and maintained at −78°C in 15% glycerol. Additional strains of *C. albicans* (AMMRL 36.42, AMMRL 36.70) were provided by the Royal North Shore Hospital, Sydney, and maintained in saline according to Muir (1988). The cultures were prepared by streaking onto Sabouraud dextrose agar (SDA) (Difco 0109-17-1) for *C. albicans* and nutrient agar (NA) (Oxoid CM3) for *E. coli*, *S. aureus*, and *P. aeruginosa*. After an overnight incubation, single colonies were used to inoculate a sterile liquid medium. The broth for the three bacteria consisted of yeast extract (5 g, ICN 103303-17), peptone (10 g, Oxoid L37), and NaCl (5 g, ICN 102892) in distilled water (1 liter). Sabouraud liquid medium (Oxoid CM147) was used for *C. albicans*. Inoculated broths were placed on an orbital shaker (150 rpm) and incubated at 37°C overnight.

Two marine pathogens, *Enterococcus sericolicida* and *Vibrio anguillarum*, were provided by the Tasmanian Department of Primary Industry and Fisheries. Cultures were prepared by streaking onto marine agar (Difco 2216; pH 7.6) and incubating overnight at 25°C. After an overnight incubation, single colonies were used to inoculate sterile liquid medium (pH 7.5) composed of nutrient broth (25 g, Oxoid No. 2), yeast extract (3 g), and NaCl (50 g) in distilled water (1 liter). Inoculated broths were placed on an orbital shaker (150 rpm) and incubated overnight at 25°C.

Antimicrobial Assay. The extracts and compounds isolated from the eggs of *D. orbita* were tested for antimicrobial activity by the fluorescein diacetate (FDA) hydrolysis method described by Chand et al. (1995). Briefly, exponentially growing cultures were transferred to a 96-well ELISA tray and incubated with the test sample for 40 min. Fluorescein diacetate (5 μl at 2 mg/ml) was added to the cultures and incubation was continued for a further 3 hr or until the production of fluorescein was visible under a UV lamp.

Weighed organic extracts or isolated compounds used in the assay were dissolved in acetone just prior to use. Water–methanol extracts were dissolved in 20% acetone in MilliQ water. The extracts were serially diluted and at least three replicates were run at each concentration to determine the minimum inhibitory concentration (MIC). At least three replicate controls consisting of 20 μl of acetone and 20 μl of MilliQ water with FDA were added to each test plate to determine effects of the acetone on the viability of the cells. Additional controls included 20 μl of test substance in 175 μl of broth with FDA to ascertain whether the test compound hydrolyzed FDA, and 195 μl of broth with FDA to check for contamination in the broth.

After the FDA assay was completed, 20 μl of culture (four replicates) from all the wells that did not show fluorescence were plated on agar to determine if the cells had been killed. The plates were incubated overnight at 37°C. Counts of visibly growing colonies were performed and compared to a dilution series of a control culture from the FDA plate containing acetone.

Cultures of cells incubated with the antimicrobial compounds also were observed under the light microscope. Slides were prepared by spinning 100 μl of the culture onto the slides in a Cytospin 2 apparatus (Shandon, 150 rpm for 3 min). The slides were Gram stained and viewed under oil with a light microscope (100×).

Isolation and Identification of Major Components. The organic extracts from the egg mass of *D. orbita* were analyzed by thin-layer chromatography on aluminum-backed silica gel plates (Merck, 60 F_{254}). Additional analysis was facilitated by mass spectrometry (MS) in a QP-5000 (Shimadzu) spectrometer with direct insertion technique or by gas chromatography–mass spectrometry (GC-MS) with a GC-17A (Shimadzu) gas chromatograph in splitless mode. The injector temperature was set at 260°C. The oven temperature was held at 40°C for 2 min and then ramped to 290°C at a rate of 4°C/min. The final oven temperature was held at 290°C for 10 min. The carrier gas was helium and the flow rate was 1.4 ml/min. The electron beam energy in the mass spectrometer was 70 eV and the source temperature was 200°C.

The isolation of antimicrobial compounds from extracts of the egg mass of *D. orbita* was guided by bioassays against *E. coli* and *S. aureus* with the FDA hydrolysis method. Chloroform extracts from the egg mass were fractionated by flash silica chromatography. The extract was chromatographed on a silica

column (25 g, 60 mesh) with redistilled dichloromethane (200 ml) followed by 10% methanol in dichloromethane (v/v, 100 ml, redistilled) as solvents, and 15-ml fractions were collected.

The major components in the active fractions were isolated by radial chromatography on a Chromatotron (Professional Technology) with 9 : 1 light petroleum (bp 40–60°C)–dichloromethane followed by 100% dichloromethane, and then 5% methanol in dichloromethane. All solvents were distilled prior to use. The separation was repeated on fractions that still contained a mixture of two or more compounds.

The mass spectrum of the isolated compounds was obtained by GC-MS, solid probe MS, and high-resolution mass spectrometry (HR-MS) in a Fisons/VG Autospec-TOF-oa mass spectrometer. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were determined at 300 MHz with a Varian Unity-300 spectrometer. The spectra were obtained on solutions in deuteriochloroform or d_6-dimethylsulfoxide and referenced to tetramethylsilane.

RESULTS

Analysis of Crude Egg Extracts. Thin-layer chromatography revealed that the extracts from fresh, frozen, and freeze-dried eggs contained a number of colored compounds (Table 1). An orange spot (R_f 0.5) was the major product present in the chloroform and ether extracts of the fresh eggs. A green spot (R_f 0.35), which turned purple in sunlight, and a yellow spot (R_f 0.05) that did not change color were present in lipophilic extracts of both the fresh and hatching eggs (Table 1). In the ethanol extract, a yellow spot (R_f 0.4) that turned green and then purple in sunlight was the dominant component. An insoluble purple spot, which remained on the baseline was the major component of the hatching eggs.

The major orange and green components of the fresh eggs were isolated successfully from a chloroform extract by silica chromatography. The yellow component broke down on silica to produce the orange and green components. This is consistent with the results of Baker and Duke (1973), who identified the yellow component as tyrindolinone (**6**).

The orange component (R_f 0.5) was identified as tyrindoleninone (**3**) (Baker and Duke, 1973) after GC-MS (retention time 38.0 min; $M^{+\cdot}$ m/z 255, 257 ^{79}Br, ^{81}Br; major fragments 240, 242 and 133, and ^{1}H NMR spectroscopy (300 MHz, $CDCl_3$; δ2.65, s, 3H, CH_3S; δ7.3–7.4, m, 3H, ArH). HR MS [electron impact (EI)] produced an accurate mass $M^{+\cdot}$ 254.9356 ($C_9H_6{}^{79}BrNOS$ requires 254.9353) (the sample of tyrindoleninone also formed another high-molecular-weight product slowly at room temperature, although there was insufficient material to characterize it). Tyrindoleninone was present in the fresh eggs but not in

TABLE 1. THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF COLORED COMPOUNDS FROM ORGANIC SOLVENT EXTRACTS OF *Dicathais orbita* EGGS[a]

		Fresh			Hatching
R_f	Color	Chloroform	Ethanol	Diethyl ether	Chloroform
0.50	Orange	+	+	+	–
0.40	Yellow	+	+	+	–
0.35	Green	+	–	+	+
0.05	Yellow	+	–	+	+
0.00	Purple	(+)	–	(+)	+

[a]The solvent system was light petroleum–dichloromethane (1 : 1). The symbols used are + for present, (+) present but minor component, and – for not detected.

the hatching eggs based on results from both the solid probe MS and GC-MS. GC-MS revealed tyrindoleninone to be the major volatile organic constituent in the fresh egg masses (Figure 2).

The green component (R_f 0.35) in the egg mass was isolated by silica chromatography. The solid probe EI-MS gave the closest match to Tyrian purple (**5**) in the mass spectrum library, with brominated isotopic ion clusters at m/z 418, 420, 422, as well as 257, 259, and 240, 242. HR-MS (electron impact) gave an accurate mass for the high-molecular-weight fragment ion at m/z 417.8954 ($C_{16}H_8{}^{79}Br_2N_2O_2$ requires 417.8952). Previous studies have shown that tyriverdin (**4**) does not produce a molecular ion under electron impact

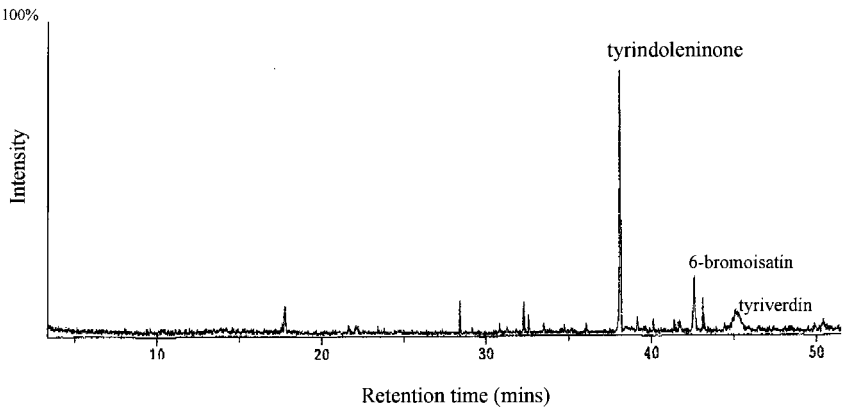

FIG. 2. A GC-MS chromatogram of the volatile organic constituents in a chloroform extract from the egg masses of the Australian muricid, *Dicathais orbita*.

conditions in the mass spectrum although Christophersen et al. (1978) have since shown that field desorption/field ionization MS does produce a molecular ion. We investigated chemical ionization and electrospray (positive ion) MS of this compound but still could not detect an MH^+ ion. However, the ^1H NMR (DMSO-d_6) of this green component demonstrated the presence of a singlet at δ1.9, corresponding to the S**C**$\mathbf{H}_3$ protons (6H), with additional peaks at δ8.2, s, 2H, N**H**; δ6.96, dd, J = 8.4 Hz, 1.5 Hz, 2H, Ar**H**; δ7.27, d, J = 1.5 Hz, 2H, Ar**H**; δ7.46, d, J = 8.4 Hz, 2H, Ar**H**). These data correspond well to values reported by Christophersen et al. (1978) for tyriverdin in the same solvent. The solid probe MS indicated that tyriverdin ($M^{+\cdot}$-2CH_3SH) was found in hatching eggs, as well as in fresh eggs.

The polar yellow component (R_f 0.05), which did not change color with time, was also isolated from the eggs by silica chromatography by eluting with 5% methanol in dichloromethane. The mass spectrum of this compound was matched to 5-bromoisatin, the only bromoisatin in the GC-MS mass spectrum library [m/z $M^{+\cdot}$ 225, 227 (^{79}Br, ^{81}Br); major fragment ions were observed at m/z 197 and 170 (^{79}Br)]. This is more likely to be 6-bromoisatin (**7**) as identified by previous research on the hypobranchial glands of muricids (Baker, 1974; Clark and Cooksey, 1997). HR-MS (electron impact) gave an accurate mass for M^+ at m/z 224.9423 ($C_8H_4{}^{79}BrNO_2$ requires 224.9425). 6-Bromoisatin was detected in trace amounts in both the fresh and hatching eggs by GC-MS.

Tyrian Purple was obtained by leaving pure tyriverdin in the presence of sunlight for several days. The product was then washed with diethyl ether and filtered to remove any remaining tyriverdin and 6-bromoisatin. The solid probe MS was used to confirm the absence of tyriverdin and 6-bromoisatin, but the extreme insolubility of Tyrian purple prevented spectra from being obtained on this compound.

Antimicrobial Activity. Extracts from the egg masses of *D. orbita* exhibited significant antimicrobial activity. Bacteriostatic activity was observed against both human and marine pathogens (Table 2). Organic extracts of the fresh eggs were much more active than the hatching eggs, and extracts prepared with lipophilic solvents showed greater activity than a polar solvent extract (Table 2). Bioassay-guided fractionation of the active compounds resulted in the isolation of three antimicrobial compounds identified as tyrindoleninone, tyriverdin, and 6-bromoisatin.

In the FDA assay, tyriverdin was the most active compound (Table 2). Antimicrobial activity was observed at a concentration of 1–0.5 μg/ml against all test microorganisms for tyriverdin, which was substantially greater in activity than the crude extract. Tyrindoleninone was mildly bacteriostatic against both human and marine pathogens, whereas 6-bromoisatin did not inhibit effectively the growth of two marine bacteria (Table 2). Tyrian purple exhibited minimal activity, which could be due to trace amounts of tyriverdin or 6-bromoisatin left in the sample. It should be noted, however, that a sonicated suspension of Tyrian purple has to be used in this assay due to the insolubility of the compound in acetone.

TABLE 2. BACTERIOSTATIC ACTIVITY OF EXTRACTS AND ISOLATED COMPOUNDS FROM EGG MASSES AND ADULT TISSUE OF *Dicathais orbita*[a]

Solvent extract or compound	Minimum inhibitory concentration (mg/ml)					
	S.a.	E.c.	P.a.	C.a.[b]	E.s.	V.a.
Fresh eggs						
Chloroform ex.	0.1–1	0.1–1	1–10	0.1–1	1	1
Diethyl ether ex.	0.1	0.1	0.1	NT	NT	NT
Ethanol ex.	10	10	10	NT	NT	NT
Methanol/Water ex.	–	–	–	–	–	–
Hatching eggs						
Chloroform ex.	10	10	10	NT	NT	NT
Tyriverdin	0.0005	0.0005	0.0005	0.001	0.001	0.001
Tyrindoleninone	0.5	0.5	1	0.1	0.1	0.1
6-Bromoisatin	0.1	1	1	>1	>1	>1
Tyrian purple	>1	>1	>1	>1	1	1

[a] The test microorganisms are *Staphylococcus aureus* (S.a.), *Escherichia coli* (E.c.), *Pseudomonas aeruginosa* (P.a.), *Candida albicans* (C.a.), *Enterococcus sericolicida* (E.s.), and *Vibrio angillarium* (V.a.). ex. = extract. NT = not tested. −: no activity at the maximum concentration tested (50 mg/ml for water/methanol extracts, 10 mg/ml for the other extracts and 1 mg/ml for isolated compounds); >: partial activity at the maximum concentration tested.

[b] The same results were obtained for *Candida albicans* strains ACM4581, AMMRL 36.42, and AMMRL 36.70. C.a., although a yeast, is included here for comparative purposes.

Plating the cultures from the FDA assay revealed that tyriverdin was only bacteriostatic and lysed only a small proportion of the microbial cells (Table 3). This was confirmed under the light microscope, where the cells were observed to cluster around crystals of tyriverdin. Only those cells in contact with the crystals were lysed or distorted. By comparison, tyrindoleninone was highly toxic at a concentration of 1 mg/ml, causing 100% cell death to most of the microorganisms (Table 3). Only a few patches of lysed or distorted cells were observed under the light microscope for cultures of *S. aureus*, *E. coli*, *V. angullarium*, and *C. albicans* after incubation with tyrindoleninone. Cultures of the Gram-positive marine pathogen *E. sericolicida* and the Gram-negative bacteria *P. aeruginosa* were more resistant to tyrindoleninone than the other test organisms (Table 3). The 6-bromoisatin, like tyriverdin, caused minimal cell death, which was detectable under the light microscope but not by plating onto agar.

DISCUSSION

The egg masses of *D. orbita* contain the well-known dye Tyrian purple (**5**) and several of its precursor molecules. The demonstrated antimicrobial activ-

TABLE 3. CONCENTRATION OF EXTRACTS AND COMPOUNDS ISOLATED FROM EGG MASSES OF *Dicathais orbita* REQUIRED TO CAUSE 100% CELL DEATH[a]

	Minimum concentration to elicit 100% cell death (mg/ml)					
Sample	S.a.	E.c.	P.a.	C.a.	E.s.	V.a.
Fresh eggs, chloroform ex.	>10	>10	–	>10	>10	>10
Tyriverdin	–	–	–	>1	–	–
Tyrindoleninone	1	1	>1	1	>1	1
6-Bromoisatin	>1	–	–	–	–	>1
Tyrian Purple	–	–	–	–	–	–

[a] Cell death was detected by incubating cultures with the extracts and then plating them onto fresh agar. These were compared to control cultures incubated with solvent only. The test microorganisms are *Staphylococcus aureus* (S.a.), *Escherichia coli* (E.c.), *Pseudomonas aeruginosa* (P.a.), *Candida albicans* (ACM4581; C.a.), *Enterococcus sericolicida* (E.s.), and *Vibrio angillarium* (V.a.). ex. = extract. >: partial activity at the maximum concentration tested (10 mg/ml for crude extract and 1 mg/ml for isolated compounds); –: no detectable activity at the maximum concentration tested. It should be noted, however, that this does not exclude the potential for some cell death because this assay will only detect a high level of cell death (ie., 90–100% cell death).

ity of the precursors tyrindoleninone (**3**) and tyriverdin (**4**) against both human and marine pathogens suggests that these compounds may be produced by *D. orbita* to keep the developing embryos free from infection. The sterilization of brood cells has been demonstrated for terrestrial invertebrates, such as honeybees (Blum et al., 1959), and a number of marine mollusks are known to protect their eggs with natural products (e.g., Paul and Van Alstyne, 1988; Pawlik et al., 1988; Paul and Pennings, 1991). This is the first study to provide evidence of a natural function for the precursor compounds of Tyrian purple. It is not presently known how these compounds get to the egg mass from the hypobranchial gland of the adult, where the ultimate precursor tyrindoxyl sulfate (**1**) and the sulfatase enzyme are produced (refer to Baker and Sutherland, 1968).

The pattern of antimicrobial activity exhibited by the brominated compounds isolated from the egg masses of *D. orbita* corresponds well to their presence during the different stages of egg development. Tyrindoleninone (**3**) is the major constituent present in the fresh eggs of *D. orbita* and has the greatest toxic effect on bacterial cells. During egg development, tyrindoleninone (**3**) is converted to tyriverdin (**4**), which has stronger antimicrobial activity but is not as toxic to microbial cells. By the time the *Dicathais* embryos hatch, the egg capsules have turned purple (authors' personal observation). This indicates that most of the tyriverdin present has been converted into Tyrian purple (**5**), which does not appear to have significant antimicrobial activity. The loss of antimicrobial activity during development of *D. orbita* eggs may protect the embryos

from autotoxicity. Many plants produce toxic compounds in immature fruits that ripen as the seeds develop (Orians and Janzen, 1974). Similar mechanisms of protecting embryos in a ripening toxic matrix have not been described in animals previously. However, the loss of antimicrobial activity with larval development has been observed in the egg mass of the sea hare *Aplysia juliana* (Kamiya et al., 1988).

A number of muricid mollusks produce Tyrian purple (Baker, 1974), but the egg masses of only one species, *Nucella lapillus*, have been tested previously for antimicrobial properties. The intracapsular fluid of *N. lapillus* did not possess antimicrobial activity against 13 strains of bacteria (Pechenik et al., 1984). However, the hypobranchial secretions of *N. lapillus* contain precursors of Tyrian purple (Clark and Cooksey, 1997), and its egg capsules turn purple in sunlight (authors' personal observation). This suggests that, like *D. orbita*, they too contain Tyrian purple and its precursors. Consequently, the egg masses of *N. lapillus* would be expected to exhibit some antimicrobial activity, unless they were tested at a late stage of embryo development in which toxicity may be lost, as demonstrated for the hatching eggs of *D. orbita*. The lack of observed antimicrobial activity in egg masses of *N. lapillus* also could be explained by a different methodology. Pechenik et al. (1984) tested the eggs of *N. lapillus* for antimicrobial activity by the traditional disk diffusion assay, and it is possible that the potential antibacterials did not diffuse adequately into the agar.

The pharmacological potential of tyriverdin and related compounds is worthy of further research. The minimum inhibitory concentration found for tyriverdin in this study was lower than that reported for penicillin G in the same antimicrobial assay (Chand et al., 1995). The fact that tyriverdin appears to be bacteriostatic rather than toxic suggests to us that it may not be cytotoxic. The relatively simple structure of tyriverdin also means that both it and its analogs can be synthesized for future research. Methods for the synthesis of Tyrian purple and tyriverdin have been described (Christophersen et al., 1978; Pinkney and Chalmers, 1979; Voss and Gerlach, 1989; Gerlach, 1991). Indeed, future research should use synthetic tyriverdin to avoid further harvesting of the important reproductive stage of *D. orbita* from the field.

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