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CHEMICAL DEFENSE OF A DORID NUDIBRANCH, *Glossodoris quadricolor*, FROM THE RED SEA

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Abstract—The nudibranch, *Glossodoris quadricolor* (Doridacea) feeds on the red sponge *Latrunculia magnifica*, which grows in the reefs of the Gulf of Aqaba, Red Sea. The ichthyotoxic substance from the sponge, latrunculin B, was also indentified in the mucous secretion of the mollusk by TLC, indicating the use of this substance as defense allomone.

Key Words—Doridacea, chemical defense, nudibranch, sponge, diet-derived toxin, ichthyotoxin, latrunculin B, *Glossodoris quadricolor*, *Latrunculia magnifica*.

INTRODUCTION

Nudibranch mollusks lacking a protective shell have developed special defensive mechanisms to escape potential predators. This includes the secretion of strong acids (Thompson, 1960) or other noxious substances that may act as feeding inhibitors or toxic agents towards fishes or other marine animals. A number of studies have demonstrated that nudibranchs use metabolites as defense allomones, which they obtain from their diet, mostly sponges (for reviews see Schulte and Scheuer, 1982; Faulkner and Ghiselin, 1983). *Latrunculia magnifica* is a red-colored branching sponge that grows exposed on the reefs of the Gulf of Aqaba (northern Red Sea). Neeman et al. (1975) had observed that, when squeezed, the sponge exudes a reddish fluid which causes escape reactions in fishes. Toxins named latrunculin A and B were isolated from sponge extracts and structurally characterized as 2-thiazolidinone macrolides, which exhibit marked ichthyotoxic properties (Kashman et al., 1980, 1982) (Figure 1).

The small dorid nudibranch *Glossodoris quadricolor* is found exclusively on the branches of this sponge. With its bright colors of yellow, white, and dark

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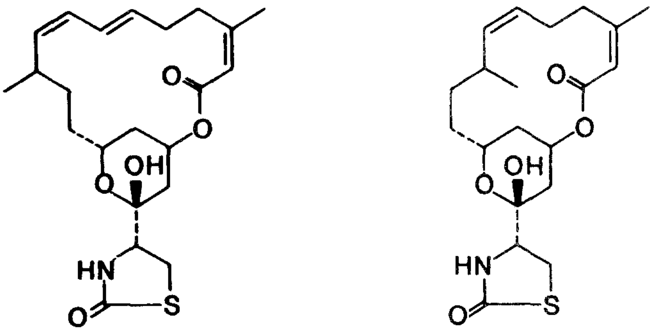

FIG. 1. Structures of latrunculins.

blue stripes contrasting with the sponge's red surface, the sea slug is easily seen from a distance of several meters. However, during numerous underwater studies, it was never observed that the nudibranch was attacked by a fish.

This paper describes the analysis of the mucous secretion of the nudibranch, confirming its defensive properties.

METHODS AND MATERIALS

Glossodoris quadricolor and sponge samples of *Latrunculia magnifica* were collected in February 1983 and 1984 in the fringing reefs of the Gulf of Aqaba along the coast of Jordan at a depth of 5–30 m. Samples of five nudibranchs were placed in a small flask containing 5 ml distilled water and slightly agitated; the mucous secretion was decanted and frozen. Nudibranch and sponge samples were kept at −20°C.

Nudibranch and sponge samples were extracted with hexane for 12 hr. and the extracts were evaporated to dryness in a water bath (60°C). These and the aqueous mucous extracts were directly applied onto a thin-layer plate (10 × 10 cm, HPTLC plate, Merck, Darmstadt). Chromatography was performed using the solvent system of benzene–ethylacetate (1:1) according to Neeman et al. (1975). Latrunculin B, kindly provided by Dr. Y. Kashman, Tel-Aviv University, Israel, was used as reference substance. Visualization of the fractions was achieved by spraying the plates with a 0.5% aqueous solution of 2′,7′-dichlorofluorescein or by UV light detection (254 nm). The fractions were marked, scratched from the plate, and eluted from the silica using EtOH.

Ichthyotoxicity was tested using killifish (*Poecilia reticulata*) of uniform size (1.5 cm) placed in beakers containing 10 ml tap water. Various concentrations of aqueous or ethanolic extracts were added; the observation time was 4 hr. Dead fish were removed and replaced by other fish to confirm the results obtained.

RESULTS AND DISCUSSION

Dissection of the nudibranch *Glossodoris quadricolor* revealed that their pharynx and stomach contain small pieces (up to 1.5 mm diameter) of the red sponge *Latrunculia magnifica*. This confirms the assumption that the mollusk feeds on the sponge. Latrunculin A and B have been isolated from different sponge samples by Kashman et al. (1980). By thin-layer chromatography, only one major and a closely associated minor fraction were detected in aqueous extracts of the nudibranch's mucous secretion; they were identical in R_f value as well as chromatographic separation to the pure latrunculin B reference substance. This component was also present in sponge as well as whole nudibranch extracts, but both extracts contained additional constituents of red, yellow, and blue color, probably the pigments of the sponge (Figure 2).

The pure latrunculin B exhibited marked ichthyotoxicity at a concentration of about 1 mg/liter. The test fish were killed within minutes, confirming earlier observations of Neeman et al. (1975), who determined the LD_{50} of 0.4 mg/liter

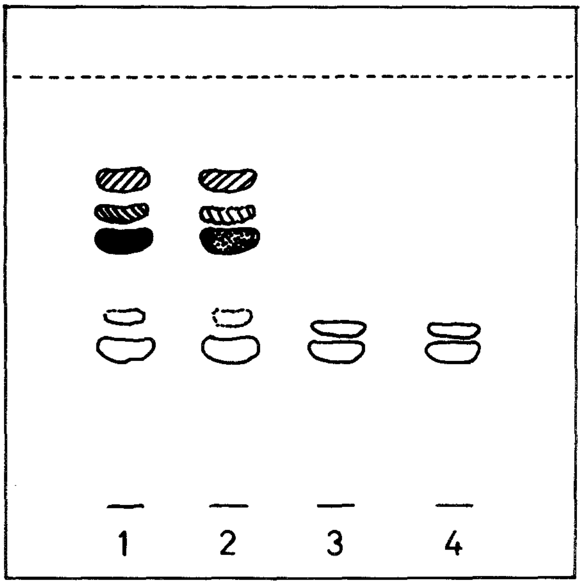

FIG. 2. Thin-layer chromatography of sponge extract (1), nudibranch extract (2), the mucous secretion of the nudibranch (3), and latrunculine B (4). Solvent system: benzene–ethylacetate 1:1, detection UV.

for the toxin fraction. The fishes show excitation, lose their balance in the water, and die rapidly. Extracts from 0.1 g sponge or one nudibranch dissolved in 0.5 ml EtOH were toxic at greater than 10,000-fold dilutions. Eluates of the fractions separated by TLC produced toxic effects to the test fish only when fractions corresponding to latrunculin B were applied. Quantitation of these results was not achieved; however, even dilutions of the eluates between 1:1000 and 1:5000 of the mucus of one nudibranch still produced toxic symptoms.

The results of TLC indicate that the active components of the sponge, the latrunculins, are sequestered by the mollusk in the mucous secretion and are still in an active form as far as toxicity to fish is concerned. Furthermore, comparison by TLC of the sponge extracts and latrunculin B with the substance in the mucus also suggests that latrunculin B is not chemically altered or metabolized by the nudibranch.

It is well known that among marine mollusks, members of the suborder Doridacea feed mainly on sponges and store metabolites from their diet in the dorsum (Schulte and Scheuer, 1982; Faulkner and Ghiselin, 1983). These noxious components provide the protection against predators that they certainly need since they are slow moving and noncryptic animals. The bright color of many reef nudibranchs may also provide a signal to potential predators that may have learned to recognize this particularly unpalatable prey.

This chemical interdependence of a Red Sea nudibranch and a sponge is another example of a prey-predator interrelationship and of the peculiar defense mechanism of this mollusk group.

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