MULTIPLE FUNCTIONS FOR SECONDARY METABOLITES IN ENCRUSTING MARINE INVERTEBRATES

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Abstract—We used three chemical fractions (spanning a wide range of polarities) from the extracts of four marine invertebrates, the sponges Crambe crambe and Hemimycale columella and the ascidians Cystodytes dellechiajei and Polysoyocrateron lacazei, to test inhibition of cell division, photosynthesis, and settlement. We used assay organisms from the same habitat, seeking to determine whether a species may display diverse, ecologically relevant bioactivities and, if so, whether the same types of compound may be responsible for such activities. Cell division was strongly inhibited by the sponge C. crambe. A dichloromethane fraction from C. crambe prevented development of sea urchin Paracentrotus lividus eggs at a concentration of 10 μg/ml, as did the butanolic fraction, but at higher concentrations (50 and 100 μg/ml). At 50 μg/ml, the aqueous fraction of C. crambe allowed cell division but prevented eggs from developing beyond the gastrula stage. Similar results were recorded with the dichloromethane fraction of P. lacazei and from the aqueous fraction of H. columella. Photosynthesis was unaffected by any of the species at 50 μg/ml. Larval settlement was inhibited by one or another fraction from the four species surveyed at a concentration of 50 μg/ml, although C. crambe exhibited the greatest amount of activity. We therefore found that various fractions displayed the same type of bioactivity, while compounds from the same fraction were responsible for multiple activities, suggesting that secondary metabolites are multiple-purpose tools in nature, which is relevant to our understanding of species ecology and evolution. Moreover, results showed that the assessment of the role of chemical compounds is significantly influenced by the assay organism, fractionation procedure, concentration, and

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duration of experiments. All these factors should be carefully considered when testing ecological hypotheses of the roles of chemically-mediated bioactivities.

**Key Words**—Secondary metabolites, chemical defense, evolution, ascidians, sponges.

**INTRODUCTION**

The field of natural products research has uncovered a vast source of new compounds in marine organisms (Faulkner, 1996, and previous reviews by the same author), many of which show antimitotic, cytotoxic, antibacterial, and antiviral properties that may play important ecological roles (Bhakuni and Jain, 1990). Despite the number of compounds reported, comparatively few of them have been properly tested for an ecological role, and so the biological significance of most secondary metabolites remains largely unknown. Indeed, the bioactivity of a metabolite has frequently been described much later than its chemical characteristics. For instance, avarol, a sesquiterpenoid hydroquinone, was first isolated from the sponge *Dysidea avara* (Schmidt) in 1974 (Minale et al., 1974), but it was not until several years later that avarol was reported to produce aberrations in the development of sea urchins (Cariello et al., 1980) and to display antibacterial, antifungal, and antiviral activities (Seibert et al., 1985; Uriz et al., 1992). Additional ecological functions of avarol have been suggested (Martin and Uriz, 1993; Uriz et al., 1992a), although only the antipredatory role has been experimentally tested (Uriz et al., 1996a). Similar stories can be told for a variety of bioactive marine natural products.

The importance, however, of predator-prey interactions in the production of chemical defenses has been highlighted both in terrestrial and benthic organisms. Chemical defenses may have evolved to avoid predation (Rosenthal and Berenbaum, 1992), resulting, in many instances, in a chemically mediated predator-prey coevolution with ecological advantages for both parties (Paul et al., 1990). However, the importance of antipredation in the evolution of chemical defenses in marine environments has been, in our opinion, overemphasized, perhaps because many predictions stem from works on chemical adaptations to herbivory in terrestrial plants. The idea that the antipredatory function is the sole or the most important selective process in the evolution of chemical defenses underlies most of the literature on seaweeds (Hay and Steinberg, 1992; Steinberg and van Altena, 1992; Yates and Peckol, 1993; Hay et al., 1994, to cite some recent examples) and also benthic animals (Van Alstyne et al., 1994; Pawlik et al., 1987, 1995; Paul et al., 1990; Uriz et al., 1996a). Historically, comparatively less attention has been given to other possible roles of chemical substances, such as competition for space or antifouling mechanisms, although more recently they have been the subject of a growing number of studies, especially