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SOME CHEMICAL ASPECTS OF SPAWNING IN ALCYONACEAN CORALS

QUELQUES ASPECTS CHIMIQUES DE LA PONTE CHEZ LES ALCYONAIRES

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ABSTRACT

The recent discovery by a group of Townsville biologists of the synchronised annual mass release of gametes from many scleratinian corals (Harrison et al., 1984) prompted chemical investigation of the corresponding phenomenon in alcyonacean corals.

Spawned eggs were collected from a number of alcyonacean species of the genera Sarcophyton, Lobophytum, and Sinularia (family Alcyoniidae) and their chemical composition investigated. This was compared with the chemical composition of the releasing colonies before (+ eggs) and after (- eggs) spawning. Some significant differences were detected.

Specifically, eggs released from Lobophytum compactum Tixier-Durivault, 1956, were shown to contain significant quantities of the diterpene thunbergol. This compound was barely detectible in the soft coral extract before spawning, and was virutally absent in the extract after spawning. By contrast, the major diterpenoid metabolite (50% of the organic extract) in the soft coral Lobophytum crassum, von Marenzeller, 1886, was 13-hydroxylobolide. This compound was undetectible in the eggs from the same colony.

These findings show that during the preparation of eggs for release, diterpenes are selectively incorporated into the egg-lipid material. In some cases concentrated several hundredfold, in other cases selectively excluded from the eggs.

Attempts to assess the ichytoxicity or antifeedant properties of the eggs were thwarted by the large numbers of fish devouring the eggs the moment they were released.

Ecological implications of these findings are discussed.

RESUME

La découverte récente par un groupe de biologistes de Townsville d'une émission synchrone de gamètes par de nombreuses espèces de Scléractiniaires (Harrison et al., 1984) a poussé à l'examen des aspects chimiques d'un phénomène analogue chez les Alcyonaires.

Les oeufs d'un certain nombre d'espèces d'Alcyonaires des genres Sarcophyton, Lobophytum et Sinularia (famille des Alcyoniidae) ont été récoltés, et leur composition chimique a été analysee. Les résultats ont été comparés avec la composition chimique des colonies reproductrices avant (avec oeufs), et après (sans oeufs) la ponte. Des différences significatives ont été détectées.

En particulier, les oeufs pondus par Lobophytum compactum Tixier-Durivault, 1956, contenaient des quantités notables de "thunbergol diterpene". Ce composé était à peine décelable dans les extraits d'Alcyonaires avant la ponte, et virtuellement absent dans les extraits après la ponte. A l'opposé, le principal métabolite diterpenoide (50% de l'extrait organique) de l'espèce L. crassum von Marenzeller, 1886, était le 13-hydroxylobolide. Ce composé n'était pas décelable dans les oeufs de la même colonie.

Ces résultats montrent que pendant la mâturation, avant la ponte, des diterpènes sont incorporés sélectivement dans le matériel lipidique des oeufs. Dans certains cas, ils sont concentrés plusieurs centaines de fois; dans d'autres cas, ils sont exclus des oeufs de façon sélective.

Des essais, en vue d'évaluer l'ichtyotoxicité ou les propriétés répulsives des oeufs ont été contrariés par le nombre important de poissons dévorant les oeufs au moment de la ponte.

Les conséquences écologiques de ces résultats sont discutées.

INTRODUCTION

Following the pioneering work of Leon Ciereszko (1960) on the presence of terpenes in gorgonian corals and the isolation of large quantities of prostaglandins in the gorgonian Plexaura homomalla by Weinheimer and Spraggins (1969), the chemical composition of alcyonarian soft corals (Coelenterata, Anthozoa) has attracted much attention. Several reviews illustrate the range and variety of the terpenoid chemistry derived from these colonial organisms (Tursch et al., 1978; Coll et al., 1980). One factor in this story which has been brought to light only after long term careful study (Kashman and Groweiss, 1980) is that the chemical composition of some soft corals varies throughout the year. This raises the possibility that terpenoid compounds may play a role in the annual life cycle of soft corals. Toxic terpenoid components have been implicated in soft coral defence (Bakus, 1981; Coll et al., 1982; Coll and Sammarco, 1983). More recently they have been shown to play an allelopathic role in competitive interspecific interactions between alcyonacean and scleractinian corals (Sammarco et al., 1983; Coll and Sammarco, 1983). Terpenoid compounds thus appear to play a multifunctional role in these invertebrates.

Detailed studies of the reproductive biology of a number of scleractinian corals by marine biologists at James Cook University culminated in the observations of synchronous mass spawning of many of these hard corals in 1981 and 1982 (Harrison et al., 1984). Preliminary investigations of several alcyonacean soft corals (R. Babcock, pers. comm.) revealed that they may follow a similar pattern of synchronous mass spawning at approximately the same time. We were subsequently able to collect eggs from a number of alcyonacean soft corals in October and November 1983 and to investigate the chemical composition of the eggs in relation to that of the intact colony.

In this paper we describe the collection and chemical analysis of the eggs released from soft corals of the genus Lobophytum and compare the range of metabolites found in the eggs with those present in the releasing colony. No novel terpenoid metabolites were isolated from the colonies under investigation.

MATERIALS AND METHODS

Preliminary methodology for egg collection was developed and tested at Magnetic Island (146° 51' E; 19° 09' S) during the predicted mass spawning in October 1983. In this way the optimum net design and collection procedures were established (Figure 1). These procedures were then applied at Orpheus Island Research Station at Pioneer Bay, Orpheus Island (146° 18' E; 19° 09' S), where Lobophytum compactum was selected for study because it was determined to be dioecious.

Female colonies of the soft coral Lobophytum compactum Tixier-Durivault 1956 were identified by tearing the tissue near a lobe (Figure 1(a)) to reveal the presence of purple eggs. Colonies so determined were tagged, and photographed, and a sample of approximately 500 g wet weight collected. At about 1600 hours on the day predicted for spawning (24-25/11/83), muslin nets approximately conical in shape (0.5 m radius; 1 m height) were placed over the corals. The nets were similar to a plankton net with small polystyrene floats for buoyancy and a light weight plastic jar as cod end. The jar, which was buoyed in place with a little air, was attached by its lid, the top of which was removed leaving only the threaded outer rim. This was screwed over the net and onto the outside of the jar. In this way there were no obstacles to trap the eggs, and they could rise as a consequence of their positive buoyancy into the jar. The base of the net was formed on a wire loop to which lead weights were attached (Figure 1(b)).

The spawning was observed at dusk (1730-1830 hours) and the jars and their contents collected at 2300 hours.

Similar sampling was carried out on the reef flat in front of the Lizard Island Research Station (145° 28' E; 14° 40' S) on the same nights (24-25/11/83). Colonies of Lobophytum crassum von Marenzellar 1886, which is dioecious, were examined in this case.

The coral samples and eggs collected at the two sites were frozen and freeze-dried prior to chemical analysis. After spawning was complete, samples of the soft corals were collected for identification and stored in 70% ethanol. A further small sample was collected for chemical reference.

Terpenoid components were extracted with dichloromethane and isolated by chromatography on silica gel (see Ahond et al., 1979). Comparative and quantitative studies utilised a Waters 6000A high performance liquid chromatograph (HPLC) on silica gel with ethyl acetate:hexane mixtures as eluant and a Waters R401 refractive index detector. Quantification of peak areas was achieved by use of a Hewlett Packard 3390A integrating recorder. Structural assignments were made by comparison of ^{1}H and ^{13}C n.m.r. data with those of reported compounds. The assignments are unambiguous.

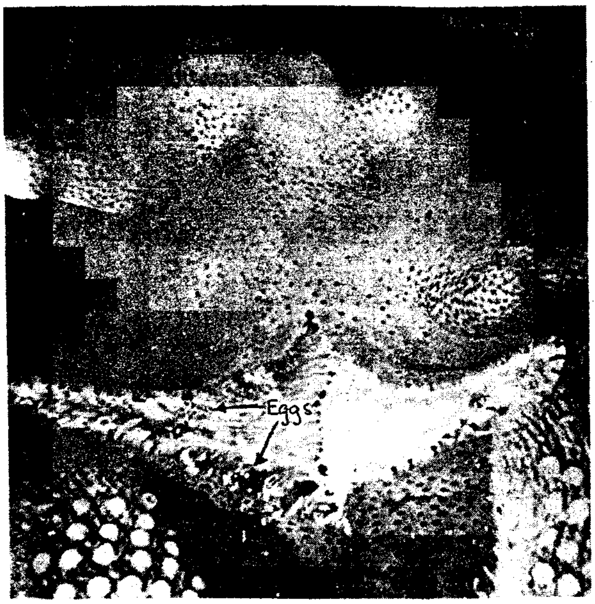
RESULTS

The eggs of both Lobophytum species were coloured purple and were buoyant, being composed of about 50% lipid, mostly wax ester (Benson, 1975). Quantitative and comparative data on the chemical composition of the tissue and spawned eggs of Lobophytum compactum appear in Table 1. The organic extracts were relatively simple, and amenable to quantification by HPLC (Fig. 2).

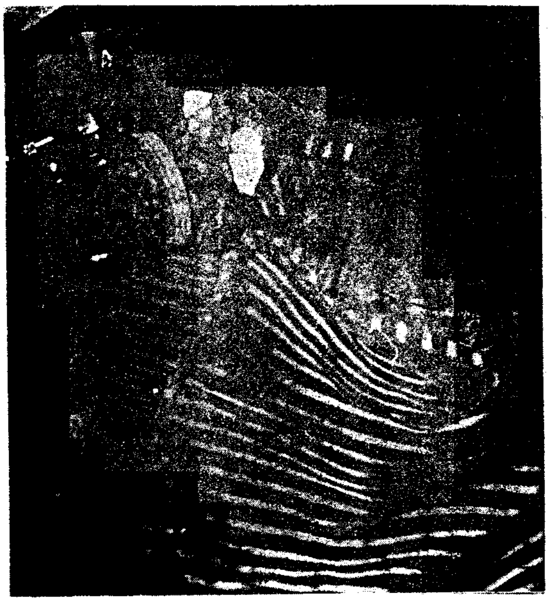
By contrast, the organic extract of Lobophytum crassum was extremely complex and could not be resolved or quantified by HPLC. Extensive chromatographic separations were carried out on the coral extract to identify the major terpenoid components, and qualitative evaluation of the presence (+) or absence (-) of these components in the eggs was carried out. The results appear in Table 2.

Significant differences were observed between the eggs and tissue in each case. Lobophytum compactum contained significantly more (10-100X) of the diterpene thunbergol than was present in the coral tissue (Figure 2). L. crassum eggs contained none of the diterpene 13-hydroxylobolide present at very high levels in the tissue of the soft coral (50% of the organic extract).

Fish were observed to feed on some eggs with apparent relish (Fig. 1(d)). There was no evi-



(a)



(b)

Figure 1.

Collection and analysis of soft coral eggs.

(a) Colony of *Lobophytum compactum*. Eggs are visible (→) in polypal canals.

(b) Net and jar covering colony of *L. compactum*.

(c) Eggs in collection jar - buoyant and purple in colour.

(d) Fish feeding on eggs released by alcyonacean coral (*Sinularia sp.*).

(c)

(d)

Table 1: *Lobophytum compactum*: Composition of total DCM extract (% dry weight)

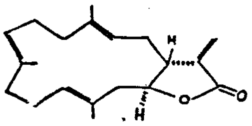
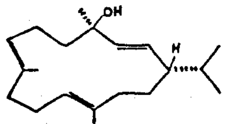
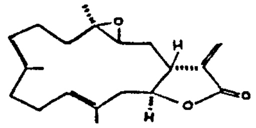
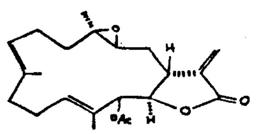
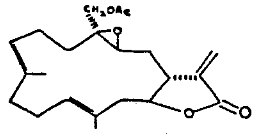
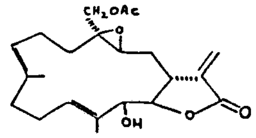
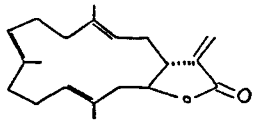
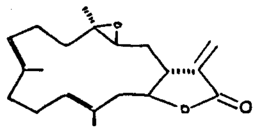
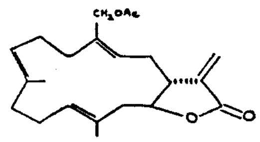
	Component	Coral	Eggs	Reference
1.	"Wax Ester"	6%	53%	Benson et al., 1975
2.		1%	0.2%	Ahond et al., 1979
3.	 thunbergol	~0%	1.3%	Bowden et al., 1981
4.		8-9%	5.4%	Ahond et al., 1979
5.	"Sterol"	<1%	0.5%	
6.		<3%	~0%	Ahond et al., 1979
	TOTAL	15-20%	~61%	

Table 2. *Lobophytum crassum*: Terpenoid composition of DCM extract (major components only) (% dry weight).

	Coral	Eggs	Reference
Total extract	1.1%	65%	
 lobolide	0.05	+	Kashman et al., 1982
 13-hydroxy-lobolide	0.5	-	Kashman et al., 1982
	0.04	+	Ahond et al., 1979
	0.004	+	Ahond et al., 1979
	0.08	+	Kashman et al., 1982

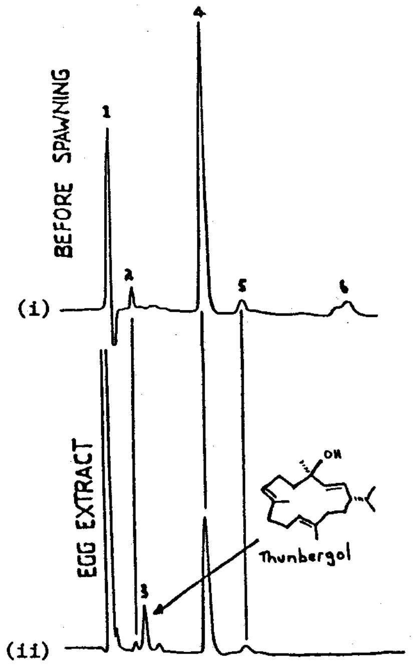


Figure 2.
HPLC analysis of (i) organic extract of L. compactum before spawning and (ii) organic extract of the eggs released from L. compactum (numbers refer to compounds shown in Table 1).

dence of feeding deterrancy or ichthyotoxicity either immediate or after many hours.

DISCUSSION

The results show significant differences between the chemical composition of the tissue of a soft coral colony and of the eggs spawned by that colony. Clearly some terpenes are selectively stored (or synthesised) in the eggs of alcyonaceans while others are selected against inclusion (or synthesis) by the eggs. As a result of our earlier studies (Coll et al., 1982) of the defensive role of terpenes in soft corals, we assumed that the presence of terpenes in the eggs of soft corals might have a defensive role. In view of our observations, they do not always seem effective. At the time of egg release, it was possible to identify spawning colonies by the presence of clouds of fish consuming large numbers of the eggs with no visible ill effects, and no rejection of the eggs as a food source (Fig. 1(d)). Nevertheless, it is difficult to abandon the thesis that the compartmentalization of terpenoid metabolites between the soft coral tissue and the eggs has ecological significance.

Several alternate hypotheses may be imagined to account for the presence of terpenoid metabolites in the eggs of soft corals.

It is possible that certain compounds may act in a regulatory capacity. Alternatively the levels of some compounds present in the eggs may be used as chemotactic agents to attract sperm to the eggs.

Despite the problem of being able to collect samples during only a brief interval each year, further studies of the chemical relationship between corals and their spawned eggs and of the reasons for the observed differences are under investigation. It is hoped that further insights into the role of terpenoid compounds in soft coral metabolism will be established.

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