by continuous light. Plants were transferred daily into fresh medium (*4*); they showed synchronization of egg discharge in 14-day intervals (*2, 3*). On days of maximal egg discharge, the harvests contained 10^5 to 10^6 eggs.

Daily harvests of eggs were suspended with 2 liters of culture medium in an extraction flask. The extraction procedure was a closed-loop technique (*5*). Volatile compounds stripped from the egg suspension were absorbed on a bed of 1.5 mg of activated carbon. After desorption with 30 μl of dichloromethane, the extracts were subjected to two-dimensional glass capillary gas chromatography. Eluates from egg suspensions of *Dictyota dichotoma* revealed one major compound and trace quantities of several others. An average of 0.5 pg of volatile substance per egg was produced in 1 hour.

The retention data (Table 1) indicate that the main fraction secreted by *Dictyota* eggs has a molecular size and polarity characteristics very similar to those of ectocarpen. Mass spectrometry confirms this finding by showing M to be 150 and by revealing a fragmentation pattern similar to that of ectocarpen. Thus, the *Dictyota* attractant may be a hydrogenated derivative of ectocarpen. Two-dimensional glass capillary gas chromatography and injection with a synthetic sample confirmed that the main product secreted by eggs of *Dictyota dichotoma* is *n*-butyl-cyclohepta-2,5-diene (Table 1 and Fig. 2b). This compound is a minor constituent of the essential oil from *Dictyopteris* and is characterized as dictyopterene C′ (*6*). Although *Dictyota* and *Dictyopteris* belong to the same family, Dictyotaceae, the compound is found in vegetative parts of *Dictyopteris*, and no connection with fertilization is apparent.

Because fertile male gametophytes of *Dictyota* were not available in cultures, we collected six male gametophytes in the intertidal zone of Aran Island (Ireland) in July 1980 and subjected them to a regime of 14 hours of light and 10 hours of darkness in the laboratory. At the onset of light on the 6th day, spermatozoids were discharged. For the bioassay, 0.1-μl droplets of fluorocarbon solvent FC-78 (*7*) were placed at the bottom of a polystyrene petri dish with culture medium, and spermatozoids were added. Droplets containing synthetic *n*-butyl-cyclohepta-2,5-diene caused massive attraction of *Dictyota* spermatozoids (Fig. 1), whereas droplets of pure solvent were ineffective in attracting spermatozoids.

The response of spermatozoids confirms that *n*-butyl-cyclohepta-2,5-diene is the sex attractant secreted by eggs of *Dictyota dichotoma*. The chemical identity of sex attractants has now been established for four genera of marine Phaeophyceae. Three genera from different orders in the subclass Phaeophycidae synthesize monocyclic unconjugated C_{11} olefines. Ectocarpen is found in two species of *Ectocarpus* (*8*) (Fig. 2a), multifiden in *Cutleria* (*9*) (Fig. 2c), and *n*-butyl-cyclohepta-2,5-diene in *Dictyota*. In the subclass Cyclosporidae, which is considered to be the most isolated branch of the Phaeophyceae (*10*), a conjugated C_8 alkene is found in two species of *Fucus* (*11*) (Fig. 2d). Thus, sexual attractant compounds seem to reflect phylogenetic relations in marine brown algae.

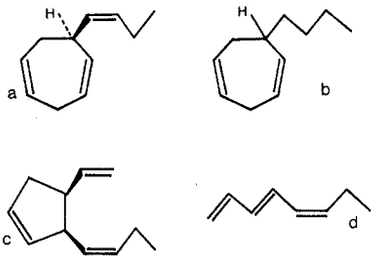

Fig. 2. Chemical structure of sex attractants in brown algae: (a) ectocarpen, (b) *Dictyota* sperm attractant (absolute configuration not determined), (c) multifiden, and (d) fucoserraten.

Note added in proof: Optical rotation established the absolute configuration of the *Dictyota* attractant as (−)-(R)-6-butylcyclohepta-1,4-diene. It is identical with dictyopterene C′ (*12*).

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Contractility of Bile Canaliculi: Implications for Liver Function

Abstract. *Dynamic contractions of bile canaliculi were observed in groups of cultured hepatocytes by time-lapse cinephotomicrography during the early stages of monolayer formation. The contractions, which were forceful and appeared to have a pumping action, may facilitate the flow of bile in the liver's canalicular system.*

Actin filaments have been found in diverse types of nonmuscle cells (*1, 2*). Their function varies with the nature of the cells (*3, 4*). In the liver there is an enhanced investment of microfilaments in the cytoplasm of cells lining the bile canaliculi (*5*). It has been speculated that these pericanalicular filaments provide support and serve a contractile function facilitating bile flow (*5*).

The presence of actin in the filaments has been demonstrated by immunohistochemical (*6–9*), biochemical (*10, 11*), and electron microscopic (*5, 12*) studies; by ultrastructural cytochemistry with heavy meromyosin (*13, 14*); and by studies in which cytochalasin B (*15*) and phalloidin (*7, 16*) were used. Myosin has also been identified (*17*). These techniques cannot, however, be used to ascertain whether bile canaliculi contract. Time-lapse cinephotomicrography bridges the gap between cell morphology and biochemistry. Using this method, we demonstrated forceful contraction of bile canaliculi in groups of freshly isolated rat hepatocytes in the early stages of monolayer formation.

The livers of adult male Wistar rats (170 to 230 g) were exposed and perfused with collagenase to obtain isolated hepatocytes (*18, 19*). The proportion of living hepatocytes obtained, as determined by trypan blue exclusion, was 85 to 95 percent. The cells were inoculated into 60 by 15 mm Falcon dishes containing L 15 culture medium supplemented with 10 percent fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). The cells were allowed to attach for

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2 to 3 hours and then examined under an inverted microscope (Zeiss ICM 405) equipped with a 16-mm movie camera (Bolex H16 RX-5) and a motor drive system with time-lapse controller (Hommel Electronics). All the experiments were carried out at 37°C. Movies were taken at a speed of one frame every 15 seconds or one frame every 30 seconds and examined with an analytic movie projector (L-W International).

Although the isolation method that we used is designed to yield single hepatocytes, a number of incompletely separated liver cell groups are always present. It was these that were selected for study. Normally, liver cells are bound together by components of their junctional complexes, especially the zonulae occludens that border bile canaliculi; these junctions must be broken to isolate cells. By selecting incompletely separated liver cell groups, we were able to examine canaliculi that remained intact between adjoining cells (Fig. 1). The structural integrity of the cells and canaliculi was confirmed through the use of an electron microscope (Philips 400) and standard techniques (*20, 21*).

At standard projection speed (24 frames per second), very active cytoplasmic movements were observed. Most appeared random, but a striking finding was the forceful contraction of the canaliculi. These contractions were associated with vacuolar movements, especially in the pericanalicular region, but appeared independent of other movements in the cytoplasm. Each contraction was rapid and resulted in the closure of open canaliculi. Relaxation of the canaliculi, on the other hand, was gradual and appeared passive. The contractions occurred either randomly, separated by long intervals, or regularly in rapid bursts. During 33 hours of continuous observation, individual canaliculi contracted 13 to 26 times. The forceful contractions expelled material from the lumen into the surrounding medium through what appeared to be the intercellular spaces but which could have been the open ends of interrupted canaliculi. This phenomenon was best seen during the later stages of observation (hours 16 to 33).

Isolated hepatocytes are capable of both synthesizing (*22*) and secreting bile acids (*23*). Canalicular contractions may facilitate the flow of this bile toward the ducts by producing contraction waves (peristalsis) (*24*). The flow may also be assisted by pressure generated by the forceful contractions.

Osmotic force generated by the active secretion of bile acids and inorganic ions has heretofore been considered the most important mechanism underlying bile flow in the canalicular system (*25–29*). Recently, interest has centered on a paracellular pathway (*30–32*). The results of our study implicate dynamic canalicular contractions mediated by actin filaments as yet another mechanism regulating the flow of canalicular bile.

Still to be determined are the means by which the contractions are controlled. Another important question is whether centrilobular canaliculi contract more forcibly than those periportally and whether the contractions provide a pressure gradient for bile flow within the liver lobule.

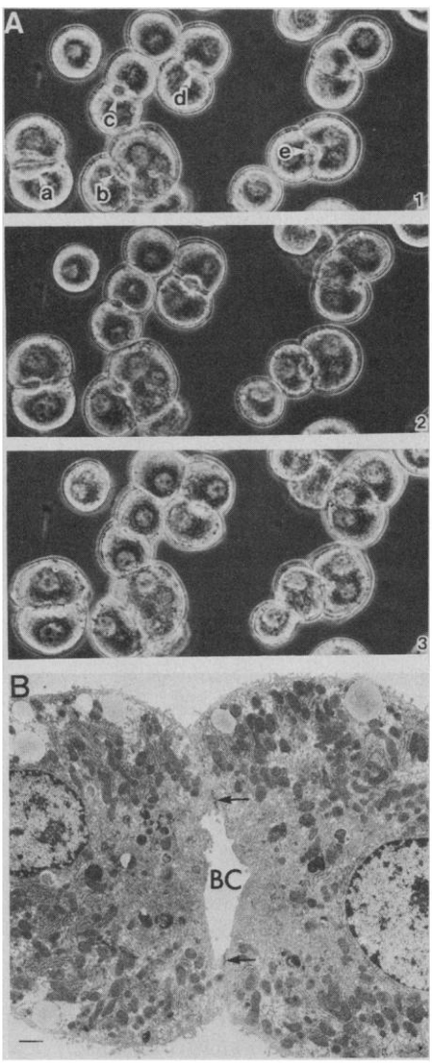

Fig. 1. (A) Phase-contrast cinephotomicrographs of freshly isolated groups of hepatocytes in the early stages of monolayer formation. Five bile canaliculi are labeled. In frame 1 all are open or partially open; in frame 2, *a*, *b*, and *c* are closing and *d* and *e* are opening wider; and in frame 3, *a*, *b*, and *c* are fully closed and *d* and *e* are closing. In the movie, the contractions are forceful (×600). (B) Transmission electron micrograph of two freshly isolated nondisrupted hepatocytes in the early stages of monolayer formation. Note the bile canaliculus (*BC*) with intact junctions (arrows) between the cells. This canaliculus is partly closed. Scale bar, 1 μm; stain, uranyl acetate and lead citrate.

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