adhesion and allows for the intimate association of the adhesive and the substrate (*19*). Onto this is applied a mixture of polyphenolic protein and collagen, with the former presumably acting as the adhesive, and the latter as a fibrous filler giving cohesive strength to the glue. Finally, the enzyme polyphenoloxidase is added to catalyze the quinone tanning of the adhesive, thus conferring permanence to the bond (*18*).

J. HERBERT WAITE
MARVIN L. TANZER
Department of Biochemistry, University of Connecticut Health Center, Farmington 06032

References and Notes

1. C. M. Yonge, *J. Mar. Biol. Assoc. U.K.* **42**, 112 (1962).
2. A. Tamarin and P. J. Keller, *J. Ultrastruct. Res.* **40**, 401 (1972); J. P. Pujol, *Bull. Soc. Linn. Normandie* **8**, 308 (1967); J. Pikkarainen *et al.*, *Eur. J. Biochem.* **4**, 555 (1968); A. Bdolah and P. J. Keller, *Comp. Biochem. Physiol. B* **55**, 171 (1976); J. P. Pujol, M. Rolland, S. Lasry, S. Vinet, *Comp. Biochem. Physiol.* **34**, 193 (1970); A. Bairati and L. Vitellaro-Zuccarello, *Mar. Biol.* **28**, 145 (1974).
3. C. H. Brown, *Q. J. Microsc. Sci.* **93**, 487 (1952).
4. A. Tamarin, P. Lewis, J. Askey, *J. Morphol.* **148**, 199 (1976).
5. G. Gerzeli, *Pubbl. Stn. Zool. Napoli* **32**, 88 (1961).
6. J. A. Allen, M. Cook, D. J. Jackson, S. Preston, E. M. Worth, *J. Mollusc. Stud.* **42**, 279 (1976); K. Glaus, *Biol. Bull. (Woods Hole, Mass.)* **135**, 420 (1968).
7. J. H. Waite and M. L. Tanzer, *Biochem. Biophys. Res. Commun.* **96**, 1554 (1980).
8. Phenol glands (from 30 to 40 specimens of *Mytilus*) were dissected (*3*) over dry ice. Glands were ground for 20 minutes by hand with a glass tissue grinder and 15 volumes of 1*M* NaCl, 0.05*M* tris (*p*H 7.5) with 0.001*M* KCN, 0.025*M* EDTA, 0.01*M* *N*-ethylmaleimide and 0.001*M* phenylmethylsulfonylfluoride at 4°C. The homogenate was briefly centrifuged (300*g*; 10 minutes), and the pellet was extracted with five volumes of neutral salt buffer and centrifuged as before. The second pellet was homogenized in two volumes of 5 percent acetic acid (4°C) for 10 minutes and centrifuged at 20,000*g* for 1 hour. The supernatant was assayed for L-dopa (*20*) and protein (*21*) and contained from 25 to 40 μg of dopa per milligram of protein. Between a third and a fourth of the proteins extracted were dopa-containing. The supernatant was dialyzed against 3.5 percent GuCl, 5 percent acetic acid, and 0.001 percent Triton X-100 overnight at 20°C and then centrifuged 20,000*g* for 30 minutes to remove insoluble material. The supernatant was applied to a column of SP-Sephadex C-50 (lot 1995) (1 by 15 cm) and eluted with the above-mentioned dialysis buffer and a linear gradient of guanidine hydrochloride. Fractions were assayed for dopa (*20*) (500 nm), protein (280 nm), and guanidine hydrochloride concentration (conductivity). The peak fractions were pooled for application onto Phenyl Sepharose CL-4B (1.5 by 90 cm) with 7.5 percent guanidine hydrochloride, 5 percent acetic acid, and 0.001 percent Triton X-100 as elution buffer. All chromatography was conducted at room temperature. Peak fractions from the Phenyl Sepharose column were pooled, chromatographed on Sephadex G-200 (1.5 by 20 cm), then dialyzed extensively against several changes of 5 percent acetic acid. A portion of the nondialyzable fraction was freeze-dried for subsequent acid hydrolysis; the remainder was used for gel electrophoresis.
9. C. H. Brown, *Nature (London)* **165**, 275 (1950).
10. R. Davies and J. L. Frahn, *J. Chem. Soc.* **20**, 2295 (1977).
11. D. R. Eyre, *Science* **207**, 1315 (1980).
12. R. R. Porter and K. B. M. Reid, *Nature (London)* **275**, 699 (1978).
13. T. L. Rosenberry and J. M. Richardson, *Biochemistry* **16**, 3550 (1977).
14. J. H. Waite, A. S. M. Saleuddin, S. O. Andersen, *J. Comp. Physiol.* **130**, 301 (1979).
15. H. S. Mason, *J. Biol. Chem.* **172**, 83 (1948).
16. S. O. Andersen, *Comp. Biochem. Physiol.* **35**, 705 (1970).
17. D. J. W. Lane and J. A. Nott, *J. Mar. Biol. Assoc. U.K.* **55**, 477 (1975).
18. J. D. Smyth, *Q. J. Microsc. Sci.* **95**, 139 (1954).
19. G. L. Schneberger, *Adhes. Age* (January 1980), p. 42.
20. L. E. Arnow, *J. Biol. Chem.* **118**, 531 (1937).
21. E. F. Hartree, *Anal. Biochem.* **48**, 422 (1972).
22. S. Panyim and R. Chalkley, *Arch. Biochem. Biophys.* **130**, 337 (1969).
23. M. Schick, *Anal. Biochem.* **63**, 345 (1975).
24. M. L. Tanzer, *J. Biol. Chem.* **243**, 4045 (1968).
25. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rogers, F. Smith, *Anal. Chem.* **28**, 350 (1956).
26. R. J. Simpson, M. R. Neuberger, T. Y. Liu, *J. Biol. Chem.* **251**, 1936 (1976).
27. We thank the National Institutes of Dental Research for a National Research Service fellowship (2F32 DE 05158) and the National Institutes of Health (grant AM 12683) for research support. R. Trelstad provided the 3-hydroxyproline standard. T. Housley performed the amino acid analyses.

5 December 1980; revised 6 February 1981

Dictyota dichotoma (Phaeophyceae): Identification of the Sperm Attractant

Abstract. *Freshly released eggs of the marine brown alga* Dictyota dichotoma *secrete a substance that attracts spermatozoids. This compound has been identified as* n*-butyl-cyclohepta-2,5-diene. It is closely related to attractants in several other brown algae and confirms that a relation exists between phylogeny and attractant compounds.*

Various details of sexual reproduction have been studied in *Dictyota dichotoma*, a well-known species of marine brown algae found on temperate North Atlantic coasts. Spermatozoids of the algae are attracted by eggs, indicating sexual chemotaxis (*1*). Furthermore, in some localities liberation of eggs and spermatozoids has a pronounced semilunar periodicity, with mass discharge occurring on specific days of the lunar cycle (*2, 3*).

A clonal culture of female gametophytes was derived from a tetrasporophyte of *Dictyota dichotoma* (Hudson) Lamour., which was collected in November 1959 at Helgoland (German Bight). The same strain was used for studies on lunar periodicity (*3*).

Female gametophytes (about 10 g, fresh weight) were placed in glass dishes and subjected to a regime of 14 hours of light and 10 hours of darkness. Every 28th cycle, the dark phase was replaced

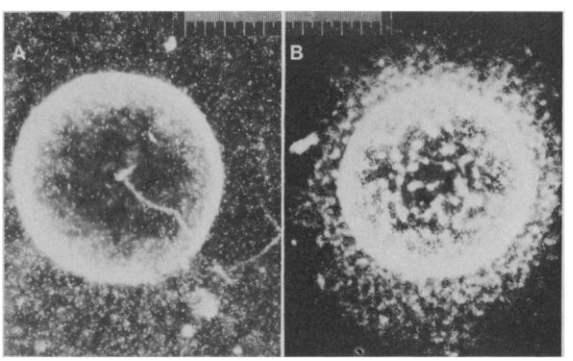

Fig. 1. Two droplets of fluorocarbon solvent FC-78 are shown 3 minutes after addition of *Dictyota dichotoma* spermatozoids in (A) blank solvent and (B) solvent containing synthetic *n*-butyl-cyclohepta-2,5-diene (1.5 mg/ml). Darkfield, microflash exposure. Total length of scale, 1 mm.

Table 1. Analytical data for *Dictyota* egg product, for ectocarpen, and for synthetic n-butyl-cyclohepta-2,5-diene.

Item	Retention index (elution temperature 80° ± 1°C)		Mass fragmentation
	OV 73	OV 61	
Dictyota egg product	1168.2 ± 0.5	1230.5 ± 0.4	150 (M^+; 12%); 135 (2%); 121 (6%); 107 (19%); 93 (87%); 91 (85%); 79 (100%)
Ectocarpen	1151.6 ± 0.4	1224.2 ± 0.2	148 (M^+; 12%); 133 (18%); 119 (15%); 105 (25%); 91 (89%); 79 (100%)
n-Butyl-cyclohepta-2,5-diene	1168.0 ± 0.6	1230.7 ± 0.3	150 (M^+; 10%); 135 (2%); 121 (4%); 107 (13%); 93 (57%); 91 (56%); 79 (100%)

0036-8075/81/0529-1040$00.50/0

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.

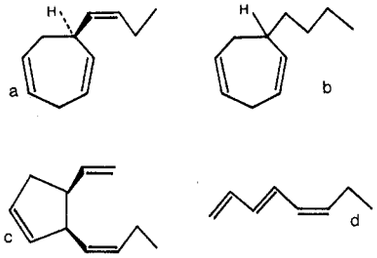

Fig. 2. Chemical structure of sex attractants in brown algae: (a) ectocarpen, (b) *Dictyota* sperm attractant (absolute configuration not determined), (c) multifiden, and (d) fucoserraten.

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*).

D. G. MÜLLER
Fakultät für Biologie der Universität, D 7750 Konstanz, Federal Republic of Germany
G. GASSMANN
Biologische Anstalt, D 2192 Helgoland, Federal Republic of Germany
W. BOLAND
F. MARNER
L. JAENICKE
Institut für Biochemie der Universität, D-5000 Köln, Federal Republic of Germany

References and Notes

1. J. L. Williams, *Ann. Bot. (London)* **18**, 183 (1904).
2. ———, *ibid.* **19**, 531 (1905).
3. D. G. Müller, *Bot. Mar.* **4**, 140 (1962).
4. Provasoli-enriched seawater [R. J. Starr, *J. Phycol.* **14** (Suppl.), 47 (1978)].
5. K. Grob and F. Zürcher, *J. Chromatogr.* **117**, 285 (1976).
6. R. E. Moore, *Acc. Chem. Res.* **10**, 40 (1977).
7. Supplied by 3M Company, Düsseldorf.
8. D. G. Müller and G. Gassmann, *Naturwissenschaften* **76**, 462 (1980).
9. L. Jaenicke, D. G. Müller, R. E. Moore, *J. Am. Chem. Soc.* **96**, 3324 (1974).
10. M. J. Wynne and S. Loiseaux, *Phycologia* **15**, 435 (1976).
11. D. G. Müller and G. Gassmann, *Naturwissenschaften* **65**, 389 (1978).
12. J. A. Pettus, Jr., and R. E. Moore, *J. Am. Chem. Soc.* **93**, 3087 (1971).
13. Dedicated to Erwin Bünning on the occasion of his 75th birthday. We thank Deutsche Forschungsgemeinschaft for financial support and Biologische Anstalt Helgoland, Abt. Gastforschung, for the use of space and facilities.

16 September 1980; revised 4 December 1980

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

0036-8075/81/0529-1041$00.50/0