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The Sperm Attractant of the Marine Brown Alga *Ascophyllum nodosum* (Phaeophyceae)

Abstract. *Spermatozoids of the intertidal seaweed* Ascophyllum nodosum *(Fucales, Phaeophyceae) are attracted to eggs prior to fertilization. The attractant has been isolated and its structure identified as 1(3*E*,5*Z*,8*Z*)-undecatetraene (finavarrene). The relation of finavarrene to sex hormones in other brown algae is discussed.*

Chemotactic attraction of spermatozoids by eggs is a well-known phenomenon in algae, mosses, and ferns (*1*). Some marine algae in the order Fucales, which clearly show sperm chemotaxis, were studied as early as 1854 (*2*). However, it was not until 1972 that advances in analytical methods permitted the identification of the pheromones involved in this reaction. Sperm attractants are now known from five genera of marine brown algae: *Ectocarpus* (*3*), *Cutleria* (*4*), *Dictyota* (*5*), *Desmarestia* (*6*), and *Fucus* (*7*) (Fig. 1). In all cases, the sperm attractants are olefinic hydrocarbons, and their molecular size and structure appear to parallel phylogenetic relationships (*8*). Since the attractant in the genus *Fucus* (Fucales) is a straight-chain octatriene and those of all other brown algae studied (in other orders) are monocyclic C_{11} molecules, we decided to study another member of the order Fucales, *Ascophyllum nodosum* (L.) Le Jol., which forms large intertidal populations in many areas of the North Atlantic. It is dioecious, and sperm chemotaxis has been reported in this species (*2*).

Mature receptacles of female *Ascophyllum nodosum* plants, collected in various sheltered locations of Galway Bay, Ireland, between 10 and 30 April 1982, were processed on the German research vessel *Friedrich Heincke*. The receptacles were soaked in water for 5 minutes and dried at 20°C. Receptacles that produced oogonial masses within a few hours were submerged in seawater (10°C) in an illuminated cold room. Eggs soon were liberated and were allowed to sediment through clean seawater to remove debris. Suspensions of clean eggs were pipetted into extraction flasks filled with 2 liters of seawater decontaminated by stripping. Low molecular hydrophobic compounds were removed from suspensions of living eggs by the closed-loop stripping technique (*9*), and adsorbed on a filter bed containing 2 mg of activated carbon. After 12 hours, substances were eluted from the filter with 30 µl of dichloromethane and subjected to analytical and preparative glass-capillary gas chromatography. The extracts contained a single compound that was produced by eggs for as long as 36 hours. In 16 trials, the average yield of this compound in an hour was 4.5 ± 2.0 ng per 10^6 eggs.

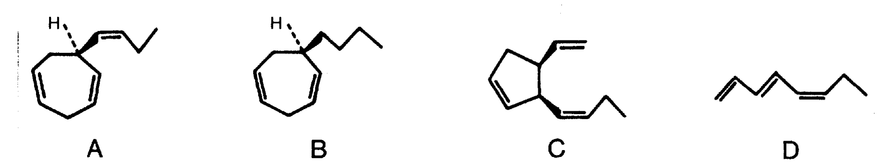
To study the biological effect of the compound, the fractions were collected after emergence from the gas-chromatographic column in a micro-ampule placed directly on the column exit and cooled with dry ice. The ampule contained a strip of cover-glass on which there were droplets of Vaseline. This preparation was tested with freshly released spermatozoids of *A. nodosum* following the procedure described for *Fucus serratus* (*10*). Spermatozoids were strongly attracted to the Vaseline droplets impregnated with the egg product, indicating that the isolated compound represented the native hormone.

Detailed analytical studies on this substance and comparison by gas chromatography–mass spectrometry with synthetic references (*11*) revealed that it is 1(3*E*,5*Z*,8*Z*)-undecatetraene (Table 1). This compound had been reported as a constituent in vegetative thalli of the brown alga *Dictyopteris plagiogramma* (*12*). It is also secreted by the gametes of another brown alga, *Spermatochnus paradoxus*, but no biological function was detected in this case (*13*). Now that a highly specific biological activity of this undecatetraene has been detected, we suggest "finavarrene" as a trivial name, referring to Finavarra, a locality in Galway Bay where the isolation work was carried out. The molecular size of finavarrene is identical to that of related substances in other brown algae in which the attractants are all C_{11} olefines. *Fucus*, with a C_8 molecule, appears to be an exception. *Ascophyllum* and *Fucus*, both members of the order Fucales in the subclass Cyclosporidae, share the straight-chain character of their attractants, whereas all sex hormones in algae of the subclass Phaeophycidae are monocyclic cyclopentane or cycloheptane derivatives.

Spermatochnus paradoxus, a member

Table 1. Analytical data for the *Ascophyllum* egg product (finavarrene), a synthetic sample (*11*), and retention indices for two additional undecatetraenes.

Compound	Retention index OV 73 (120°C)	Retention index UCON 75H (160°C)
Gas chromatography		
Ascophyllum (finavarrene)	1182.0 ± 0.1	1389.2 ± 0.7
1(3*E*,5*Z*,8*Z*)-undecatetraene	1181.8 ± 0.2	1389.0 ± 0.2
1(3*E*,5*E*,8*Z*)-undecatetraene	1193.1 ± 0.4	1409.0 ± 0.2
1(3*Z*,5*E*,8*Z*)-undecatetraene	1190.0 ± 0.1	1400.0 ± 0.1
Mass spectrometry		
Mass/charge 148 (M^+), 119, 105, 91, 79 (100 percent)		



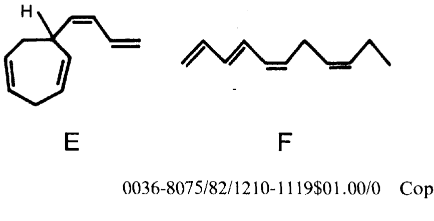


Fig. 1. Structural formulas of finavarrene and other sexual hormones of brown algae: (A) ectocarpene in the genus *Ectocarpus*, (B) dictyopterene C′ in *Dictyota dichotoma*, (C) multifidene in *Cutleria multifida*; (D) fucoserratene in the genus *Fucus*; (E) desmarestene in the genus *Desmarestia*, and (F) finavarrene, the spermatozoid attractant of *Ascophyllum nodosum*.

of the order Chordariales, is not closely related to *Ascophyllum*. The identity of the *Spermatochnus* gamete product with the *Ascophyllum* attractant is probably only a coincidence. This interpretation is supported by the occurrence of the same substance in vegetative parts of *Dictyopteris*, a member of yet another order, Dictyotales. Whether *Spermatochnus* interferes with fertilization in *Ascophyllum*, however, is not known. Nor is it known to what concentration finavarrene may build up in a densely populated *Ascophyllum* habitat.

From the few sexual hormones known from marine brown algae, it appears that the marine environment contains numerous substances that function as intraspecific or even interspecific information carriers. The thresholds for biological effects can be extremely low, less than 10^{-11} molar, as has been shown for male gametes of *Cutleria multifida* (*14*). More thorough knowledge of chemical messenger systems in other species is a prerequisite to understanding the interactions in the marine environment.

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Leaf Closure in the Venus Flytrap: An Acid Growth Response

Abstract. *The rapid closure of leaves in the Venus flytrap* (Dionaea muscipula) *involves irreversible cell enlargement, which can be initiated by acidifying the cell walls to* p*H 4.50 and below. Leaves infiltrated with neutral buffers that keep the* p*H above 4.50 to 4.75 will not close in response to stimulation of their trigger hairs even though the action potentials that ordinarily cause closure are produced. During the 1 to 3 seconds required for closure about 29 percent of the cellular adenosine triphosphate is lost. It is likely that this adenosine triphosphate is used in very rapid transport of hydrogen ions from the motor cells and that the movement is due to a mechanism of "acid growth."*

Rapid leaf movements are usually assumed to be the result of changes in turgor pressure following a loss of solute (*1–3*). The best-studied rapid movement, closure of the *Mimosa* leaf, is accomplished by just such a mechanism (*4, 5*). There is no evidence, however, that this mechanism is responsible for rapid movements of leaves that lack pulvini (jointlike thickenings), such as Venus flytrap (*Dionaea muscipula* Ellis) leaves. The limited information available on Venus flytrap leaves indicates that the movements may be due to very rapid growth (*6*).

As early as 1916, Brown (*7*) demonstrated that closure of Venus flytrap leaves results from an irreversible expansion of the outer surfaces of the trap lobes in the lateral direction and that reopening results from an irreversible expansion of the inner surface. More recently it has been demonstrated that the lobes expand longitudinally as well (*8*). While Brown proposed that this movement is due to a rapid gain in turgor by the outer trap surface, analogy with rapid movements of sundew tentacles indicates that the movement may be due to rapid changes in plasticity of the cell walls and a resulting cell expansion, causing a decrease rather than an increase in turgor (*6*).

Table 1. Response of Venus flytrap leaves whose extracellular spaces were perfused with 50 m*M* acetate or 2-(*N*-morpholino)ethanesulfonic acid buffers in the absence of mechanical stimulation and in the presence of stimulation of trigger hairs at 6-second intervals with a camel's hair brush. Sap was expressed from the cut petiole in a pressure chamber and the leaves were rehydrated in the appropriate buffer solution. All buffer solutions were adjusted with sorbitol to approximately 70 milliosmoles per kilogram. Values are means ± standard errors. N.D., not determined.

*p*H*	Number of stimuli necessary to cause trap to close 50 percent	Closure rate of unstimulated traps (percent per minute)
Control†	7 ± 3	0.0 ± 0
3.0	N.D.	0.27 ± 0.01
4.0	N.D.	1.38 ± 0.20
4.5	11 ± 5	0.32 ± 0.16
4.75	60 ± 24	N.D.
5.0	90 ± 7‡	0.0 ± 0
6.0	N.D.	0.0 ± 0

*Buffers had a buffering capacity 30 m*M* above the *p*H listed. †Perfusion with 70 m*M* sorbitol. ‡Traps requiring more than 100 stimuli for 50 percent closure were recorded as >100 and averaged with the data as 100.

Many plants exhibit rapid cell expansion when cell wall components are acidified (*9*). This suggests a plausible mechanism for the rapid expansion of trap cells. If hydrogen ions are released from the cells after the action potential that triggers closure, rapid changes in wall plasticity could occur. The release of H$^+$ would necessarily be active, since the H$^+$ gradient in nearly all plant cells favors passive movement into the cell, and would most likely occur through the action of the postulated electrogenic H$^+$ pump in plant cell plasma membranes (*10*). Experiments that show a loss of adenosine triphosphate (ATP) in trap midribs during closure indicate the presence of an energy source for rapid H$^+$ extrusion in the midribs (*11*). If such activity also occurs in the trap lobes, where movement takes place (*7, 12, 13*), rapid, ATP-dependent H$^+$ transport may be involved in closure.

We propose that the rapid closure of Venus flytrap leaves is due to acid-stimulated growth triggered by a very rapid H$^+$ pump. If this hypothesis is true, then (i) Brown's (*7*) observation of cell expansion during closure should be reproducible, (ii) turgor in outer epidermal cells should diminish with closure, (iii) leaves whose intracellular spaces are infiltrated with neutral buffers should not respond to a series of action potentials resulting from stimulation of the trigger hairs, (iv) leaves whose intracellular spaces are infiltrated with acid buffers should close spontaneously, and (v) trap lobes should lose large amounts of ATP or some other high-energy compound during the 1 to 3 seconds required for trap closure.

Cell expansion during closure was directly measured by using a pounce wheel to mark the surface of the lobes with a series of evenly spaced ink dots and measuring the change in distance be-