FUCOSERRATEN, THE FEMALE SEX ATTRACTANT OF *FUCUS SERRATUS* L. (PHAEOPHYTA)

Dieter G. MÜLLER
Max Planck-Institut für Züchtungsforschung, Abteilung Straub, 5 Köln-Vogelsang, Germany

and

Lothar JAENICKE
Institut für Biochemie der Universität zu Köln, 5 Köln, Germany

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1. Introduction

Sexual reactions in algae, archegoniates and certain fungi take place in liquid medium. For many species it is known that gametes of one sex may be attracted by secretions of the gametes of the opposite sex [1]. In *Ectocarpus siliculosus* the receptive female gametes secrete a volatile, odorous substance which attracts male gametes and induces them to change their normal locomotion pattern to initiate the sexual reaction. This substance, ectocarpen, is a hydrocarbon and was identified as S-1-but-1'-enyl-2, 5-cycloheptadiene [2].

Since the original work of Thuret [3], it is well known that spermatozoids of the brown seaweed *Fucus serratus* are attracted by the eggs (fig. 1a). After unsuccessful attempts by several workers to isolate the attractant, Cook and Elvidge [4] succeeded when they realized that the compound was volatile; however, the analytical techniques available at that time were insufficient for the identification of the extremely small quantities of attractant. They reported that a large number of hydrocarbons C_5 to C_{11} as well as several aliphatic aldehydes and alcohols acted as attractants. Using the techniques which had been successful in the collection, isolation and chemical characterization of the *Ectocarpus* substance [2], we are now able to identify the *Fucus* attractant. It is the conjugated hydrocarbon 1, 3, 5-octatriene, C_8H_{12}.

2. Materials and methods

Biological assay of the substance was achieved by using its lipophile character and its volatility. Vaseline droplets easily extract the substance from a stream of air passing over them. When such vaseline droplets are placed into a suspension of active spermatozoids, the droplets containing the attractant act like eggs and become surrounded by a halo of spermatozoids [5].

Female plants of *Fucus serratus* L. were collected daily in the tidal flats at the Marine Station of Roscoff (France). The fruiting tips of the plants were detached and a daily average of 6.5 kg of mature receptacles was thoroughly washed in tap water and placed moist in polyethylene bags. After 3 to 4 days storage in 8° the material was soaked in sea water until eggs and oogonia were released. The strongly mucilaginous supernatant was decanted and eggs and oogonia washed several times with sea water. After removal of debris the suspension of eggs and oogonia was placed in a 1 ℓ gas washing bottle at 12°. No attempt was made to separate the eggs from oogonia, since it had been observed that oogonia with unreleased eggs attracted spermatozoids in the same manner as eggs did (fig. 1b). The amount of eggs was estimated by measuring their volume when they were allowed to settle on the bottom of a measuring cylinder. During the handling of the egg masses a slight odor reminiscent of linseed oil was perceptible.

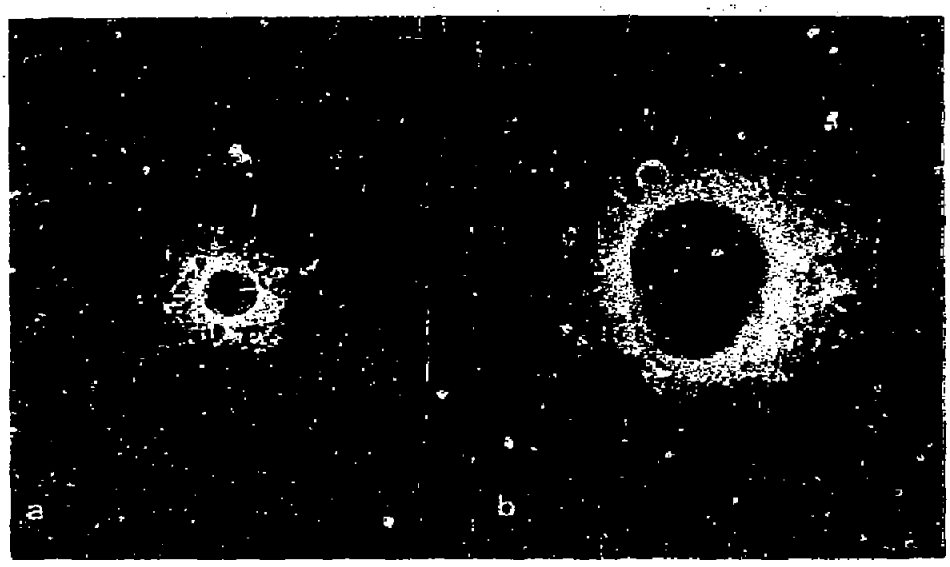

Fig. 1. *Fucus serratus*: (a) egg and (b) oogonium containing 8 unreleased eggs, both attracting a halo of spermatozoids. Dark field. Scale unit: 0.1 mm.

3. Results and discussion

The attractant was collected as follows: fresh air was drawn from outside the laboratory and pressed by a membrane pump through a copper oxide bed in a steel pipe heated to red glow in a furnace. Stainless steel, teflon and glass tubing were the only materials in contact with the purified air, which was pressed at the rate of 40 ml/min through the egg suspension agitated by a magnetic stirrer. A series of three cold traps was used to condense the volatiles emerging from the egg suspension as detailed in fig. 2. Traps 1 and 2 condensed water vapour and some attractant, while the solvent FC-78 in trap 3 washed the attractant very effectively from the air stream and contributed up to 90% of the total harvest. Each batch of egg suspension was extracted for 24 hr, the cold traps then flame sealed and stored at −20°. Daily crops of eggs were extracted from Jan. 9 through March 10, 1972. During this time 252 kg of fresh female receptacles yielded 9.8 ℓ of eggs, from which 690 μg of attractant were obtained. The condensate dissolved in FC-78 was subjected to gas chromatography for purification and further characterization [5]. The attractant emerges as a homogeneous peak from an Apiezon-Chromosorb (WAW, 5% KOH) column at 90°, where its Kovats Index is 880.3.

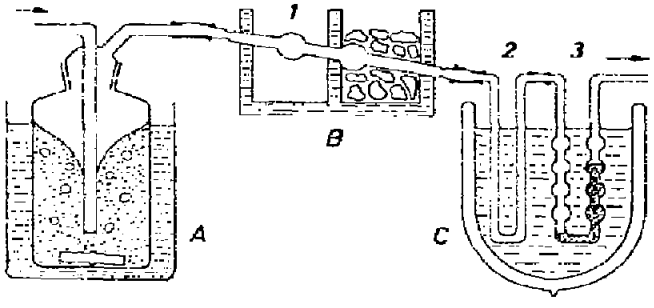

Fig. 2. Extraction setup. (A). Gas washing bottle with magnetically stirred egg suspension in water bath. (B). Two-chamber styrofoam box, right part filled with dry ice. Trap 1 designed to remove water vapour. Water is precipitated in the left bulb as liquid, in the right bulb as ice. (C). Cold bath containing a dry-ice–ethanol mixture. Trap 2 condenses small amounts of active material; trap 3 contains 200 μl of FC-78 (an inert fluorinated hydrocarbon solvent supplied by 3M Company, Düsseldorf), which effectively washes the attractant from the air stream.

Mass spectrometry of the purified attractant gives the molecular peak at *m/e* 108. The ratio of the molecular peak to the isotope peak at *m/e* 109 is close to

8:1. Further prominent features of the mass spectrum are the peaks at *m/e* 91 $(C_7H_7)^+$, *m/e* 79 (M-29) and *m/e* 77 $(C_6H_5)^+$, and *m/e* 29 $(C_2H_5)^+$.

Solutions of the attractant in n-hexane have a very characteristic UV-absorption spectrum, showing three peaks at 256 nm (log ε 3.46), 266 nm (log ε 3.58) and 276 nm (log ε 3.48), strongly suggesting a conjugated triene. Three double bonds are also indicated by the uptake of 3 moles of hydrogen during catalytic hydrogenation (Pt/H_2 in FC-78). Retention time of the hexa-hydrogenated attractant corresponds to that of n-octane on an OV-17 column at 50°. Cochromatography and mass-spectrometry demonstrate the identity of n-octane and hexa-hydro-attractant. This means that the attractant of *Fucus serratus* eggs is an octatriene. The mass-spectrum indicates that the double bonds are located at positions 1, 3 and 5. We synthesized the 1, 3, 5-octatriene by the Wittig-reaction, using *trans*-2 pentenal and the ylid from bromopropene. The product is identical with the natural compound in chemical and biological properties. The stereochemistry of the double bonds has not yet been finally established. However, we assume that the natural product represents the all-*trans*-1, 3, 5-octatriene, and we propose to name it "fucoserraten".

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