Diversification of Chemoreceptors in *Ectocarpus, Sphacelaria,* and *Adenocystis* (Phaeophyceae)

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Z. Naturforsch. **40c,** 457–459 (1985);
received January 23, 1985

Chemotaxis, Phaeophyceae, *Ectocarpus siliculosus, Sphacelaria rigidula, Adenocystis utricularis*

Ectocarpene, (+)-(6S)-(1Z-butenyl)-2,5-cycloheptadiene, is the mating pheromone of the three distantly related seaweeds *Ectocarpus siliculosus, Sphacelaria rigidula* and *Adenocystis utricularis*. Analogues of this pheromone, differing in position and π-electron density of the double bond in the C_4-side chain, were synthesized and assayed for biological activity. The results suggest, that the fundamental molecular recognition process is conservative in all species, while receptor dynamics and -topologies have been modified by individual developments and expressions during plant phylogeny.

Coordination of cellular activities during sexual reproduction by pheromones [1] has been thoroughly studied in marine brown algae. At present, ten such molecules are identified, most of which are highly unsaturated linear or alicyclic C_{11} hydrocarbons [2]. They are produced by mature female gametes. Spermatozoids react to these signals by modification of their flagellar beat pattern and are thus directed to and trapped by the female cell [3]. Ectocarpene (**1**), which seems to represent the phylogenetically most ancient molecule, is used as a sperm attractant in three species from different taxonomic groups. It may be expected that differences in the pheromone-recognition systems evolved during phylogenetic diversification. Quantitative studies of sperm chemotaxis with the genuine pheromone and systematically altered synthetic compounds offer a possibility to probe potential stages in chemoreceptor evolution in closely or distantly related species.

Ectocarpus siliculosus is isogamous and considered to represent the most primitive type of the class Phaeophyceae. Its female gametes secrete as their attractant one single compound, ectocarpene (**1**). Members of the more highly evolved orders Cutleriales, Desmarestiales and Laminariales, in addition to their specific pheromones, form **1**, as a non-active by-product [2]. The pheromone systems of *Adenocystis* (isogamous, order Dictyosiphonales) and *Sphacelaria,* (anisogamous, order Sphacelariales, *cf.* Fig. 1) were recently examined. Both genera are not closely related neither to each other nor to Ectocarpus. *Adenocystis* secretes **1** only, whereas *Sphacelaria* has a more complex bouquet with 76% **1**, and 12% each of multifidene and its trans-substituted isomer. In all three species male gametes react to **1** in the way described above though less pronounced than *Ectocarpus siliculosus*. This behaviour enabled us to compare receptor specificities in these three species.

Sphacelaria rigidula (Kutz) has been used for culture studies under the name *Sphacelaria furcigera* [4] and renamed by Prud'homme van Reine [5]. Life history and culture conditions of *Adenocystis utricularis* (Bory) Skottsberg [6] and *Ectocarpus siliculosus* (Dillw.) Lyngb. [7] have been described previously. Male gametes were collected by their phototactic response (positive in *Ectocarpus* and *Sphacelaria,* negative in *Adenocystis*) from mature gametophytes at the beginning of the daily light phase.

In order to detect potential differences in the binding mechanism of **1** within the three species, the $C_{(6)}$-substituted 1.4-cycloheptadienes **3–8** with a side chain, varying in electron density and topology, were

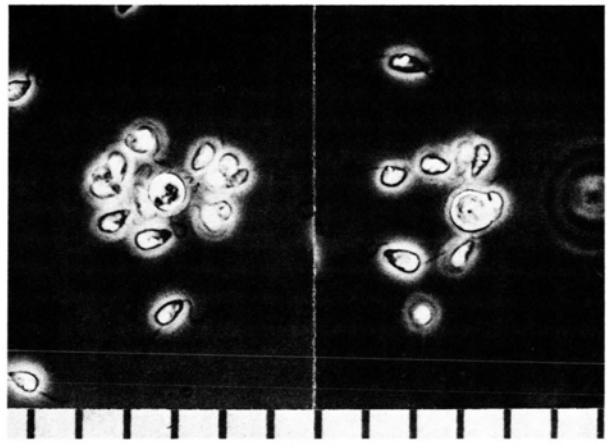

Fig. 1. Anisogamy and fertilization in *Sphacelaria rigidula.* Left: settled female gamete surrounded by motile male gametes. Right: plasmogamy, lateral fusion, male gamete = may still be seen. Scale units: 10 μm.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/85/0500–0457 $ 01.30/0

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synthesized [8] and assayed for biological activity by the FC 78 droplet technique [9, 10].

S-(+)-ectocarpene (**1**) (100% ee [11]) has the lowest threshold concentration for all three species; hence the absolute configuration of the secreted pheromone is S-(+) throughout. Chiral discrimination is low but clearly corresponds to the fair optical yield of the synthetic R-(−)-enantiomer (**2**) (ca. 66% ee [12]).

Although *Ectocarpus* as well as *Adenocystis* and *Sphacelaria* use **1** as the male-attracting pheromone, the response of their male gametes to alterations of π-electron density and -topology within the C_4-side chain of the lure is different. Desmarestene (**3**), at 5×10^{-10} M, triggers a remarkable but transient response in *Ectocarpus,* which is insignificant under conditions of the standard bio-assay. Higher concentrations inhibit the recognition system [13]. *Sphacelaria* and *Adenocystis* gametes exhibit no such pecularities below the threshold concentration. The enhanced π-electron density of the conjugated system is obviously not sensed by their receptors.

The shift of the double bond from the 1′- to the 2′-position greatly diminishes the response of *Ectocarpus-* and *Sphacelaria* gametes, while for *Adenocystis* spermatozoids this synthetic semiochemical is even more effective than the natural pheromone. Shifting the double bond further to the 3′-position decreases biological activity in all three species (Table I and Figure 2).

The behaviour of *Ectocarpus* and *Adenocystis* gametes towards the terminal bromide **6** is striking. The biological activity of **1** is largely restored for the former and partially for the latter, but the molecular basis for this effect is presently not understood. It

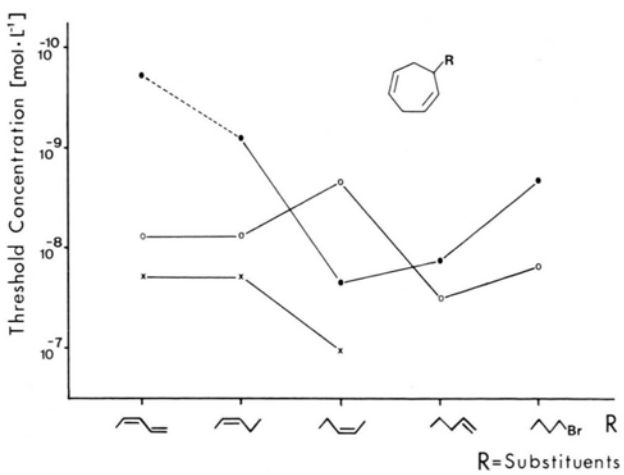

Fig. 2. Plot of threshold concentrations of the various alkenylcycloheptadienes in the three brown algae *Ectocarpus, Sphacelaria* and *Adenocystis*. (•) *Ectocarpus siliculosus;* (o) *Adenocystis utricularis;* (x) *Sphacelaria rigidula.* The dotted line for desmarestene and *Ectocarpus* indicates a statistically insignificant threshold concentration based on visual observation after 2 min of preincubation in the dark [13].

Table 1. Biological activities of extocarpene and related structures.

Ref.	Compound	Species	Threshold concentration mol/l seawater
11	S-(+) **1**	E	8.6×10^{-10}
		S	2.9×10^{-8}
		A	8.9×10^{-9}
12	R-(−) **2**	E	8.8×10^{-9}
		S	——
		A	2.8×10^{-8}
15	**3**	E	2.8×10^{-10}
		S	2.8×10^{-8}
		A	9.1×10^{-9}
8	**4**	E	3.4×10^{-8}
		S	1.1×10^{-7}
		A	3.4×10^{-9}
8	**5**	E	1.4×10^{-8}
		S	——
		A	4.5×10^{-8}
8	**6**	E	2.2×10^{-9}
		S	——
		A	2.2×10^{-8}
8	**7**	E	5.6×10^{-8}
		S	1.8×10^{-6}
		A	5.6×10^{-8}
8	**8**	E	——
		S	10.0×10^{-4}
		A	1.0×10^{-5}

Table I. Threshold concentrations were determined using the FC 78 droplet technique [9, 10]. The intrinsic partition coefficients of all compounds [10, 13] were determined experimentally and used for calculation of the ligand concentration in the immediate solvent/water interphase. E = *Ectocarpus siliculosus;* S = *Sphacelaria rigidula;* A = *Adenocystis utricularis;* —— = no response with stock solutions corresponding to 1 mmol attractant in FC 78.

may be compared to the enhancing effect of a bromomethyl- or iodomethyl-group instead of the vinyl-group in multifidene, which is attributed to an induced dipolar interaction [14].

The two other derivatives checked, the terminal allene **7** and alkyne ketone **8**, support the tendencies observed. Enhanced electron density in the side chain, remote to the ring, lowers the ligand action in the *Ectocarpus* system and causes low response in *Sphacelaria* gametes. Thus, one polarizing group of the receptor seems to correspond geometrically to the butenyl terminus of the pheromone. Spermatozoids of *Adenocystis* show nearly inverse behaviour. They seem to have an entirely different three-dimensional architecture and charge distribution in the receptor matrix at least for this particular point of interaction.

Summarizing these and earlier results [14] we conclude, that the molecular recognition mechanism by mutual polarization of receptor and messenger [10], has been modified by individual developments and expressions during phylogeny. In the three taxa studied, the biological response to their common lure **1**, demonstrates clearly expressed differences towards a standard set of systematically altered pheromones.

[1] P. Karlson, and M. Lüscher, Nature **183,** 55–56 (1959).
[2] L. Jaenicke, and W. Boland, Angew. Chem. **94,** 659–669 (1982); Angew. Chem. Int. Ed. **94,** 643 (1982).
[3] A. Geller, and D. G. Müller, J. Exp. Biol. **92,** 53–66 (1981).
[4] C. van den Hoek, and A. Flintermann, Blumea **16,** 193–242 (1968).
[5] W. F. Prud'homme van Reine, A taxonomic revision of the European Sphacelariaceae (Sphacelariales, Phaeophyceae). E. J. Brill/Leiden Univ. Press (Leiden Botanical Series No. 6, X + 293) (1982).
[6] D. G. Müller, Phycologia **23,** 87–94 (1984).
[7] D. G. Müller, Planta **75,** 39–54 (1967).
[8] W. Boland, and K. Mertes, Helv. Chim. Acta **67,** 616–624 (1984).
[9] D. G. Müller, Z. Pflanzenphysiol. **80,** 120–130 (1976).
[10] W. Boland, K. Jakoby, L. Jaenicke, D. G. Müller, and E. Fölster, Z. Naturforsch. **36c,** 262–271 (1981).
[11] W. Boland, L. Jaenicke, and A. Brauner, Z. Naturforsch. **37c,** 5–9 (1982).
[12] T. Schotten, Diploma Thesis, University of Cologne 1982.
[13] W. Boland, L. Jaenicke, D. G. Müller, and A. Peters, Eur. J. Biochem. **144,** 169–176 (1984).
[14] W. Boland, R. Terlinden, L. Jaenicke, D. G. Müller, and E. Fölster, Eur. J. Biochem. **126,** 173–179 (1982).
[15] W. Boland, K. Jakoby, and L. Jaenicke, Helv. Chim. Acta **65,** 2355 (1982).