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Spermatozoid-attracting substance in hermaphrodite brown algae, *Pelvetia wrightii* and *Fucus evanescens*

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Summary. A spermatozoid-attracting substance of the hermaphrodite brown algae, *Pelvetia wrightii* and *Fucus evanescens*, was identified as 1, trans-3, cis-5-octatriene, respectively, by ^{1}H-NMR and ^{13}C-NMR data and biological activities.

Müller and Jaenicke[3,4], in 1973, isolated the volatile attractant of spermatozoids; 1, trans-3, cis-5-octatriene (fucoserratene, **1**) from the eggs and oogonia of a dioecious brown alga, *F. serratus*. However, spermatozoid-attracting substances of hermaphrodite brown algae have not been explored so far.

Receptacles of the hermaphrodite brown algae, *P. wrightii* and *F. evanescens* were collected in the tidal flat along the Charatsunai coast of Muroran, Hokkaido, in October and June respectively. The mature receptacles were detached from the plants, wiped with gauze and rinsed in filtered sea water to remove diatoms and microorganisms[5]. The cleaned receptacles were kept at 18 °C in a growth chamber illuminated with white fluorescent lamps at about 2500 lx for 8 h and then transferred to Yamaguchi University, in a container at 5 °C with dry ice. The receptacles (22.4 kg), treated in the cold, were soaked in MeOH saturated with pentane for 3 days and the pentane-soluble portion was chromatographed on an alumina gel and florisil gel with pentane. Early fractions were further separated by $AgNO_3$-silica gel column chromatography (pentane and increasing amounts of diethyl ether) followed by preparative GC (Varian Model 920 gas chromatograph; 5% PEG 20M 1 m × 5 mm, column temp. 70 °C, flow rate 35 ml/min) to give a spermatozoid-attracting substance (6.3 mg). The structure of the attractant was fully substantiated by comparison of UV (λ_{max}^{hexane} 252, 261 and 272 nm), MS [m/z 108 (39%, M^+); m/z 79 (100%, M-29)][3,4], ^{1}H-NMR and ^{13}C-NMR data (table 1)[3,6] with those of authentic **1** which was prepared by a Wittig reaction between 1, trans-3-pentadienal and propyltriphenyl phosphonium bromide in THF in solid-liquid 2 phase using 18-crown ether - *t*-BuOK[7]. The natural **1** from *P. wrightii* was shown to contain a small amount (~5%) of 1, trans-3, trans-5-octatriene[7-9] (**2**) by ^{13}C-NMR[6] (table 1). From a hermaphrodite alga, *F. evanescens*, the male-gamete attractant, **1** (4×10^{-5}% based on the weight of fresh receptacles), was separated according to the procedure described above. The separated attractant was found to be contaminated with a trace of 2-methyl-1,3,5-heptatriene (**3**) (tentatively identified) by gas chromatography (Shimadzu GC-6A, flame ionization detector, 15% TCEP Chromosorb WAW, 6 m × 3 mm, column temp. 70 °C, N_2 flow rate 80 ml/min) and mass spectral analysis; **1** [15.5 min. 98% (based on peak area)] and **3** (18.2 min. 2%); m/z 108 (40%, M^+), 93 (base peak, M^+-15), 77 (51%), 65 (11%), 39 (17%), 27 (11%).

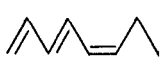
This procedure, including fluorescent illumination and cold treatment is an effective method of attractant extraction. We have demonstrated that synthetic **1** attracts spermatozoids of *F. evanescens*[7-10]. When we compared directly the biological activities of **1** and **3** for the males of *F. evanescens* and *P. wrightii* according to the method of Müller[11,12], the preference of the males for **1** was quite unambigurous (table 2). The spermatozoid-attracting activity of **2** for

Table 1. ^{13}C-NMR and ^{1}H-NMR data of fucoserratene from *P. wrightii*

Carbon No.	^{13}C-NMR* δ (ppm)	^{1}H-NMR* δ (ppm), Multiplicity, J (Hz)
1	116.8	A 5.08 dd $J_{1A,2}=10$, $J_{1A,B}=1.5$ B 5.19 dd $J_{1B,2}=17$, $J_{1A,B}=1.5$
2	137.2	6.15 m
3	135.0**	5.72 m
4	132.9**	6.44 m
5	127.7**	5.98 t $J_{4,5}=J_{5,6}=10$
6	128.5	5.42 dt $J_{5,6}=10$, $J_{6,7}=6$
7	21.2 25.9***	2.21 quin. $J_{6,7}=J_{7,8}=6$
8	14.2	1.01 t $J_{7,8}=6$

* ^{13}C-NMR and ^{1}H-NMR spectra in $CDCl_3$ were recorded on a JEOL EX-60 and FX-100 NMR-spectrometer. TMS served as an internal reference ($\delta=0$). ** Assignments for values marked may be interchanged. *** The ^{13}C-NMR chemical shift of C-7 in **2** characteristically appears downfield compared with C-7 in **1**[6].

Table 2. The response of spermatozoid-attractants and related compounds

Attractant		Activity* *P. wrightii*	*F. evanescens*
	natural fucoserratene (**1**)	+ + +	+ + +
	synthetic fucoserratene (**1**)	+ + +	+ + +
	synthetic 1, trans-3, trans-5-octatriene** (**2**)	+ + +	+ + +
	synthetic 2-methyl-1,3,5-heptatriene*** (**3**)	±	±

* The assay for spermatozoid-attracting activity was carried out at a concentration of 10^{-2} and 10^{-3}M according to Müller's method[11,12]; + + +, very active; ±, slightly active or inactive. ** Over 95% purity. *** A mixture of geometrical isomers[9].

P. wrightii and *F. evanescens* was only slightly less than that of **1**. Müller et al.[13] have demonstrated that fucoserratene (**1**) attracts the male gametes of dioecious brown algae, *F. serratus* and *F. vesiculosus*. Recently, we have reported that the male-attracting substance of a dioecious brown alga *Sargassum horneri* consists of **1** (15%), **2** (10%), 1, cis-3, trans-5-octatriene (65%) and cis-2, cis-4, trans-6-octatriene (10%) which have been shown to attract the male strongly[7,9]. Experiments to clarify the roles of **2** and **3** in the seriated sex behavior of hermaphrodite brown algae, particularly an examination of the synergistic and inhibitory effects and of the specificity of sexual chemotaxis, are still in progress.

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Effects of hypochlorite on thiamine and its derivatives

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Summary. Thiamine and its phosphorylated derivatives reacted with hypochlorite with reaction rates following the order: thiamine > thiamine monophosphate > thiamine diphosphate from pH 4.0 to 6.5. At least one unknown transient intermediate was formed and at least one non-thiochrome product was fluorescent. Chemiluminescence was also observed.

Recently Yagi and Itokawa[2] made an important report that chlorinated tap water and hypochlorite solution destroyed thiamine. In the reaction the hypochlorite ion was considered to be the species that cleaved thiamine mainly into its 4-amino-2-methyl-5-hydroxymethylpyrimidine (**I**) and 5-hydroxyethyl-4-methylthiazole (**II**) moieties. Only the reaction with thiamine was reported and there was no indication of any reaction intermediates or other products.

Here we wish to report our comparative studies on the hypochlorite destruction of thiamine (Th) and its 2 commonly-found biological phosphorylated derivatives thiamine monophosphate (ThMP) and thiamine diphosphate (ThDP). In addition, evidence is presented to show that at least one transient intermediate and product other than **I** and **II** above occurred in the hypochlorite reaction with thiamine. Chemiluminescence and fluorescence were also observed.

Materials and methods. Th, ThMP and ThDP were from Sigma. **I** and **II** were gifts kindly provided by Dr Y. Ito of Takeda Chemical Co. The chlorine concentration of sodium hypochlorite from BDH and that present in the tap water was determined by titration with sodium arsenite and the end point indicated by the iodide and starch system[3]. A Beckman Acta V, a Gilford 2000 and a Perkin-Elmer (Coleman 55) spectrophotometers were used for spectral scanning, absorbance vs time plots and absorbance measurements respectively. An Aminco Bowman spectrofluorometer was used for obtaining the fluorescence spectra and quantitating free thiamine in the form of thiochrome (excitation at 375 nm, emission at 430 nm) produced by