Male-attracting Substance in Marine Brown Algae the Genus *Dictyopteris*Tadahiko KAJIWARA^{*1}, Kazuya KODAMA^{*2}, and Akikazu HATANAKA^{*1}

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The occurrence of ectocarpene, which is the attractant of male-gamete excreted by the female gametes of the brown marine alga *Ectocarpus siliculosus*, was first confirmed by the odoriferous essential oils of *Dictyopteris prolifera* and *Dictyopteris undulata*.

The "ocean smell" of the essential oils from both species of Japanese *Dictyopteris*, consisted of mainly non-isoprenoids C₁₁-hydrocarbons [dictyopterenes (A, B and C') and ectocarpene]. In striking contrast to *D. prolifera*, the constituents of "beach odor" from *Dictyopteris divaricata* were sesquiterpenes (hydrocarbons) instead of the non-isoprenoids (hydrocarbons).

In the previous paper¹⁾, sargohornene and the isomers were identified as the attractants of male-gamete of a marine brown alga *Sargassum horneri*.

Earlier, MÜLLER *et al.* had identified ectocarpene²⁾, (S)-(+)-6-(*cis*-but-1-enyl)-cyclohepta-2,5-diene, as the male-attracting substance of the odoriferous oil from female cultures of the marine brown alga *Ectocarpus siliculosus*. The pheromone, ectocarpene, was isolated also from Hawaiian *Dictyopteris*³⁾.

This paper describes the occurrence of ectocarpene and dictyopterenes in *Dictyopteris prolifera* and *D. undulata*, and sesquiterpenes in *D. divaricata*.

Materials and Methods

Dictyopteris divaricata was collected at Funka bay, Muroran, Hokkaido in early June, and *D. prolifera* and *D. undulata* at Yoshimo, Shimono-seki, Yamaguchi at the end of September.

Freshly collected, wet brown seaweeds *D. prolifera* (83 g), *D. undulata* (93 g) and *D. divaricata* (380 g) were extracted with *n*-pentane saturated with methanol. The pentane layer were separated and the solvents were evaporated to give 1.3 g, 0.8 g and 3.1 g of essential oils, respectively. These essential oils were chromatographed on an alumina column (200 mesh activated for column chromatography, Wako Pure Chemical Ind. Ltd.; eluent, *n*-pentane). The pentane eluates were carefully concentrated, and the concentrates were re-chromatographed on a silica-gel column (100 mesh

Mallinckrodt Chemical Co. analytical reagent; eluent, *n*-pentane) to give 0.6 g, 0.5 g and 1.2 g of odoriferous essential oils, respectively.

The infrared spectra (a Hitachi EPI-G2 grating infrared spectrophotometer) of these odoriferous essential oils showed that these consisted of unsaturated hydrocarbons. These hydrocarbons were further characterized by GLC (condition-a: 3 m × 3 mm column of 5% PEG 20 M on Shimalite W; column temp., 90°C; injector temp., 120°C; N₂ flow rate, 60 ml/min and condition-b: 2 m × 4 mm glass column of 10% Silicone GE-SE-30 on Chromosorb W; column temp., 80°C–160°, 2°C/min; injector temp., 200°C; N₂ flow rate, 60 ml/min). Some peaks were identified by comparison of their mass spectra (a Hitachi M-60 at 70 eV with ion source temp. 140°C) and retention data with those of authentic compounds synthesized through unequivocal routes⁴⁾ and authentic sesquiterpenes.

Results and Discussion

The characteristic odoriferous oils from freshly wet *D. prolifera*, *D. undulata* and *D. divaricata*, were found to be a mixture of sesquiterpenes and/or non-isoprenoids C₁₁-hydrocarbons by combined GLC-mass spectrometry as shown in Fig. 1 and Table 1.

Retention data and mass spectra of Peaks-4 in Fig. 1 were consistent with those of ectocarpene synthesized through unequivocal routes⁴⁾ as shown in Fig. 2 and Table 1.

Thus, the occurrence of the male-gamete at-

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Fig. 1. Gas chromatograms of the odoriferous essential oils from *D. prolifera*, *D. undulata* and *D. divaricata*.

tractant^{2,3)}, was first confirmed in Japanese species *D. prolifera* and *D. undulata*.

Dictyopterene A [(+)-(R,R)-trans-1-(trans-hex-1'-enyl)-2-vinylcyclopropane], dictyopterene B [(-)-(R,R)-trans-1-(trans,cis-hexa-1',3'-dienyl)-2-vinylcyclopropane] and dictyopterene C' [(-)-(R)-6-butylcyclohepta-1,4-diene], had been isolated as "ocean smell" from Hawaiian species *D. plagio-gramma* and *D. australis*^{5,6)}. Peaks 3, 4, 5 and 7 in Fig. 3 were first identified with dictyopterene A, dictyopterene C', ectocarpene and dictyopterene B, respectively, in Japanese species *D. prolifera*⁷⁾ and *D. undulata* by comparison of GLC retention data and mass spectra using authentic samples⁴⁾⁸⁾.

However, dictyopterene B was not detected by GLC analysis at higher temperature (condition-b)

as shown in Fig. 1. The fact suggests that dictyopterene B was thermally converted to the enantiomer of ectocarpene during GLC analysis as shown in Fig. 4. Comparison of gas chromatograms at lower temperature (condition-a) (Fig. 3) of the non-isoprenoids C₁₁-compounds in essential oils from both *Dictyopteris*, indicated that dictyopterene A and dictyopterene B were the major constituents as summarized in Table 2.

The characteristic "ocean smell" of *D. prolifera* consisted of non-isoprenoids C₁₁-compounds, and *D. undulata* a mixture of the C₁₁-compounds (hydrocarbons) and sesquiterpenes (hydrocarbons).

In striking contrast to *D. prolifera* the constituents of "beach odor" of freshly wet *D. divaricata* was found to be a mixture of sesquiterpenes of

Fig. 2. Gas chromatograms of non-isoprenoids C_{11} -compounds in the essential oils of *D. prolifera* and *D. undulata*.

Fig. 3. Mass spectra of ectocarpene.

Table 1. Constituents of the odoriferous essential oils from *D. prolifera*, *D. undulata* and *D. divaricata*

Peak No.	Components	MS data m/e (intensity %)	GLC* t_R (min)
1	$C_{11}H_{18}$	150(16), 93(42), 79(100), 67(25), 66(45), 41(50), 27(16)	9.5
2	Dictyopterene A	150(6), 93(36), 79(100), 67(27), 66(22), 41(45), 27(19).	11.3
3	Dictyopterene C'	150(23), 93(70), 79(100), 67(20), 66(19), 41(37), 27(17).	13.8
4	Ectocarpene	148(18), 133(7), 119(31), 105(39), 91(97), 79(100), 66(41), 41(60).	14.3
5	$C_{15}H_{24}$	204(10), 161(31), 136(83), 121(100), 93(85), 41(32).	24.2
6	$C_{15}H_{30}$	210(10), 125(12), 97(51), 83(69), 69(80), 55(94), 53(100), 43(97).	26.0
7	α -Copaene	204(61), 189(69), 105(100), 91(94), 43(92).	28.7
8	β -Cubebene	204(55), 189(38), 161(100), 119(47), 105(64), 81(32), 41(40).	30.8
9	$C_{15}H_{24}$	204(83), 189(20), 161(100), 119(47), 105(41), 81(96), 41(90).	32.3
10	$C_{15}H_{24}$	204(83), 189(38), 161(100), 119(40), 105(94), 81(32), 41(90).	33.3
11	δ -Cadinene	204(64), 189(38), 161(100), 119(32), 105(36), 81(57), 41(29).	35.9

* condition-b.

Table 2. Comparison of constituents in "ocean smell" from *D. prolifera* and *D. undulata*

Peak No.	Components	Peak area (%)		GLC* t_R (min)
		<i>D. prolifera</i>	<i>D. undulata</i>	
1	$C_{11}H_{18}$	3	3	5.5
2	$C_{11}H_{18}$	8	9	5.9
3	Dictyopterene A	65	69	8.1
4	Dictyopterene C'	3	5	11.3
5	Ectocarpene	9	6	12.4
6	Undecatriene	tr	tr	13.0
7	Dictyopterene B	10	9	17.4

* condition-a.

Fig. 4. Rearrangement of dictyopterene B to the enantiomer of ectocarpene.

the cadinene type⁸⁾ instead of the C_{11} -hydrocarbons, by GLC and mass analysis (Fig. 1 and Table 1).

On the other hand, MOORE *et al.*⁹⁾ reported that in contrast to the Hawaiian species, the species of *D. zonarioides*¹⁰⁾ that grown along the coasts of Lower California, elaborates the sesquiterpenes instead of the C_{11} -compounds.

Further investigation is required to discuss whether or not the remarkable difference of components (non-isoprenoids and sesquiterpenes) between the species of *Dictyopteris*, depends on the seasonal variation and the growth of the *Dictyopteris*.

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