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Evidence of ectocarpene and dictyopterenes A and C' in the water of a freshwater lake¹

Abstract—During a study of volatile organic substances in the waters of eutrophic shallow Federsee, ectocarpene and dictyopterenes A and C' were detected for the first time in a freshwater lake. These substances were found in only one fortnightly sample during the year, indicating that marked short term chemical changes occur in small lakes. The origin of these substances could not be determined, although a common source from one organism seems likely due to their synchronous appearance and biosynthetic relationship.

The most intensively studied chemical interactions between different organisms in the aquatic environment are those mediated by sexual hormones. Such substances have been isolated from a large number of marine Phaeophyceae and their structures determined as C₈ and cyclic C₁₁ olefins (Müller et al. 1981, 1982). Very low concentrations of these substances released by mature eggs effectively attract spermatozooids for plasmogamy (Boland et al. 1981). These substances have been isolated from collected algal material and cultivated plants. Attempts have been made to establish the existence of such substances under natural conditions in the aquatic habitat. In an interference test, the existence of a lipophilic fraction with sexual hormone activity was shown to be common in seawater samples (Derenbach et al. 1980). However, the components responsible remained unknown as did their actual concentrations. During a study of the seasonal dynamics of low molecular weight compounds in a eutrophic shallow freshwater lake in southwest Germany (Federsee), we have now found evidence of the occurrence of some of these substances in a freshwater environment.

We express thanks to L. Jaenicke, W. Boland, and F. Marner for supplying reference samples of ectocarpene and several other C₁₁ olefins. B. Hickel identified the diatoms.

The volatile organic substances (up to a molecular weight of ca. 200) were stripped from 10 liters of lake water after the addition of 24% (w/w) NaCl, adsorbed on Tenax GC, then desorbed thermally and separated on a glass capillary column (Jüttner 1984). Samples of lake water were taken at 14-day intervals. In the samples from the end of July three components were present not detected before in freshwater. Their retention times (Fig. 1) together with their mass fragmentation patterns were identical to those of authentic samples of dictyopterene A [*trans* 1-(*trans* 1-hexenyl)-2-vinylcyclopropane], dictyopterene C' (6-butylicyclohepta-1,4-diene), and ectocarpene [6-(*cis* 1-butenyl) cyclohepta-1,4-diene]. However, the absolute configuration of the natural products was not established. Dictyopterene A was synthesized via 2-vinylcyclopropyl-carbaldehyde, which was obtained by using in part the methods of Kajiwara et al. (1980). From this intermediate the dictyopterenes were synthesized according to Ohloff and Pickenhagen (1969).

Assuming a similar response to the flame ionization detector as for undecane and using the recovery values obtained with the authentic substances, we found concentrations as high as 485, 129, and 326 ng·liter⁻¹ of lake water for dictyopterene A, dictyopterene C', and ectocarpene. When the particulate matter was removed from the water by continuous flow centrifugation before addition of the salt the concentrations of the three components were reduced to 34, 12, and 15 ng·liter⁻¹ in the particle-free fraction. This indicates that the major part of these compounds was either adsorbed to or dissolved in the particulate matter or released from organisms on addition of salt to the raw water. The concentrations found in the lake water considerably exceeded threshold concentrations for positive chemotaxis for similar substances with male gametes of *Syringoderma* and *Cutleria* (Boland et al. 1981; Müller et al. 1982).

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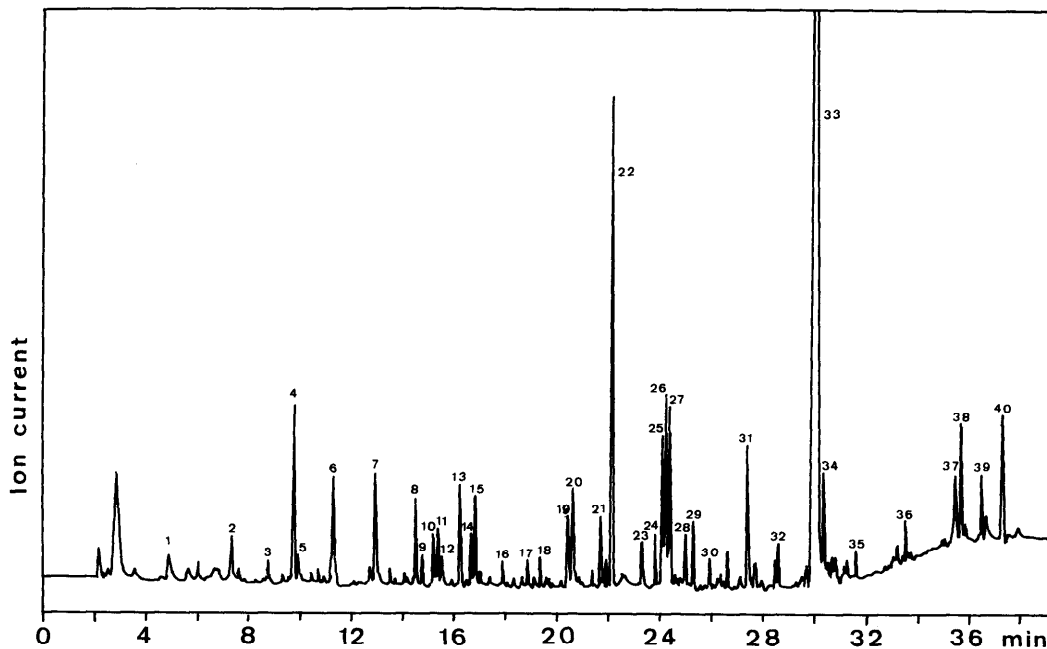


Fig. 1. Gas chromatogram of the volatile substances isolated from 10 liters of lake water (Federsee) separated on a 50-m UCON 50 HB 5100 coated glass capillary column (0° – 180°C , $5^{\circ}\text{C}\cdot\text{min}^{-1}$, He carrier gas). 1. Acetone; 2. benzene; 3. pentanal; 4. penten-3-one; 5. 1,3,3-trimethylcyclohexene; 6. toluene; 7. hexanal; 8. pent-1-en-3-ol; 9. ethylbenzene; 10. *p*-xylene; 11. *m*-xylene; 12. 3-methylbut-2-enal; 13. unknown; 14. *o*-xylene; 15. heptanal; 16. hex-1-en-3-ol; 17. 6-methylheptan-2-one; 18. mesitylene; 19. pseudocumene and octan-2-one; 20. octanal; 21. 2,2,6-trimethylcyclohexanone; 22. dictyopterene A; 23. *m*-dichlorobenzene (internal standard); 24. 2,6,6-trimethylcyclohex-2-en-1-one; 25. nonanal; 26. dictyopterene C; 27. ectocarpene; 28. benzaldehyde (contaminant); 29. α -cyclocitral; 30. 2-ethylhexan-1-ol; 31. decanal; 32. acetophenone (contaminant); 33. β -cyclocitral; 34. pentadecane; 35. naphthalene; 36. unknown; 37. unknown (M^+m/z 166); 38. heptadecane; 39. heptadec-1-ene; 40. 2,6-di-*tert*.butyl-4-methylphenol (internal standard).

Although lake water samples were analyzed fortnightly throughout the year, only the samples from the end of July had these high concentrations of the C_{11} components. Samples taken earlier or later in 1982 had peaks with the same retention times, but these did not surpass the heights of background substances. However, in general the values before the end of July were slightly higher than those after this date, which were too low for detection (Fig. 2). Since the three substances have exactly the same dynamics it seems most likely that they have a common source.

The sharp peak of the occurrence of the C_{11} olefins did not coincide with the distribution of any organism in the phytoplankton. There was an intense bloom of *Microcystis viridis* and *Microcystis wesenbergii* from June to September, representing the major part of the total biomass during that time. In the late July sample there were only

small amounts of accompanying phytoplankton species such as *Melosira granulata* var. *angustissima*, *Stephanodiscus*, *Cyclotella meneghiniana*, *Oscillatoria redekei*, *Pediastrum*, *Chodatella*, *Micractinium*, and numerous species of *Scenedesmus*. The differences in the dynamics of their occurrence make it seem unlikely that *Microcystis* was the source of the C_{11} olefins; the fact that cultures of *Microcystis* gave no evidence of the production of these substances points in the same direction. Therefore, one of the minor organisms (the identity of which remains unknown) must be responsible for these substances. Freshwater Phaeophyceae and Rhodophyceae can be ruled out since no members of these algal classes are found in the lake.

The short term occurrence of the C_{11} compounds within only one fortnightly period during the whole year indicates some physiological or reproductive change in an

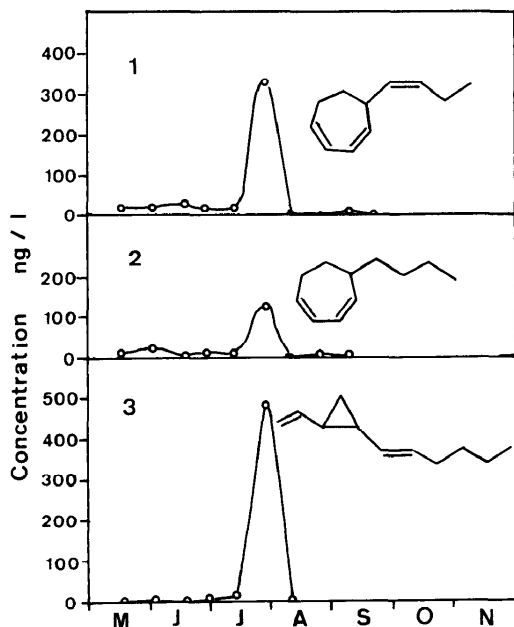


Fig. 2. Concentrations of ectocarpene (1), dictyopterene C' (2), and dictyopterene A (3) in raw water of Federsee from May to November 1982.

organism or a rapidly reproducing agent rather than the appearance of a new phytoplankton species. This behavior is clearly distinct from the dynamics of most other volatile substances observed in the lake water. The concentrations of these other components rose slowly to a maximum value which was followed by a steady and in some cases a sharp decrease to background levels (Jüttner 1984).

The unique behavior of the C_{11} olefins among the many other substances such as unsaturated alcohols, aldehydes, ketones, and nor-carotenoids may point to the fact

that C_{11} olefins play a physiological role in the water body although their source and effects remain unknown. In addition, we have shown that short term chemical changes can be detected in small lakes.

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