that were hardly soluble in the cleavage cocktail were washed off the solid support with a suitable solvent. Before concentration cleavage solutions were neutralized with pyridine. Analytical HPLC was carried out on C18 reversed-phase columns (250×4 mm) with linear gradients of acetonitrile in water/0.1% TFA and a flow of $1\,mL\,min^{-1}$. Product peaks were characterized by ESI-MS. Experimental details for the synthesis of **8**, **9**, **11**, and **12** are found in the Supporting Information.

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Wound-Activated Chemical Defense in Unicellular Planktonic Algae**

Georg Pohnert*

Diatoms are highly successful unicellular algae occurring in ocean and fresh water phytoplankton, as well as in biofilms on solid substrates. They are exceedingly abundant and are among the most important primary sources sustaining the marine food chain. Despite this, little is known about the chemical defense of these micro algae. Two of the few reported examples are the aldehydes decadienal **5** and decatrienal **6** (see Scheme 1) from the diatom *Thalassiosira rotula*, which reduce the hatching success from eggs of copepods (zooplankton grazers).[1] This observed activity explains the paradox that herbivorous copepods are less successful feeding on diatoms, although these algae are considered as high-quality food.

Here I provide biosynthetic and kinetic data on the formation of fatty acid derived metabolites in planktonic diatoms, demonstrating that the release of $\alpha,\beta,\gamma,\delta$-unsaturated dienals is widespread among this class of algae. The enzymatic mechanism to produce these metabolites is efficiently activated seconds after cell disruption and leads to high local concentrations of the defensive metabolites **5** and **6** or of structurally related potentially active aldehydes like **9**.

The simultaneous production of C_{11} hydrocarbons and 9-oxonona-5*Z*,7*E*-dienoic acid from C_{20} fatty acids was demonstrated with the benthic diatom *Gomphonema parvulum*.[2, 3] The polar dienoic acid contains the same aldehydic

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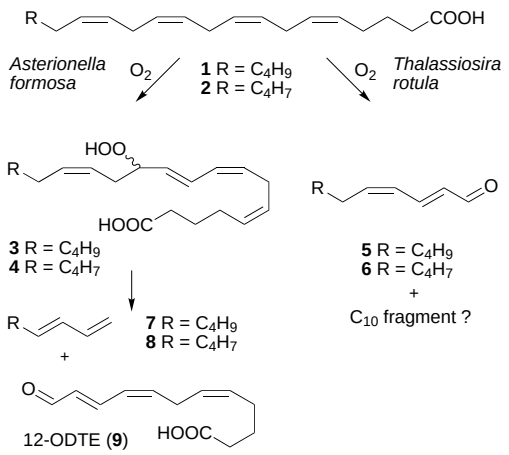
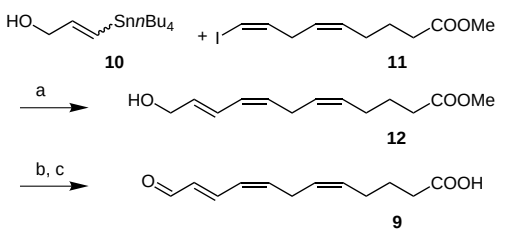
structural element as **5** and **6** and is likely to play an important role in the chemical defense of *G. parvulum*.[2] To establish if the production of frequently detected volatile hydrocarbons is generally indicative of the release of $\alpha,\beta,\gamma,\delta$-unsaturated dienals, I investigated the biosynthesis of C_8 hydrocarbons from the planktonic freshwater diatom *Asterionella formosa*. (3*E*,5*Z*)-Octa-1,3,5-triene (**8**) and (3*E*)-octa-(1,3)-diene (**7**) were detected in suspensions of broken cells of *A. formosa* or under osmotic stress conditions.[4, 5] In analogy to the biosynthesis of C_{11} hydrocarbons,[2] we reasoned that (5*Z*,8*Z*,10*E*)-12-oxododeca-5,8,10-trienoic acid (**9**; 12-ODTE) could be released as a second product, besides **7** or **8**, after oxidative cleavage of the fatty acids **1** or **2** (Scheme 1).



Scheme 1. Suggested mechanism for the oxidative transformation of eicosanoic fatty acids by damaged diatom cells.

Direct investigation of cell-free *A. formosa* extracts, without extra extraction or enrichment steps, by high pressure liquid chromatography/mass spectrometry (HPLC/MS) revealed the rapid production of a dominant medium-polarity compound.[6] The molecular species (*m*/*z* 209 $[M+H^+]$), the main fragment ion (*m*/*z* 191 $[M+H^+-H_2O]$), and the UV absorption ($\lambda_{max}=279$ nm) were consistent with the structure of 12-ODTE (**9**). To facilitate identification of this metabolite and to provide material for bioassays, an authentic sample of 12-ODTE (**9**) was prepared according to Scheme 2.

Addition of [5,6,8,9,11,12,14,15-$^2H_8$]arachidonic acid (**1**) to the cell-free solution of *A. formosa* resulted in 12-ODTE (**9**)



Scheme 2. Synthesis of 12-ODTE (**9**): a) **11**,[17] **10** (1.5 equiv; *E*/*Z* = 75/25), $[(CH_3CN)_2PdCl_2]$ (0.1 mol %), DMF, RT, 86 % (5*Z*/*E* > 97/3, 8*Z*/*E* > 97/3, 10*Z*/*E* = 5/95); b) MnO_2, CH_2Cl_2 5 min, RT, 84 %; c) PPL, phosphate buffer (pH 7), reverse-phase MPLC, 81 % (5*Z*/*E* > 97/3, 8*Z*/*E* > 97/3, 10*Z*/*E* = 5/95). For spectroscopic data, see ref. [18]. DMF = dimethylformamide, RT = room temperature, PPL = porcine pancreatic lipase.

labeled with six deuterium atoms (*m*/*z* 215 $[M+H^+]$), which indicates that the intact C1–C12 segment of the substrate was transformed into **9**. The second fragment, comprising the aliphatic part of [$^2H_8$]-**1**, [$^2H_2$]-(1,3)-octa-(3*E*)-diene (**7**), was detected as a major volatile product by GC/MS analysis.

Since the production of **7** and **8** is oxygen dependant[4] and 12-hydroperoxyeicosatetraenoic acid (**3**) could be trapped as a biosynthetic intermediate,[7] the formation of C_8 hydrocarbons in *A. formosa* is most likely to be catalyzed by a lipoxygenase/hydroperoxide lyase. Thus, the hydrocarbons frequently detected during algal blooms may indeed be considered as indicators for the simultaneous production of reactive Michael acceptors like 12-ODTE (**9**) or 9-oxonona-5*Z*,7*E*-dienoic acid.[2]

Interestingly, decadienal **5** and decatrienal **6** from *T. rotula* were also found to be derived from eicosanoic (C_{20}) fatty acids. Addition of [$^2H_8$]arachidonic acid (**1**) to broken cells of *T. rotula* led to rapid formation of [$^2H_4$]decadienal **5**.[8] In contrast, linoleic acid (C_{18}) did not serve as precursor for **5**. Unlike terrestrial plants, where volatile aldehydes are derived from C_{18} fatty acids,[9] the alga uses the pool of eicosanoids for the production of **5** and **6**. Therefore *T. rotula* follows the general observed substrate preference of eicosanoic fatty acids for production of oxylipins in other algae.[10]

Despite the fact that both unsaturated aldehydes and hydrocarbons are reported from numerous diatoms, quantification experiments with carefully isolated intact algae gave no detectable oxygenated fatty acid degradation products.[4, 11] This situation changes dramatically seconds after mechanical damage of the cells. A rapid onset of aldehyde production is observed and levels of 49 fmol of **9** per cell of *A. formosa* (Figure 1 a) and 4.1 fmol of **6** per cell of *T. rotula* (Figure 1 b) are produced after only 1–3 minutes.[11] The rapid enzymatic fatty acid degradation to **5** and **6**, therefore, represents an exceptionally fast and efficient mechanism of wound-activated chemical defense in *T. rotula.*

Although the role of **5** and **6** in the defense chemistry of *T. rotula* has already been described,[1] the ecological role of 12-ODTE (**9**) and related products remains to be established in more detail. Preliminary studies show that (5*Z*,7*E*)-9-oxonona-5,7-dienoic acid and **9** are active fungicides against *Schizophyllum commune* and *Aspergillus nidulans*.[12] This observation still has to be validated in field experiments with parasitic fungi that are known to control the spring increase of *A. formosa*.[13] Encouraging results from bioassays with herbivorous marine amphipods have already been obtained with (5*Z*,7*E*)-9-oxonona-5,7-dienoic acid, which proved highly active as a deterrent against *Amphitoe longimana*.[14]

The strategy of rapid transformation of eicosanoic fatty acids after cell damage allows *A. formosa* and *T. rotula* to build up a remarkably high local concentration of the defensive metabolites **5** or **6** and other related Michael acceptors, like **9**. These reactive aldehydes are targeted very efficiently against the herbivores that activate their production and they might even be formed in the digestive tract of those grazers.[15] Such mechanisms could account for the formation the high local concentrations (approximately 10 μM of **5** or **6**) which are required for the reduction of copepod hatching success.[1]

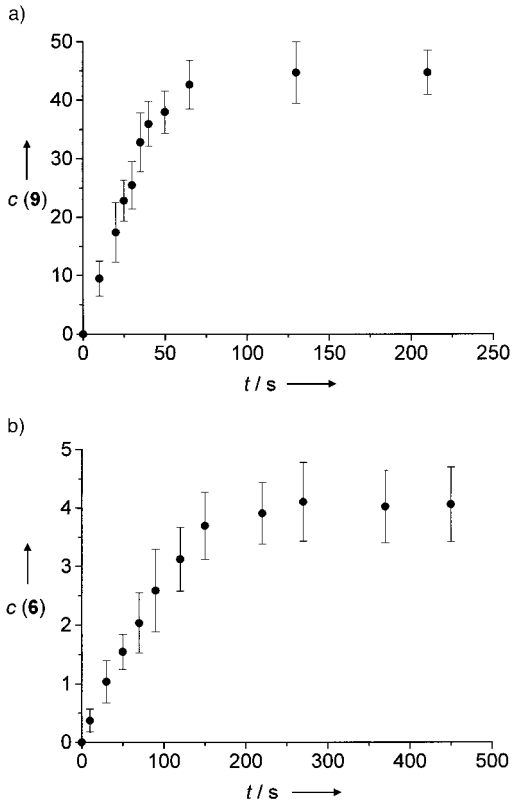


Figure 1. Kinetic data for $\alpha,\beta,\gamma,\delta$-unsaturated aldehyde production after mechanical damage of planktonic diatoms. a) 12-ODTE (**9**) production [fmol per cell] after mechanical damage of *A. formosa*. b) Decatrienal **6** production [fmol per cell] after mechanical damage of *T. rotula*. Control experiments with undamaged cells showed no detectable **9** or **6**.

In the diatoms investigated, cellular resources are invested in the production of C_{20} fatty acids that are activated only upon demand for chemical defense. The production of costly constitutive defensive secondary metabolites can thus be minimized and the risk of self-toxicity is simultaneously reduced. The exploration of chemical defense in planktonic microbes is still in its infancy, and the discovery of this highly dynamic defensive strategy might contribute to a more detailed understanding of the complex connections in this community.

Experimental section

General: *A. formosa* (SAG, University of Göttingen, Germany) was grown as previously described;[5] *T. rotula* (obtained as a gift from S. Poulet, Roscoff, France) was grown in artificial sea water,[16] as standing cultures at 19 °C (day:night 14:10). Cells were harvested through centrifugation and experiments were carried out with the concentrated cell suspensions in the culture medium. Preparation of cell-free extracts was performed as previously described.[2]

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[7] Trapping experiments were performed as previously described[2] and the identity of the intermediate was confirmed through comparison of the HPLC/MS properties with authentic 12-hydroxyeicosatetraenoic acid (SIGMA, Deisenhofen, Germany).
[8] Compounds **5** and **6** were extracted from cell-free preparations of *T. rotula* in the presence and absence of 6 μg mL^{-1} of [$^2H_8$]arachidonic acid (**1**) by solid-phase microextraction (SPME) and were analyzed by GC/MS according to: D. Spiteller, G. Spiteller, *Angew. Chem.* **2000**, *112*, 595 – 597; *Angew. Chem. Int. Ed.* **2000**, *39*, 583 – 585.
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[11] Cells were concentrated through centrifugation. De novo production of lyase products was prevented by adjustment of the cell suspension to pH 1 with 2 N HCl (no production of **9** was observed in experiments at pH 1; recovery of **9** was 96 % after treatment of reference samples at pH 1). This suspension (6.4×10^6 cells mL^{-1}) was sonicated (80 % cell disruption as judged by light microscopy), centrifuged, and investigated by HPLC/MS. No detectable amounts of **9** were found in intact cells. Kinetic experiments were performed after sonication of cells in the medium; production of **9** was stopped during the time course by adjustment of aliquots to pH 1. For kinetic experiments with *T. rotula* the metabolites **5** and **6** were extracted with CH_2Cl_2 and quantified using GC/MS.
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