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Biosynthesis of the Algal Pheromone Fucoserratene by the Freshwater Diatom *Asterionella formosa* (Bacillariophyceae)

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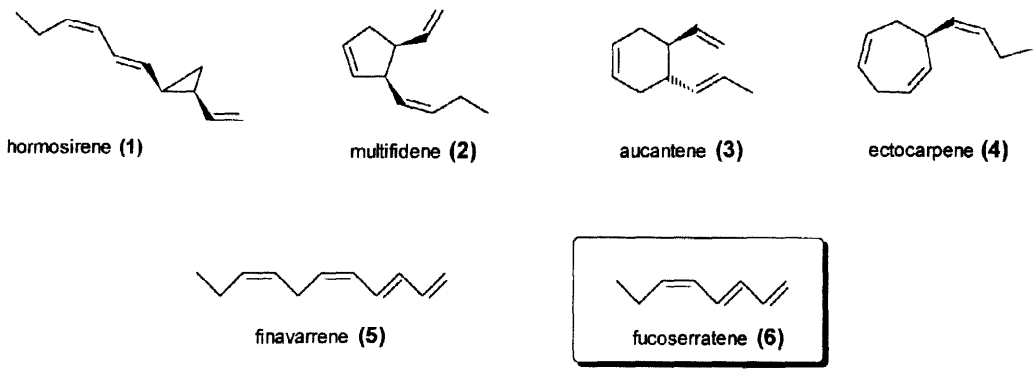
Abstract: The freshwater diatom *Asterionella formosa* (Bacillariophyceae) produces octa-1,3*E*,5*Z*-triene **(6)** (fucoserratene), previously identified as the sexual pheromone of the brown seaweed *Fucus serratus*. Fucoserratene is biosynthesised from eicosa-5,8,11,14,17-pentaenoic acid **(7)** by oxidative cleavage of the corresponding 12-hydroperoxy intermediate **8**. The biosynthetic sequence was established using eicosa-5,8,11,14-tetraen-17-ynoic acid **(18)**, a structural analogue of **7**, which was converted to octa-1,3*E*-dien-5-yne **(21)**, a structural analogue of **6**, by homogenates of *Asterionella formosa*. In addition, a general approach to highly unsaturated eicosanoids from arachidonic acid is described.

Introduction.- The sexual reproduction of many marine brown algae is controlled by environmental and chemical factors.[1,2] In general, the flagellated male spermatozoids of many marine brown algae have to cover a certain distance prior to fusion with their female counterparts. Orientation of the motile cell is governed by chemical signals released from the sedentary calling females. Most of the chemical signals are unsaturated, acyclic and/or alicyclic C_{11} hydrocarbons of different ring size and different degrees of unsaturation.[1,2] The genus of the Fucales provides an exception, since the members of this group utilise the C_8 hydrocarbon fucoserratene (**6**) for chemical communication.[3,4]



Scheme 1. C_{11} and C_8 metabolites from marine brown algae and diatoms

With the exception of **2** and **3**, most algal hydrocarbons have also been isolated from the leaves of plants,[5,6] or from certain tropical fruits.[7,8] The occurrence of **1**, **4**, **5** and **6** in diatoms is especially interesting.[9,10] Hormosirene is produced by most of the members of the family of Gomphonema (e.g. *Gomphonema parvulum*, 24 members analysed),[11] while fucoserratene has been reported to occur among the volatiles released from cells of the freshwater diatom *Asterionella formosa*.[12] Due to their ease of cultivation, these micro-organisms have

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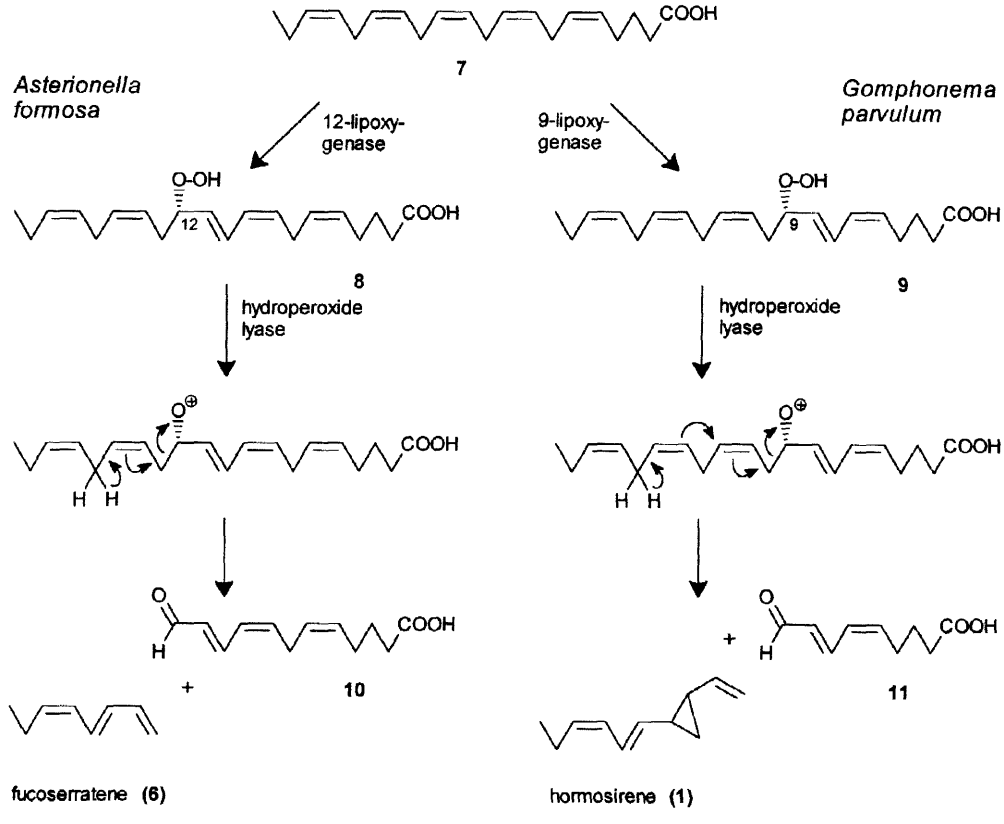
become a valuable tool for studies of algal pheromone biosynthesis. As a matter of fact, in the biosynthesis of hormosirene (**1**), for example, the same pool of highly unsaturated C_{20} fatty acid precursors is exploited by both microalgae (diatoms) and macroalgae (phaeophytes). According to Pohnert et al.,[13] eicosapentaenoic acid is activated by a 9-lipoxygenase to yield the corresponding hydroperoxide **9** which is subsequently cleaved, by a novel type of a hydroperoxide lyase, to give the C_{11} hydrocarbon and 9-oxononadienoic acid (**11**), as the second fragment. The overall sequence, including the origin of the oxygen atom in **11** have been successfully addressed using the diatom *Gomphonema parvulum*. A buffered homogenate of this organism was able to transform deuterium labelled arachidonic acid into the 9-oxo acid **11** and *trans*-1-hex-1*E*-enyl-2-vinylcyclopropane (=dictyopterene A), a more saturated C_{11} hydrocarbon structurally related to hormosirene (**1**).[13]

Following this general concept of lipid peroxidation, and subsequent oxidative cleavage of the carbon skeleton, the biosynthesis of C_{11} and C_8 hydrocarbons could start from a single precursor. The polyunsaturated fatty acid substrate could be activated, either by a 9-lipoxygenase or by a 12-lipoxygenase, and the resulting 9- or 12-hydroperoxides cleaved oxidatively, as outlined in Scheme 2. In the case of *G. parvulum* eicosapentaenoic acid (**7**) is converted into hormosirene (**1**) and the oxo acid **11**, while the enzymes of *A. formosa* could convert the same precursor into fucoserratene (**6**) and the 12-oxo-5,8,10-trienoic acid (**10**).

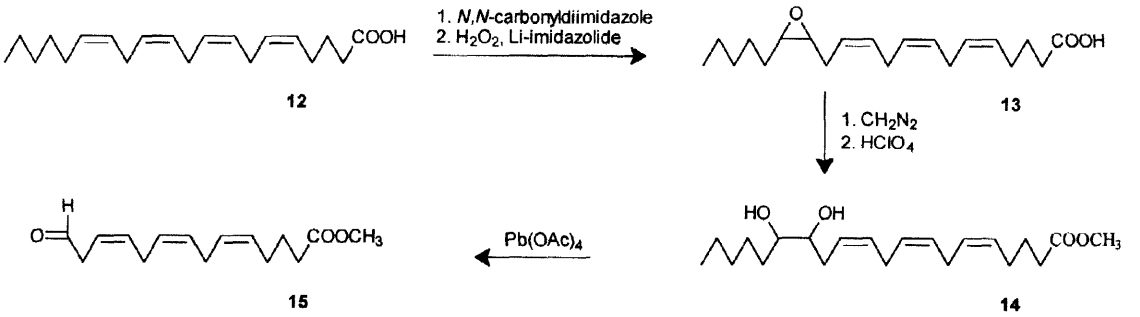


Scheme 2. Proposed biosynthesis of hormosirene (**1**) and fucoserratene (**6**) from eicosa-5,8,11,14,17-pentaenoic acid (**7**) as a common precursor.

To prove this biosynthetic hypothesis, either isotopically labelled eicosa-5,8,11,14,17-pentaenoic acid (**7**) or a structurally related substrate needs to be synthesised and administered. Here we report a simple and straight-

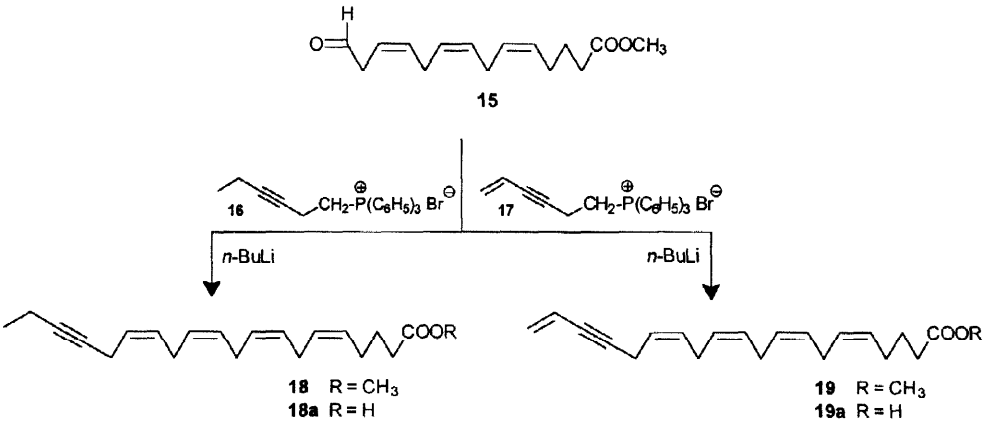
forward approach to such highly unsaturated fatty acids, utilising commercial arachidonic acid as a building block. The successful conversion of the synthetic substrate analogue eicosa-5,8,11,14-tetraen-17-ynoic acid (18) to octa-1,3-dien-5-yne (21), by a buffered homogenate from cells of *Asterionella formosa*, corroborates the postulated pathway and the involvement of 12-hydroperoxide 8.

Synthesis of Substrate Acids.- To establish whether eicosapentaenoic acid (7) is the genuine precursor of fucoserratene, either an isotopically labelled substrate, or a structural analogue, possessing all relevant double bonds in the same spatial positions as 7, was required. Wittig olefination of the aldehyde 15 promised to be the most direct approach to such fatty acids. This key intermediate is available from arachidonic acid (12) by remote intramolecular epoxidation of the terminal double bond, according to the protocol of Corey et al. (Scheme 3).[14] Here we disclose the details of the synthesis of eicosa-5,8,11,14-tetraen-17-ynoic acid (18a) and eicosa-5,8,11,14,19-pentaen-17-ynoic acid (19a). Due to their coincident double bond location with the natural precursor, these acetylenic acids were expected to mimic 7, providing substrate analogues for biosynthetic studies with the diatom *A. formosa*.



Scheme 3. Preparation of the key intermediate 15 via remote functionalisation of arachidonic acid.

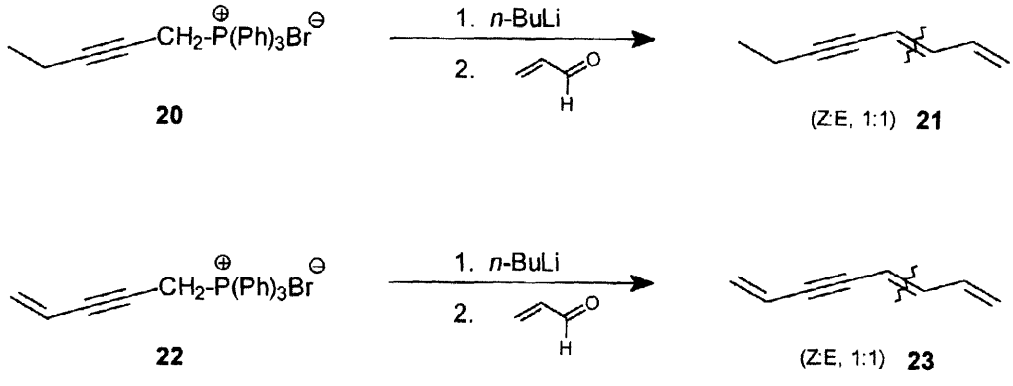
Thus, following the protocol of Corey et al., arachidonic acid (12) was first converted to the 14,15-epoxytrienoic acid (13) by remote intramolecular epoxidation of peroxyarachidonic acid, obtained from the imidazolide of 12 upon treatment with anhydrous hydrogen peroxide.[14] Following esterification with diazomethane, the epoxy ester 13 was purified by chromatography on silica gel. Subsequent exposure of the epoxy ester to perchloric acid in a binary solvent mixture of THF/water[15] afforded the diol 14 which, unlike the unstable alde-



Scheme 4. Synthesis of the eicosanoids 18 and 19 by Wittig olefination of the ester-aldehyde 15

hyde **15**, could be stored for prolonged periods. Cleavage of the diol was achieved most effectively with lead tetraacetate followed by a rapid, non-aq. work-up of the sensitive aldehyde (cf. Experimental).[14] Attempts, to cleave the diol **14** with aqueous solutions of $NaIO_4$ were unsatisfactory. Immediate olefination of **15** with the ylides derived from the acetylenic phosphonium salts **16** or **17** afforded the highly unsaturated eicosanoids **18** and **19** (Scheme 4). The Wittig reagents were obtained from hex-3-yn-1-ol and hex-5-en-3-yn-1-ol following standard procedures. From diol **14**, the two highly unsaturated esters **18** and **19** were obtained in 45 % and 43 % overall yield after chromatography. To avoid isomerisation of the highly unsaturated substrates by treatment with base, the esters were cleaved by enzymatic hydrolysis using pig liver esterase. The resulting aq. solutions of the acids **18a** and **19a**, still containing the active esterase could be used for biosynthetic studies.

The expected acetylenic C_8 metabolites **21** and **23**, from oxidative degradation of **18a** and **19a** by the diatom *A. formosa*, were synthesised as references following the protocol outlined in Scheme 5.



Scheme 5. Syntheses of octa-1,3,-dien-5-yne **20** and octa-1,3,7-trien5-yne **21**

The phosphonium salts **20** and **22** were obtained from pent-2-yn-1-ol or pent-4-cn-2-yn-1-ol by successive treatment of the alcohols with $(Ph)_3PBr_2$ and $(Ph)_3P$ without isolation of the intermediate bromides.[16] Pent-4-en-2-yn-1-ol was synthesised in 32 % yield from vinyl bromide and prop-2-yn-1-ol, following the protocol of Sonogashira et al.[17] Deprotonation of the phosphonium salts with *n*-BuLi and subsequent reaction of the resulting ylids with prop-2-enal afforded the two required hydrocarbons **21** and **23** in moderate yields, and as a mixture of 3*E*/*Z*-isomers (ca. 1:1). The spectral data were in agreement with literature data.[18,19]

Administration experiments with broken cells of *Asterionella formosa*.- Ca. 10^6 to 10^7 cells of *A. formosa* were harvested and sonicated in a phosphate buffer (0.1 M KH_2PO_4, pH 7). The suspension, containing the broken cells, was then treated with aqueous solutions of the free acids **18a** or **19a**. Approximately 20 min after addition of the substrates, the metabolites were collected from the solution by solid-phase microextraction (SPME),[13,20] and directly analysed by GC-MS. As anticipated, the crude homogenate of *A. formosa* cells catalysed oxidative degradation of the acetylenic eicosanoids to acetylenic C_8 hydrocarbons. **18a** was converted to octa-1,3*E*-dien-5-yne (**21**), and the more unsaturated precursor **19a** yielded octa-1,5*E*,7-trien-2-yne (**23**). Both compounds were identified by comparative GC-MS with the synthetic references. As shown in Figure 1, the metabolites and reference compounds displayed identical mass spectra.

The successful conversion of the highly unsaturated eicosanoids **18a** and **19a**, which resemble the double bond pattern and location of eicosa-5,8,11,14,17-pentaenoic acid **7**, clearly establish this acid as the natural precur-

sor of fucoserratene (**6**) in the diatom *A. formosa*. This view is further supported by analysis of the fatty acid pattern of a related species, *A. glacialis*, which shows the presence of several highly unsaturated eicosanoids (ca. 32% of **7**), but lacks larger amounts of linolenic and arachidonic acids.[21] Moreover, application of lower and higher homologues of **7** (e.g. octadeca-5,8,11,14-tetraenoic acid and docosa-5,8,11,14-tetraenoic acid) to broken cells of *G. parvulum* did not result in the production of olefinic metabolites. Whether or not other diatoms and brown algae producing C_8 and C_{11} hydrocarbons display the same narrow substrate selectivity remains to be established.

By analogy with the well established conversion of arachidonic acid into dictyopterene A and 9-oxonona-5Z,7E-dienoic acid (**11**),[13] it is reasonable to assume that a 12-lipoxygenase from the homogenate *A. formosa* first activates the eicosapentaenoic acid (**7**) to give the 12-hydroperoxide **8**, which is cleaved by a hydroperoxide lyase yielding fucoserratene (**6**), and 12-oxododeca-5,8,10-trienoic acid (**10**) as the polar fragment. Whether or not the mode of action of the hydroperoxide lyases can be attributed to the "homolytic" or "heterolytic" type is unknown,[22] and requires further studies with isolated enzymes.

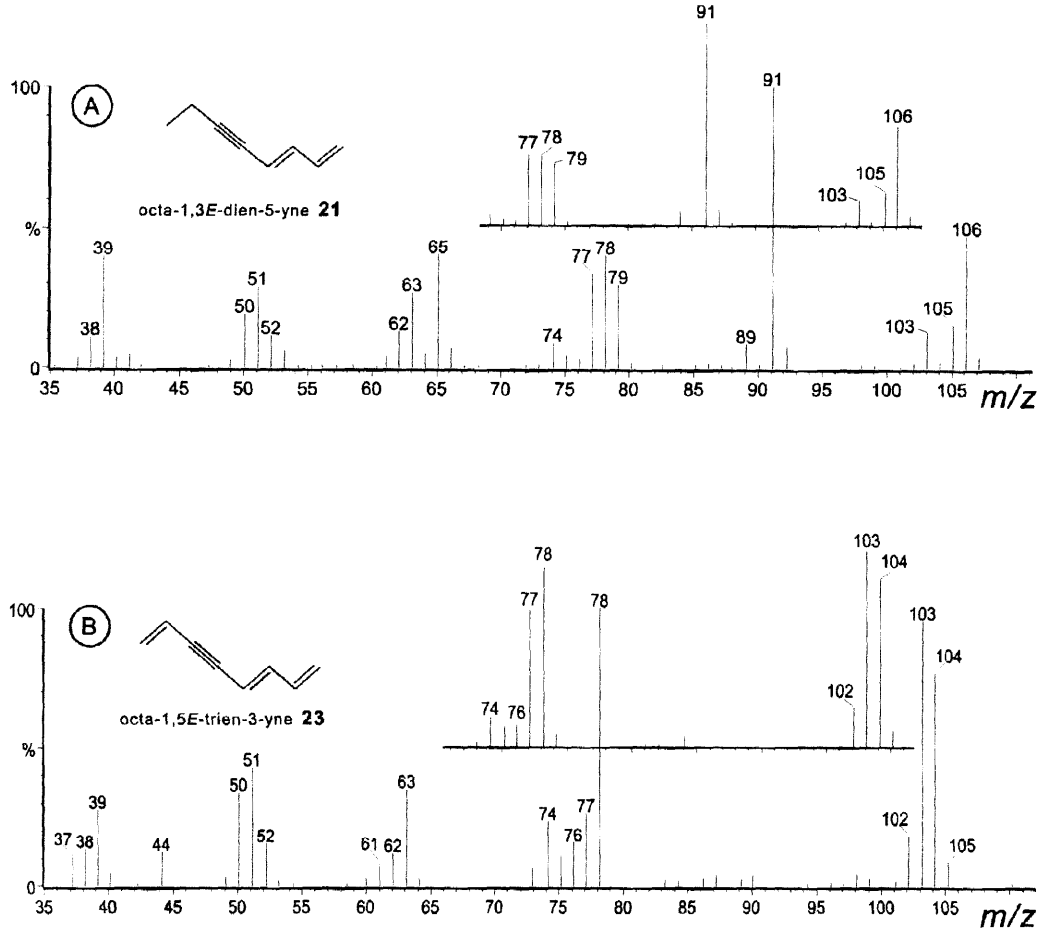


Figure 1. Mass spectra of the metabolites obtained from the incubation of broken cells of *A. formosa* with the acetylenic eicosanoids (A) **18a** and (B) **19a**. Inserts show the region of the molecular ions and first fragments of the synthetic references.

The ecological significance of the polar metabolite 12-oxo-dodeca-(5*Z*,8*Z*,11*E*)-trienoic acid (**10**) is, as yet, unknown. However, it appears is reasonable to suggest that this compound, structurally related to 9-oxonona-5,8-dienoic acid (**11**) from *G. parvulum* (cf. Scheme 2), serves as a chemical defense for the micro-organism.

Previous studies with synthetic **11** established this metabolite as a powerful feeding deterrent against a certain amphipod (*Ampithoe longimana*) which usually feeds on algae and diatoms.[23] An even more fascinating aspect is that the eggs of the brown algae, of the genus *Fucus*, may utilise eicosa-5,8,11,14,-17-pentaenoic acid **7** in a highly economic fashion, by cleaving a single precursor **7** into the sexual attractant fucoserratene (**6**) and the defensive oxo acid **10**.

Experimental

General: Reactions were performed under Ar; solvents were dried according to standard methods. IR: Perkin-Elmer Series 1600 FTIR Spectrophotometer. ^1H and ^{13}C NMR: Bruker AC 250 or Bruker AC 400 Spectrometer; $CDCl_3$ as solvent. Chemical shifts of ^1H and ^{13}C NMR are given in ppm (δ) downfield relative to TMS as internal standard. GC-MS (70eV): Fisons MD 800 or Finnigan ITD 800 coupled with a Carlo Erba GC 6000, Model Vega, equipped with a fused silica column, coated with DB1 or SE 30 (15m x 031mm); helium served as carrier gas. HR-MS: Kratos MS 50. Silica gel, Si 60 (0.200-0.063 mm, E. Merck, Darmstadt, Germany) was used for chromatography. Thin layer chromatography was performed with silica gel plates from Kodak, Rochester, N.Y. Stock cultures of diatoms were obtained from Prof. Dr. D. Czarnecki, Loras College, Dubuque, USA.

14,15-Epoxy-eicosa-5Z,8Z,11Z-trienoic acid (13)

A well stirred and chilled soln. of 1.0 g (3.30 mmol) arachidonic acid in dichloromethane (4.0 ml) was treated with a soln. of *N,N'*-carbonyldiimidazole (0.614 g, 0.38 mmol) in the same solvent (50.0 ml). Stirring was continued for 20 min followed by slow addition of a dry soln. of hydrogen peroxide (25.0 ml, 3.3 M soln. in ether) and 2.0 mg of sodium imidazolate as a base. The ethereal soln. of H_2O_2 was obtained by extraction of an aqueous soln. of hydrogen peroxide (0,5 l, 35%-soln.) with ether (100 ml) followed by thorough drying with $MgSO_4$.[24] After complete addition of the ethereal hydrogen peroxide, stirring was continued for 3 min and finely powdered $KHSO_4$ was added. The heterogeneous mixture was briefly stirred (3 min) at rt. and the solids were removed by filtration. The solution was dried over Na_2SO_4, and the reaction was allowed to go to completion at rt. After 12 h the solvents were removed *in vacuo*. The residue was redissolved in ether (20.0 ml) and esterified by slow addition of an ethereal soln. of diazomethane. Stirring was continued for 1 h, $MgSO_4$ was added for drying. Following filtration, and removal of solvents *in vacuo*, the crude ester was purified by chromatography on silica gel using a solvent mixture of pentane:ether:methanol (100:10:1, v:v:v) for elution. Yield: 0,63 g (57%). IR (Film, cm^{-1}): 2929(s); 1738(s); 1437(m); 1378(m); 1169(m); 989(m). ^1H-NMR ($CDCl_3$, 250 MHz) δ(ppm): 5,47(m, 2H, C(11,12)); 5,35(m, 4H, C(5,6,8,9)); 3,64(s, 3H, C(21)); 2,92(m, 2H, C(13)); 2, 81(t, 2H, C(10)); 2,77(t, 2H, C(7)); 2,38(dt, 1H, C(14)); 2,30(t, 2H, C(2)); 2,19 (dt, 1H, C(15)); 2,09(q, 2H, C(4)); 1,68(quint., 2H, C(3)); 1,51(m, 2H, C(16)); 1,32(m, 6H, C(17,18,19)); 0,88(t, 3H, C(20)). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 174,05; 130,37; 129,01; 128,70; 128,44; 127,75; 124,52; 57,19; 56,38; 51,49; 33,40; 31,71; 27,73; 26,53; 26,27; 26,25; 25,78; 25,59; 24,73; 22,58; 13,99. MS (70 eV) *m/z* (%): 303($M^{-31,\bullet}$, 4); 220(14); 189(13); 167(9); 149(32); 133(21); 119(50); 105(64); 91(89); 79(100); 67(84); 55(90).

14,15-Dihydroxy-eicosa-5Z,8Z,11Z-trienoic acid methyl ester (14)

The epoxyester **13** (0.63 g, 1.9 mmol) was hydrolysed by stirring with $HClO_4$ in aqueous THF (2.0 ml aq. $HClO_4$ (10%) in THF/H_2O (30 ml, 2:1, v:v)). Stirring was continued for 5 h and the product was thoroughly extracted with ether (5 x 10 ml). The combined organic layers were dried ($MgSO_4$) and the solvent was removed *in vacuo*. The diol was purified by chromatography on silica gel using a ternary solvent mixture of ether: pentane:methanol (64:32:4, v:v:v) for elution. Yield: 0.47 g (70%). IR (Film, cm^{-1}): 3385(s); 2931(s); 1736(s); 1438(m); 1369(m); 1170(m); 1059(m). ^{1}H-NMR ($CDCl_3$, 250 MHz) δ(ppm): 5,53(m, 1H, C(11)); 5,46(m, 1H, C(12)); 5,35(m, 4H, C(5,6,8,9)); 3,65(s, 3H, C(21)); 3,45(m, 2H, C(14,15)); 2,83(t, 2H, C(10)); 2,78(t, 2H, C(7)); 2,31(t, 4H, C(2,13)); 2,09(q, 2H, C(4)); 1,68(quint., 2H, C(3)); 1,48(m, 2H, C(16)); 1,39(m, 6H, C(17,18,19)); 0,87(t, 3H, C(20)). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 174,22; 131,23; 128,96; 128,76; 128,44; 127,79; 125,36; 73,77; 73,74; 51,55; 33,65; 33,42; 31,86; 31,81; 26,54; 25,77; 25,64; 25,37; 24,72; 22,61; 14,05. MS (70 eV) *m/z* (%): 352($M^{+\bullet}$, 3); 334(1), 316(1); 303(3); 253(8); 235(7); 222(15); 202(5); 180(10); 161(10); 135(18); 119(33); 113(20); 105(43); 99(20); 93(75); 87(23); 79(91); 74(43); 67(83); 55(100). HR-MS *m/z* calculated for $C_{21}H_{36}O_4$: 352,2614, found: 352,2608.

***cis*-Eicosa-(5*Z*,8*Z*,11*Z*,14*Z*)-tetraen-17-ynoic acid methyl ester (18)**

Lead tetraacetate (33.0 mg, 0.075 mmol) was washed with diethyl ether (1 x 2 ml), dried *in vacuo* and resuspended in dry dichloromethane (2.0 ml). This solution was slowly added to a cold (0°) and well stirred solution of 14,15-dihydroxyeicosa-(5*Z*,8*Z*,11*Z*)-trienoic acid methyl ester (**14**) (24.0 mg, 0.068 mmol) in the same solvent (4.0 ml). Progress of the cleavage reaction was monitored by TLC (petrol ether:diethyl ether:MeOH, 64:32:4). After complete conversion the suspension was passed through a small bed of Na_2SO_4 and Celite. The solids were rinsed with two portions of dichloromethane (2 x 2 ml) and, then, the combined eluants were evaporated in vacuo. The crude aldehyde was redissolved in dry THF (2 ml) and slowly added to a cold (-78°) solution of hex-3-ynylidenetriphenylphosphorane (0.34 mmol) in the same solvent (10.0 ml), prepared by deprotonation of hex-3-ynyl-triphenylphosphonium bromide[25] (160.0 mg, 0,343 mmol) with *n*-BuLi (0,23 ml of an 1,5 M solution in hexanes, 0,37 mmol) at 0°. Stirring was continued for 20 min at -78° before the reaction mixture was allowed to reach rt. Aq. NH_4Cl (10 ml, sat. aq. solution) was added, and the resulting reaction mixture was thoroughly extracted with ether (4 x 10 ml). The combined ether extracts were dried (Na_2SO_4), evaporated in vacuo, and the resulting crude ester was purified by chromatography on silica gel using a ternary solvent mixture (petrol ether:ether:methanol, 100:10:1) for elution. Yield: 9.5 mg (45%). IR (film, cm^{-1}): 3014, 3014, 2934, 2214, 1739, 1435, 1364, 1319, 1155, 1063, 697. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 5.40 (m, 8H-C(5),C(6),C(8),C(9),C(11),C(12),C(14),C(15)); 3.68 (s, 1H-COOC$\underline{H}_3$); 2.93 (m, 2H-C(16)); 2.81 (m, 6H-C(7),C(10),C(13)); 2.32(t, 2H-C(2)); 2.17 (q, 2H-C(17)); 2.12 (m, 2H-C(4)); 1.70 (quint., 2H-C(2), 1.11 (t, 3H-C(18)). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.01, 129.21, 129.08, 128.93, 128.66, 128.39, 128.14, 127.81, 125.61, 81.75, 77.38, 51.81, 33.55, 26.66, 25.73, 25.63, 24.08, 17.80, 14.32, 12.55. MS (70 eV): 314($M^{+\bullet}$;1), 199(10), 180(12), 157(20), 149(27), 131(40), 117(63), 105(50), 91(100), 70(52), 67(36), 50(19). HR-MS: *m/z* calcd. for $C_{21}H_{30}O_2$: 314.2262, found: 314.2244.

cis-Eicosa-(5,8,11,14,19)-pentaen-17-ynoic acid methyl ester (19)

Prepared from 14,15-dihydroxyeicosa-(5*Z*,8*Z*,11*Z*)-trienoic acid methyl ester **14** (23.8 mg, 0.067 mmol) and hex-5-en-3-ynyltriphenylphosphonium bromide[26] (158.5 mg, 0.34 mmol) as described for (**18**). Yield: 9.1mg (43%). IR (film, cm^{-1}): 3013, 2927, 2228, 1739, 1608, 1435, 1364, 1159, 974, 917, 694. ^1H NMR ($CDCl_3$, 250 MHz) δ: 5.78 (ddt, 1H-C(19)); 5.40 (m, 10H-C(5),C(6),C(8),C(9),C(11),C(12),C(14), C(15),C(20)); 3.67 (s, 3H-COOC$\underline{H_3}$); 3.09 (m, 2H-C(16)), 2.81, (m, 6H-C(7),C(10),C(13)); 2.31 (t, 2H-C(2)); 2.08 (ps-q, 2H-C(4)); 1.70 (quint., 2H-C(3)). ^{13}C NMR ($CDCl_3$, 250 MHz) δ: 174.12, 129.89, 129.05, 128.82, 128.73, 128.60, 127.99, 127.42, 125.99, 124.32, 117.45, 88.80, 79.19, 51.54, 33.45, 26.57, 25.64, 25.57, 24.78. MS (70 eV): 312($M^{+\bullet}$,2), 167(15), 157(18), 143(29), 129(64), 117(80), 108(40), 91(100), 79(47), 67(31), 55(11). HR-MS: *m/z* calcd. for $C_{21}H_{28}O_2$: 312.2078, found: 312.2067.

1,3*E*-Octadien-5-yne (21)

A chilled suspension of pent-2-ynyltriphenylphosphine bromide (613.0 mg, 1.5 mmol) in dry ether (40 ml) was slowly treated with stirring with n-BuLi (1.0 ml, 1.6 mmol, 1.6 molar soln. in hexanes). The resulting red solution was stirred for 30 min at rt., cooled to 0°, and, then, freshly distilled prop-2-enal (84,0 mg, 1,5 mmol) was slowly injected by a syringe. The faint beige suspension was stirred at rt. for another 30 min, and aq. NH_4Cl (10 ml, sat. aq. solution) was added. Extractive work-up with ether (2 x 2 ml), drying (Na_2SO_4) and slow removal of solvent via a Vigreux column afforded a concentrated solution of the highly volatile octadienynes which were further purified by chromatography on silica gel (pentane for elution). Final purification of the compounds was achieved by preparative GC. Yield: 40.5mg (26%) of (3*E*)-**20**. IR (film, cm^{-1}): 3088, 3927, 2977, 2937, 2210, 1817, 1622, 1432, 1318, 1165, 1061, 1000, 942, 908, 784. ^1H NMR ($CDCl_3$, 250 MHz) δ: 6.45 (dd, 1H-C(3)); 6.28 (ddd, 1H-C(2)); 5.55 (dd, 1H-C(4)); 5.18 (dd, 1H-C(1)); 5.06 (dd, 1H-C(1)); 2.28 (dd, 2H-C(7)), 1.10 (t, 3H-C(8)). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 141.00, 136.34, 118.69, 112.55, 94.84, 78.80, 29.73, 13.88. MS(70 eV): 106($M^{+\bullet}$, 52), 91(100), 78(35), 61(35), 51(10). HR-MS *m/z* calcd. for C_8H_{10}: 106.0781, found: 106.0783.

1,5*E*,7-Octatrien-3-yne (23)

Prepared as described for **21** from pent-4-en-2-ynyl-triphenylphosphonium bromide (611.0 mg, 1.5 mmol) and prop-2-enal (84.0 mg, 1.5 mmol). Yield: 42.0 mg (27%) of (5*E*)-21. IR (film, cm^{-1}): 3091, 3009, 2181, 1830, 1620, 1416, 1288, 1188, 998, 968, 912, 844, 783, 664. ^1H NMR ($CDCl_3$, 250 MHz) δ: 6.59 (dd, 1H-C(6)); 6.38 (ddd, 1H-C(7)); 5.93 (ddd, 1H-C(2)); 5.74 (dd, 1H-C(5)); 5.64 (dd, 1H-C(1)); 5.48 (dd, 1H-C(1)); 5.31 (dd, 1H-C(8)); 5.20 (dd, 1H-C(8)). ^{13}C NMR ($CDCl_3$, 250 MHz) δ: 142.31, 136.18, 126.79, 119.94, 117.22, 111.76, 94.44, 89.22. MS (70 eV): 104($M^{+\bullet}$, 68), 103(67), 78(100), 63(22), 51(32). HR-MS *m/z* calcd. for $C_{12}H_8$: 104.0623, found: 104.0626.

Cultivation of *Asterionella formosa*

The diatoms were cultivated in WC media[27] as a standing culture in Petri dishes (250 ml) at 17°. The dishes were illuminated with a photon flux of about 30 μmol m^{-2} sec^{-1} for 14 h followed by a 10 h dark period. The cells were harvested towards the end of the logarithmic growth period after 14-21 days. The WC media were prepared according to literature.[27]

Collection of Volatiles by Solid-Phase Microextraction (SPME)

The volatile metabolites were directly sampled from the incubation medium containing the sonicated cells, soluble enzymes and the metabolic probe by inserting an SPME fibre into the gently stirred solution for 20 min.[13,20] Best results were obtained with an SPME fibre, coated with polydimethylsiloxane (100 μm). For GC-MS analysis, the adsorbed compounds were directly evaporated from the fibre by inserting the coated tip of collection device into the injection port of a GC-MS system for 1.0 min. The metabolites were identified by comparison of their chromatographic and mass spectroscopic data with the synthetic references.

Incubation Experiments with Broken Cells of *Asterionella formosa* and Synthetic Eicosanoids. Biosynthesis of Fucoserratene (6)

Preparation of free fatty acids: The methyl esters **18** or **19** (3.1 mg, 0.01 mmol) were dissolved in ethanol (0.3 ml) and added to a suspension of porc liver esterase (PLE, 0.5 mg, 60 U) in a phosphate buffer (1.0 ml, 0.1 M KH_2PO_4, pH 7). The suspension was gently shaken and NaOH (0.1 M) was added very slowly until a pH 8.5 was reached. After 1h the methyl esters were completely hydrolysed (GLC). The solution of the acids **18a** or **19a** were used without further purification for the incubation experiments.

Incubation experiments: For a typical incubation experiment ca. 10^7-10^8 cells of *Asterionella formosa* were harvested, centrifuged, resuspended in a phosphate buffer (5.0 ml, 0.1 M KH_2PO_4, pH 7) and sonicated at 0° for 30 sec (70W). Then, a solution of either **18a** or **19a** was added, and the mixture was gently stirred for 20 min at rt. The non-polar fatty acid metabolites were directly sampled from the incubation medium by SPME and analysed by GC-MS. The chromatographic and mass spectroscopic data of the metabolites proved to be identical with those of the synthetic references **21** and **23**.

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