The isolation and structure of bacillariolide III, an extracellular metabolite of the diatom, *Pseudo-nitzschia multiseries*

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The marine diatom, *Pseudo-nitzschia multiseries* was found to excrete a metabolite formed by the cleavage of an eicosanoid, and its structure is determined.

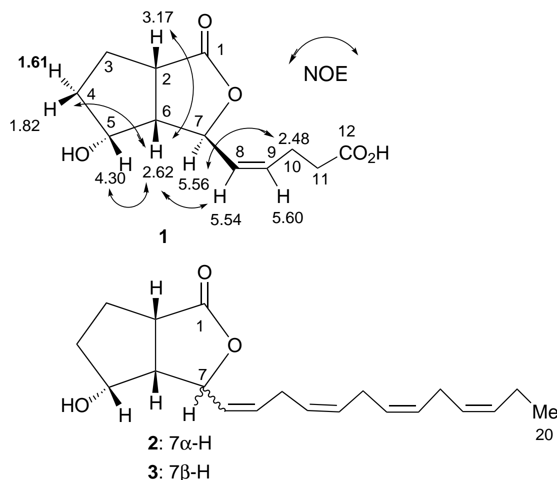
In the previous papers,[1,2] we reported the isolation and structure determination of two novel eicosanoids from the wild and cultured cells of the diatom, *Pseudo-nitzschia multiseries,*† a causative organism of so-called amnesic shellfish poisoning (ASP).[3] In the continuing study, we have noticed that the organism also produces extracellular metabolites. In this communication, we report the chemical characterization of one of the metabolites, bacillariolide III, **1** and its biosynthetic relationship with the previously reported compounds.

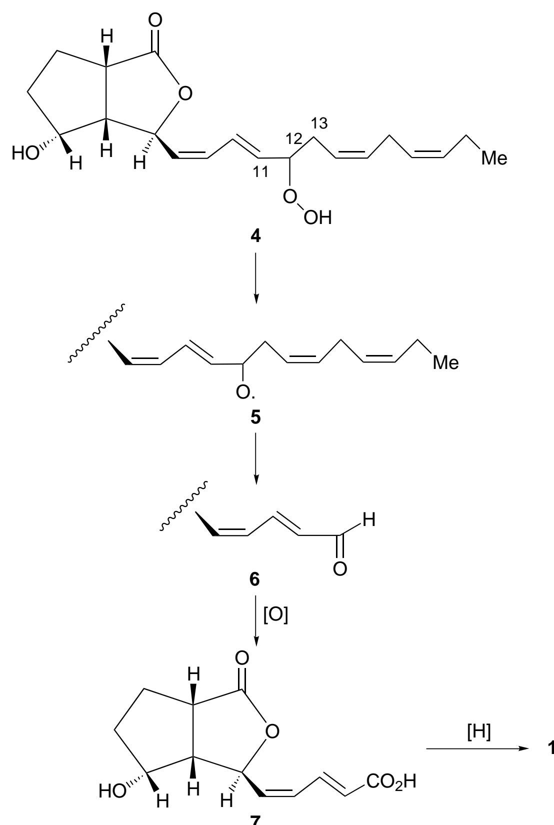
The clonal strain of *P. mutiseries* S2-184-1,‡ which had been isolated from Narragansett Bay in October, 1995 by N.Z., was cultured in F/2 + Si medium at 23 °C under fluorescent illumination (16 h/8 h L/D cycle) and sterile conditions. After centrifugation, the acidified culture medium (pH 3.0) was passed through silica gel C18. The C18 layer was washed with water and extracted with MeOH. The MeOH eluate was successively chromatographed on LH-20 (MeOH) and on silica gel C18 (10% MeCN + 0.5% acetic acid). The fraction which showed a single spot on TLC was passed through Chelex 100 Na^+ form to give the Na^+ salt of **1**. The free acid was obtained as an amorphous solid after acidification with 0.1 **m** HCl and extraction with $CHCl_3$. About 2 mg of pure bacillariolide III was obtained from a 20 l batch of culture.

Table 1 ^{1}H and ^{13}C NMR data of Bacillariolide III, **1** (^{1}H: 400 MHz and ^{13}C: 100 MHz in CD_3OD)

Position	δ_C	δ_H	^{1}H–^{1}H COSY cross peaks/δ
1	183.6		
2	44.8	3.17, dt (9.6, 6.6)	1.98, 2.62
3	27.3	1.98, m	3.17, 1.61, 1.82
4	34.7	a. 1.61, m	1.82, 1.98, 4.30
		b. 1.82, m	1.61, 1.98, 4.30
5	74.7	4.30, ddd (1.5, 5.1, 6.3)	1.61, 1.82, 2.62
6	52.1	2.62, ddd (10.0, 6.4, 1.8)	3.17, 4.30 5.56
7	76.6	5.56,[b] dd (3.1, 12.0)	2.62, 5.54
8	131.0	5.54[b], dd (6.4, 3.3)	5.56, 5.60
9	133.5	5.60, dt (10.4, 6.0)	5.54, 2.48
10	24.8[a]	2.48, dt (6.5, 10.7)	5.60, 2.41
11	36.0[a]	2.41, t (6.5)	2.48
12	183.3		

[a] Assigned from the HMQC spectrum. [b] Overlapped signals.





Scheme 1 A possible biosynthetic pathway of bacillariolide III, **1**

Bacillariolide III, $[\alpha]_D^{24}$ −25.3 (*c* 0.28, MeOH), UV: end absorption, has the molecular formula of $C_{12}H_{16}O_5$ [*m/z* calc. 240.099774; found (EIMS) 240.099646]. The IR spectrum showed absorption bands, ν/cm^{-1} ($CHCl_3$): 3500 (OH), 1770 (γ-lactone) and 1730 (γ-lactone). The ^{1}H, ^{13}C and HMQC NMR spectra (Table 1) suggested the presence of two carbonyl groups, one disubstituted double bond, four methylene groups and four methine groups, of which two are bearing oxygen atoms. Thus the compound must be bicyclic. From the ^{1}H–^{1}H COSY and HMQC spectra, the entire spin system was easily established as shown in the structure **1**. The relative stereochemistry were established by NOE difference and NOESY experiments (shown on the structure). The geometry of the double bond was concluded as *Z* from the coupling constant of

the olefinic protons (6 Hz), which was also supported by the NOE data. The absolute configuration was assumed by the perceivable biosynthetic relationship of **1** with the co-occurring bacillariolide I, **2**, whose absolute configuration was unequivocally established by the X-ray crystallography of a derivative having a known chirality.[2]

The biosynthetic origin of **1** would never have been easily speculated, had it been a sole metabolite found with the diatom. However, the structures of the two previously isolated compounds, bacillariolide I, **2**, and II, **3**, suggest that **1** is a metabolite derived from bacillariolide I, **2** by the cleavage of the carbon chain at C-12 and C-13. It can be explained by the formation of 12-hydroperoxide **4** and alkoxyl radical **5**, followed by fragmentation to the aldehyde **6**, oxidation to carboxylic acid **7** and reduction to **1** (Scheme 1).§ Such cleavage of the polyene chain by 'homolytic-type' hydroperoxide lyases has been previously found in the algae.[4] Here, we can not rule out the possibility that these 12-hydroperoxide formation and chain cleavage may precede the bicyclic lactone ring formation. However, the large amount of **2** found in the cell, and late appearance of **1** in the medium seems to support the former case.

Interestingly, **1** is strictly an extracellular metabolite and not found in the cells. On the other hand bacillariolide I, **2** and II, **3** are found only in the cells and not in the culture medium, which indicates that **1** is discharged to the outside of the cells as soon as it is biosynthesized. Diatoms are the most abundant microalgae in the oceans in both quantity and number of species. However, in contrast to the case of some other microalgal families, only a very few secondary metabolites have been found in the cells of diatoms.[5,6] We have speculated that those organisms with solid siliceous skeletons tend not to store the metabolites but rather excrete them outside. The finding of a fairly large quantity of **1** and some other metabolites in the medium certainly supports this assumption.

The biological function of this extracellular metabolite is still under investigation, but its structural resemblance with the well-known plant growth regulators, jasmonates[7,8] and cucurbic acid[9] draws our attention. Bacillariolide I is known to possess significant inhibitory activity against phospholipase A_2.[6]

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Footnotes

† This organism was previously called *Nitzschia (Pseudo-nitzschia) pungens f. multiseries*, but its name has been recently changed to *Pseudo-nitzschia multiseries* (Hasle) Hasle by the original namer: G. R. Hasle, *J. Phycol.,* 1995, **31**, 428.

‡ The electron microscopic identification of this species was done by Professor Paul E. Hargraves, University of Rhode Island, who is gratefully acknowledged.

§ The proposed biosynthetic pathway was modified according to a suggestion by Professor William Gerwick, Oregon State University, who is thanked for his advice.

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