Bacillariolides I and II, a New type of Cyclopentane Eicosanoids from the Diatom *Nitzschia pungens*

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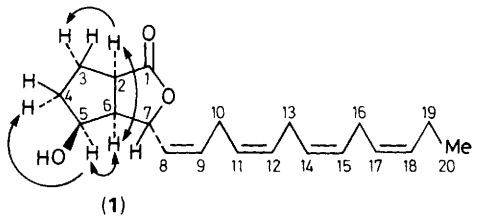
New arachidonic acid derivatives with cyclopentane and lactone rings have been isolated from the pennate marine diatom *Nitzschia pungens*, and their structures, including relative stereochemistry, have been established.

In 1987, a new type of shellfish poisoning, which was caused by the mussels shipped from Prince Edward Island, was reported in Canada.[1] The toxic principle was identified as an excitatory amino acid, domoic acid.[2,3] The circumstantial evidence suggested that diatom blooms were the source of the toxic substance.[4] In fact, diatoms such as *Nitzschia pungens* and *Amphora coffaeiformis* were later found to produce domoic acid in culture.[3,5,6] Since domoic acid is the major active principle in the red alga, *Chondria armata*, which was used as an anthelmintic in Japan without any reported incident,[7] a question has been raised regarding unexpectedly severe symptoms (including deaths) observed with the patients. While examining the algal components for compounds which may have exerted synergic effects, we found a rather high content of a novel type of eicosanoid in the algal cells of *N. pungens*.

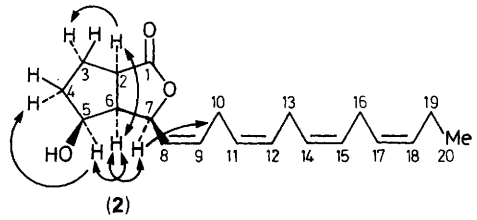
The wet diatom cells (predominantly *N. pungens*) collected at Prince Edward Island, Canada, in 1988 were extracted with methanol. The methanol extract was partitioned between hexane and 90% methanol. The methanol fraction was subjected successively to flash chromatography (silica gel, hexane–ethyl acetate, 5:1), reversed phase chromatography (C18, 80% methanol), and flash chromatography (silica gel, hexane–ethyl acetate, 27:10), which afforded two oily compounds, bacillariolide I and bacillariolide II (yields 50 and 7.7 mg from 200 g wet cells, respectively). The compounds were also isolated from the unialgal cultured cells.

Bacillariolide I (**1**), $[\alpha]_D^{24}$ −25.9° (*c* 0.21, methanol), has the molecular formula $C_{20}H_{28}O_3$ (*m/z* calc. 316.2039; found 316.2025). The IR spectrum showed the presence of a γ-lactone [$\nu_{C=O}$ ($CHCl_3$) 1770 cm^{-1}] and a hydroxy group (ν_{O-H} 3500 cm^{-1}). The NMR spectra (^1H, ^{13}C, and DEPT)† revealed the presence of four disubstituted double bonds, one carbonyl, six methylene, two ordinary methine, two oxygen-bearing methine, and one methyl groups, which accounted for all twenty carbons in the molecule. The structure (**1**) was easily derived from the connectivities of carbon and hydrogen atoms established by tracing the spin systems with homonuclear and heteronuclear COSY and decoupling experiments. The relative stereochemistry was established by NOE difference spectroscopy, which showed strong NOEs between H-2 and H-6, and H-5 and H-6. The geometries of the double bonds were determined to be all (*Z*), by comparison of the carbon chemical shifts[8] and proton coupling constants[9] with those of known structures.

The second compound, bacillariolide II (**2**), $[\alpha]_D^{23}$ −59.2° (*c* 0.33, methanol) has the molecular formula $C_{20}H_{28}O_3$ (*m/z* calc. 316.2039; found 316.2027), and thus is isomeric with (**1**). The NMR spectra of (**2**) were very similar to those of (**1**),† indicating that they are stereoisomers. The difference NOE spectra showed strong NOEs between the proton pairs, H-2 ~ H-6, and H-5 ~ H-6, indicating that (**2**) also has the same *cis*-ring juncture and stereochemistry of the hydroxy group as (**1**). However, prominent NOEs were also observed for the nuclear sets: H-6 ~ H-7, H-2 ~ H-7, and H-7 ~ H-10, which

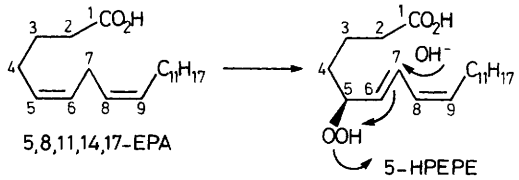


(**1**)



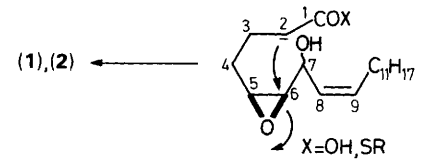
(**2**)

(Arrows depict prominent NOE)



5,8,11,14,17-EPA

5-HPEPE



(**1**),(**2**)

X=OH,SR

Scheme 1

† *Spectroscopic data* for (**1**): ^1H NMR (CD_3OD, 300 MHz) δ 0.96 (t, *J* 7.6 Hz, H-20), 1.60, 1.83 (m, H-4), 1.98 (m, H-3), 2.08 (d, q, *J* 7.6, 7.6 Hz, H-19), 2.64 (ddd, *J* 2.3, 6.4, 9.2 Hz, H-6), 2.83 (t, *J* 5.7 Hz, H-16), 2.88 (t, *J* 5.6 Hz, H-13), 2.99 (m, H-10), 3.18 (d t, *J* 9.5, 6.5 Hz, H-2), 4.29 (dt, *J* 4.9, 6.6 Hz, H-5), 5.25—5.45 (m, H-11, -12, -14, -15, -17, -18), 5.52 (dd, *J* 8.1, 10.6 Hz, H-8), 5.55 (dd, *J* 2.3, 8.1 Hz, H-7), 5.57 (dt, *J* 10.6, 7.3 Hz, H-9); ^{13}C NMR (CD_3OD, 75.43 MHz) δ 14.65 (Me, C-20), 21.49 (CH_2, C-19), 26.42, 26.52, 26.92 (CH_2, C-10, -13, -16), 27.20 (CH_2, C-3), 34.62 (CH_2, C-4), 44.66 (CH, C-2), 51.83 (CH, C-6), 74.55 (CH, C-5), 76.50 (CH, C-7), 128.13, 128.18, 128.76, 129.65, 130.06, 130.09, 132.85, 133.02 (CH=CH, C-8, -9, -11, -12, -14, -15, -17, -18).

suggested that (**2**) is the epimer of (**1**) at C-7 with the orientation for the side chain *cis* to the cyclopentane ring. Another noteworthy feature in the NMR spectrum of (**2**) is a large downfield shift (δ −0.5) for H-8, which the model shows to be in close proximity to the hydroxy group.

A number of eicosanoids including prostaglandins have been discovered in marine invertebrates and algae, and considerable interest has been aroused in the origin and biosynthesis of marine prostanoids which are not formed *via* the normal endoperoxide pathway.[10,11,12] The biosynthesis of bacillariolides seems to be closely related to the leukotriene biosynthesis,[13] and can be explained by the initial formation of 5-hydroperoxide on eicosapentaenoic acid (EPA), rearrangement to the hydroxy epoxide, and carbon ring closure by anionic opening of the epoxide (Scheme 1).‡ At present, the absolute configuration of the compounds has not been established, but generally 5-lipoxygenases are known to introduce (5*S*)-hydroperoxides.[13] As far as we know, this is the second example of cyclopentane eicosanoid resulting from ring closure at other than the normal C-8 ~ C-12. The other example is hybridalactone with a cyclopropane ring at C-10 and C-14.[14,15] There is a report which implicates prostanoids as the culprit in food poisoning by a red alga,[16] but at present we have no data on the pharmacology of bacillariolides or their possible harm.

This work was supported by NIH grant GM 28754, which is greatly appreciated.

Received, 20th November 1989; Com. 9/04954A

‡ In the algal cells, the compounds are probably in the open carboxylate forms, because the fresh water extract afforded (**1**) and (**2**) upon acidification.

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