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BIOREDUCTION OF HYDROPEROXY FATTY ACID BY CYANOBACTERIUM *PHORMIDIUM TENUE*

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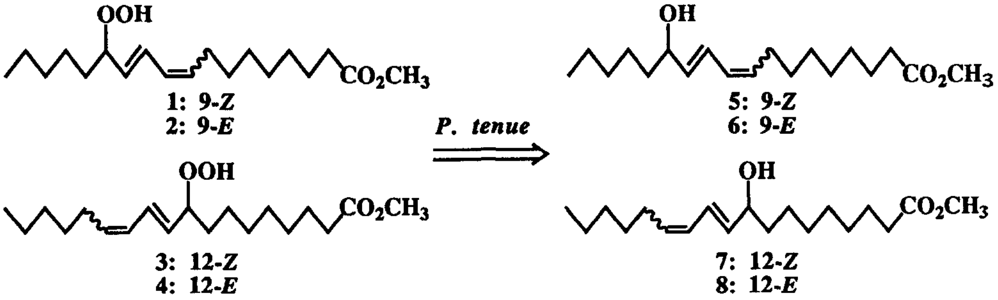
Abstract: Bioreduction of hydroperoxy fatty acids by a cyanobacterium *P. tenue* was found: 9-hydroperoxy and 13-hydroperoxyoctadecadienoic acids elaborated from linoleic acid were reduced to the corresponding hydroxyoctadecadienoic acids by the cyanobacterium.

Besides our previous paper,1) there were a few reports concerning that unsaturated fatty acids were more potent growth inhibitor of some alga than saturated ones.2) These findings allow us to presume products derived from unsaturated fatty acids to be genuine antialgal substances, taking it into consideration that unsaturated fatty acids are readily subjected to oxidation and some hydroxy fatty acids were shown to possess biological activity.3) However, the mechanism how only unsaturated fatty acids inhibited the growth of alga have never been investigated and was unclear. Therefore, we have investigated changes of linoleic acid in the presence of the cyanobacteria and the antialgal activity of the modified compounds.4) This investigation resulted in finding out bioreduction of hydroperoxy fatty acids mediated by the cyanobacterium. Here we present the bioreduction of hydroperoxyoctadecadienoic acids derived from linoleic acid to hydroxyoctadecadienoic acids by the cyanobacterium *P. tenue*.



After a culture of *P. tenue* was grown axenically for 10 days, linoleic acid was injected to the culture medium (800 ml) at the concentration of 100 ppm. The culture medium was incubated for 3 days under the same condition as precultivation of the alga with continuous shaking. Remaining alga was removed by centrifugation at 4,000 x g followed by filtration with a membrane filter (pore size: 0.4 µm), then the resulting filtrate was extracted CH_2Cl_2-ether (1:2). The organic extract was treated with CH_2N_2 to give corresponding methyl esters, which was successively subjected to silica gel column chromatography, normal phase HPLC, and reversed phase HPLC to furnish eight compounds **1** (5.9 mg), **2** (4.0 mg), **3** (4.1 mg), **4** (2.4 mg), **5** (6.6 mg), **6** (2.9 mg), **7** (6.6 mg) and **8** (2.6 mg).5) The UV spectrum of **1** showed the absorption maximum due to the diene moiety at 232 nm (ε=28,000) and the EI-MS spectrum showed a fragment ion peak at

308 (M^+-H_2O, $C_{19}H_{32}O_3$). Compounds **2**, **3** and **4** were very similar to **1** in their physicochemical data. These compounds (**1**-**4**) were, therefore, elucidated to be methyl hydroperoxyoctadecadienoate. Compound **5** showed the presence of the diene moiety at 234 nm (ε=26,000) in its UV spectrum, while the molecular ion was observed at 310 (M^+, $C_{19}H_{34}O_3$) in its EI-MS spectrum. Taking it into consideration that the physicochemical data of **6**-**8** had the nearly same properties as that of **5**, and compounds (**5**-**8**) were prepared by $NaBH_4$ reduction of the corresponding hydroperoxides (**1**-**4**), the products (**5**-**8**) were found to be methyl hydroxyoctadecadienoate. Coupling constant analyses of the olefinic proton signals in the ^1H NMR spectra determined the geometry of diene portion of eight compounds. The position of hydroperoxyl and hydroxyl groups was elucidated by fragmentation pattern in the GC-MS spectra of their trimethylsilyl ethers prepared from **5**-**8**.[6] In order to investigate participation of lipoxygenase in generating the hydroperoxides, optical purities of the eight compounds (**1**-**8**) were determined by the following procedure. $NaBH_4$ reduction of the hydroperoxide (**1**) afforded the hydroxy methyl ester, which was treated with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride to yield the corresponding Mosher's ester.[7] The ^1H NMR spectrum of the Mosher's ester in the presence of Europium tris(heptafluorobutanoylpivaloylmethanate) (Eu(FOD)$_3$) exhibited two signals attributed to the methoxyl group in α-methoxy-α-trifluoromethylphenylacetyl moiety having an equal integral value,[8] the compound **5**, therefore, proved to be a racemate. Similarly, the ^1H NMR spectra of the Mosher's esters derived from **2** to **4** had the same properties. Because the lipoxygenase catalyzed hydroperoxidation was show to proceed enantioselectively,[9] the hydroperoxides (**1**-**4**) was elucidated to be generated not by lipoxygenase but autooxidation of linoleic acid.

When linoleic acid was injected to the filtrate prepared from 10 day culture medium by removing the alga and subsequently the medium incubated for 3 days, the hydroperoxides (**1**-**4**) were yielded in nearly the same ratio as an above described experiment, but no hydroxy compounds (**5**-**8**) were detected. Thus, the former was generated by autooxidation of linoleic acid and the latter was shown to be formed by bioreduction of the hydroperoxides with the cyanobacterium. Furthermore, injection of 9-hydroperoxy and 13-hydroperoxy octadecadienoic acids in the culture medium of *P. tenue* at the concentration of 25 ppm also afforded the corresponding alcohols in 32 % yield.

In conclusion, we found out the bioreduction of the hydroperoxyocatdecanoic acids leading to the corresponding hydroxy derivatives by the cyanobacterium, *P.tenue*. Although there are a few reports on the presence of reductive enzyme for peroxide lipid in a cyanobacterium,[10] this is the first example demonstrating that the hydroperoxide was reduced to the alcohol by the cyanobacterium.

References and Notes

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4) The antialgal activity of **1**-**8** will be published elsewhere in the near future.

5) The compounds **1**-**8** were separated and characterized as the corresponding methyl esters because of their unstability.

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7) ^1H NMR (400 MHz, $CDCl_3$, δ): 7.50-7.38 (5H, m, arom.-H), 6.60 (1H, dd, J=10.8, 15.2 Hz, 11-H), 5.93 (1H, m, 10-H), 5.63-5.45 (3H, m, 9, 12, 13-H), 3.66 (3H, s, CO_2CH_3), 3.55 (3H, s, OCH_3), 2.29 (2H, t, J=7.4 Hz, 2-H), 2.13 (2H, m, 8-H), 0.85 (3H, m, 18-H)

8) In spite of a mixture of two diastereomers, a pair of signals were little diffentiated without the shift reagent.

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