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Biosynthetic Formation of Cyclic Bromo-ethers Initiated by Lactoperoxidase

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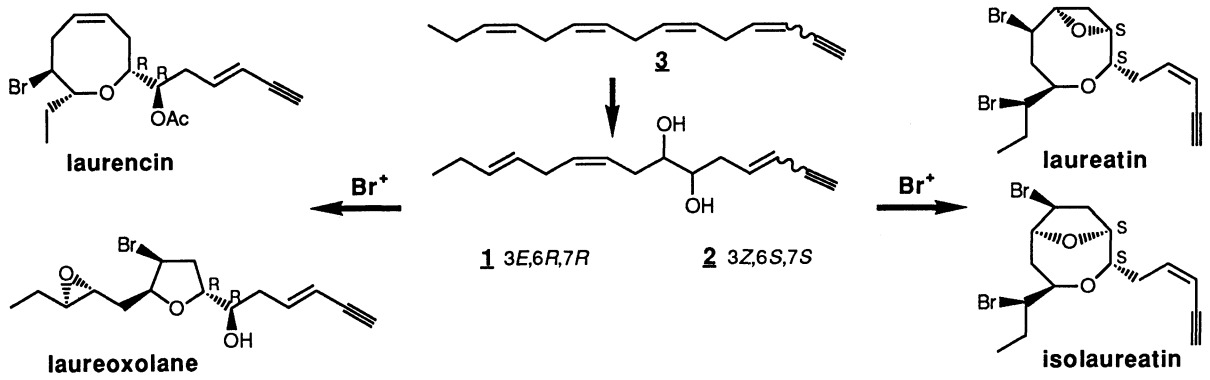
The first biosynthetic *in vitro* evidence for marine natural bromo-ethers by using lactoperoxidase is reported.

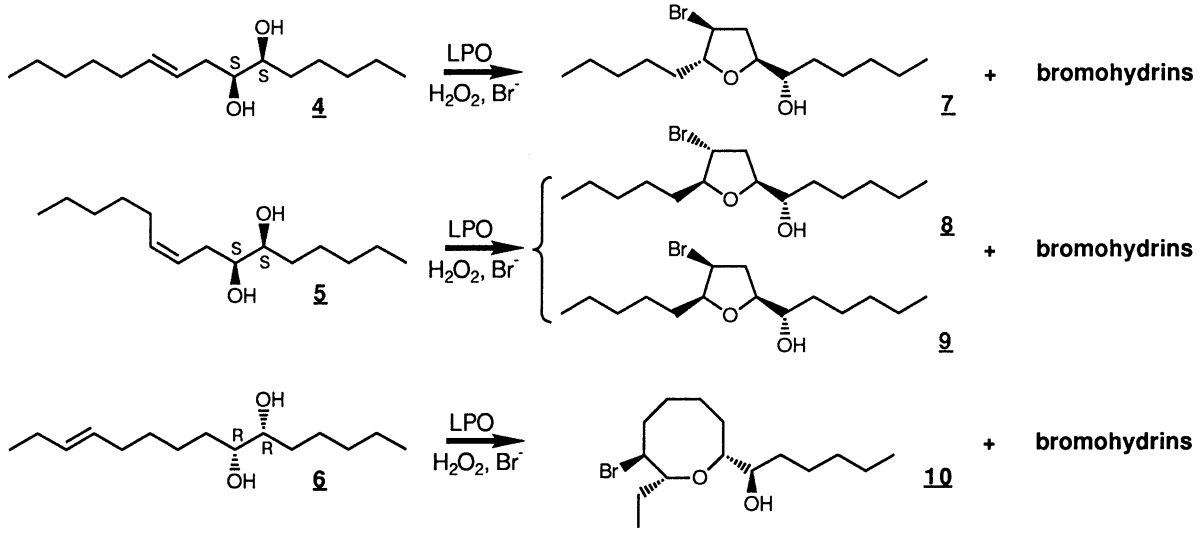
More than fifty compounds of small and medium sized cyclic halo-ethers having straight C_{15}-chained skeletons have been isolated from marine origins such as red algae, *Laurencia* species[1] and sea hare, *Aplysia* species.[2] Highly labile *trans*- and *cis*-lauradiols (1 and 2)[3] isolated from *L. nipponica* have been assumed to be the common biogenetic precursors of the bromo-ethers and to be generated from laurencenynes 3[4] (Scheme 1). However, any attempts to establish 1 and 2 as the real precursors have not yet been reported.[5] In this paper we describe as the preliminary experiments that the olefin diols (4, 5, and 6) were internally cyclized by lactoperoxidase (LPO) in the presence of hydrogen peroxide (H_2O_2) and bromide ion (Br^-) to yield cyclic bromo-ethers. The results would provide *in vitro* model studies for clarifying the biosynthetic role of 1 and 2.



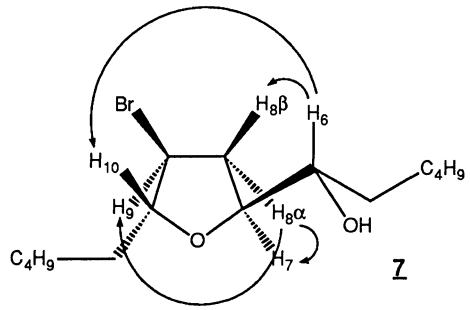
Scheme 1. Plausible biogenesis for cyclic bromo-ether compounds.

Although we could detect the activity of bromoperoxidase[6,7] in crude fractions extracted from L. nipponica, it has been found to be rather difficult to purify the enzyme at the present stage. Accordingly, we employed commercially available LPO[8] for our biosynthetic studies, because of its known enzymatic properties as well as its character corresponding to bromoperoxidase.

(6S,7S,9E)-9-Pentadecene-6,7-diol 4[9] (18 mg, 74 nmol, 3.7 mM) as the substrate was dissolved in dimethyl sulfoxide (0.2 ml) and the solution was injected into phosphate buffer (pH 5.5, 50 mM, 20 ml) containing sodium bromide (60 nmol, 3 mM). To the mixture was added an aliquot of each solution of H_2O_2 (16 nmol, 0.80 mM)[10] and LPO (32 nM) dividing into twelve portions during 2 h. The mixture was stirred in the dark at 23 °C for 24 h and extracted twice with ethyl acetate. The extracts were washed with distilled water, dried, and concentrated. The residue was then chromatographed over silica gel and purified by HPLC to give the bromo-ether 7[11] (2.1 mg, 8.8%, 40.6%*),[10] the recovered starting material (13 mg, 72%), and a mixture of bromohydrins (3.1 mg, 12.3%, 56.9%*), which was not further characterized (Scheme 2). The bromohydrins, when repeatedly subjected to the same reaction conditions, gave no bromo-ethers. The results eliminate the possibility of bromo-ether cyclization via a bromohydrin pathway.[12] On the other hand, when the (9Z)-isomer 5[9] (17.0 mg, 35 nmol, 3.5 mM) was treated under the same conditions, two bromo-ethers, 8[13] (0.53 mg, 2.35%, 10.3%*) and 9 (0.27 mg, 1.2%, 5.25%*), were obtained along with bromohydrins (4.5 mg, 18.6%, 81.2%*) and the recovered starting material (11.0 mg, 64.7%). The structures of three bromo-ethers 7, 8, and 9 were established by ^1H NMR spectra combined with homo spin decoupling measurements.



Scheme 2. Bromo-ether cyclization of olefin alcohols (4, 5, and 6).



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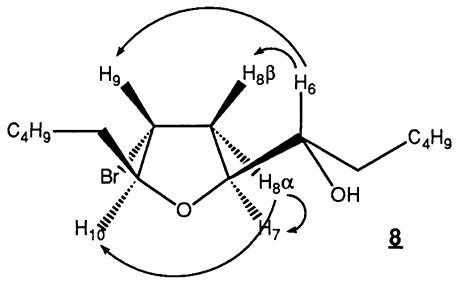

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Fig. 1. The NOE measurement of the cyclized products (7 and 8).

Absolute configurations at C-9 and C-10 were determined based on the NOE experiments depicted in Fig. 1. The whole structures of 7 and 8 are thus represented as (6S,7S,9S,10R)- and (6S,7S,9R,10S)-9-bromo-7,10-epoxy-6-hydroxypentadecanes, respectively. The compound 9 was identical with the known oxolane derived from isolaureatin.[1)]

Finally, the substrate 6, (6R,7R,12E)-12-pentadecene-6,7-diol,[9)] (16.1 mg) was subjected to enzymatic reaction under the same conditions as mentioned above to afford the desired eight-membered bromo-ether 10 (0.3 mg, 1.4%, 5.8%*) and bromohydrins (3.9 mg, 17.5%, 72.2%*) as major products with the recovered starting material (8.7 mg, 54.0%). Compound 10 was identical in all respects with deacetyloctahydrolaurencin prepared from natural laurencin.

In view of the chemical reaction mechanisms, bromo-cationic etherification of the substrates, 4 and 5, via the corresponding bridged bromonium intermediates would possibly give rise to four cyclic bromo-ether products, respectively. However, the enzymatic reactions produced only endocyclic bromo-oxolanes from these olefin diols, respectively. It is to be noted that (E)-olefin alcohol cyclizes exclusively in an anti-addition manner, while the (Z)-isomer both in anti- and syn-addition manners. The results indicate that LPO has a high regio- but a low stereo-selectivity for these enzymatic reactions. Conversion of 6 into the eight-membered bromo-ether 10 reveals that there should be a conformationally implicit role of LPO on the enzymatic reaction, since the internal bromo-cyclization of 6 never proceeded so far with any chemical reagents generating positive bromine (i.e., N-bromosuccinimide or 2,4,4,6-tetrabromocyclohexane-2,5-dien-1-one).

Consequently, we conclude that LPO clearly recognized the olefin alcohols 4, 5, and 6 as analogues of laurediols, and that laurediols 1 and 2 would be strongly suggested to be the real biosynthetic precursors of various cyclic bromo-ether metabolites in marine origins. The corresponding enzymatic reactions with highly unstable laurediols 1 and 2 as the ultimate goals are now in progress.

References

1) Cf., K. L. Erickson, "Constituents of Laurencia," in "Marine Natural Products, Chemical and Biological Perspectives," ed by P. J. Scheuer, Academic Press, New York (1983), Vol. 5, Chap. 4, pp. 131-257; A. Fukuzawa, Mya Aye, Y. Takaya, H. Fukui, T. Masamune, and A. Murai, Tetrahedron Lett., 30, 3665 (1989).

2) Y. Gopichand, F. J. Schmitz, and J. Shelly, J. Org. Chem., 46, 5192 (1981), and references cited therein.

3) E. Kurosawa, A. Fukuzawa, and T. Irie, Tetrahedron Lett., 1972, 2121.

4) H. Kigoshi, Y. Shizuri, H. Niwa, and K. Yamada, Tetrahedron Lett., 22, 4729 (1981); 23, 1475 (1982).

5) Cf., D. J. Faulkner, Tetrahedron, 33, 1421 (1977).

6) J. Geigert, S. L. Neidleman, and D. J. Dalietos, J. Biol. Chem., 258, 2273 (1983).

7) The activity of bromoperoxidase in L. nipponica was detected by the decrease in absorbance of chlorodimedone at 278 nm in the presence of H_2O_2 and sodium bromide.

8) Oxidation of Br^- by LPO was examined by stopped flow method. LPO was oxidized with H_2O_2 to form "Compound I." Compound I was then converted directly to ferric form on reaction with Br^- (rate constant 2.2 x 10^6 $M^{-1}s^{-1}$). The results reveal that Br^- was oxidized by the enzyme by way of two electron transfer in a similar manner as iodine [cf., M. Nakamura, I. Yamazaki, T. Kotani, and S. Ohtaki, J. Biol. Chem., 260, 13546 (1985)]. Reaction species generated by iron porphyrin in LPO have been established to be enzyme-bound bromonium ion [porphyrin-Fe(III)-O-$Br^{\delta+}$].

9) The substrates were prepared from natural laureatin or laurencin and confirmed to be completely homogeneous by MS and ^1H NMR spectra.

10) High concentration of H_2O_2 is harmful for the enzyme and decreases the yields of the respective bromo-ethers. The star marked percentages (%*) denote the stoichiometric yields based on H_2O_2.

11) 7: FD-MS, m/z 320 and 322 (M^+); ^1H NMR, δ2.04 (1H, dt, J=13.2, 7.5 Hz, $H_8\beta$), 2.58 (1H, dt, J=13.2, 7.5 Hz, $H_8\alpha$), 3.51 (1H, br m, H_6), 3.80 (1H, q, J=7.5 Hz, H_7), 3.85 (1H, q, J=7.5 Hz, $H_9\alpha$), and 3.90 (1H, dt, J=3.6, 7.5 Hz, $H_{10}\beta$).

12) E. Kurosawa, "Kagaku Sosetsu No. 25," ed by Chem. Soc. Jpn., Gakkai Shuppan Center, Tokyo (1979), pp. 191-200.

13) 8: FD-MS, m/z 320 and 322 (M^+); ^1H NMR, δ1.87 (1H, ddd, J=14.7, 9.3, 5.4 Hz, $H_8\alpha$), 2.39 (1H, ddd, J=14.7, 9.3, 6.4 Hz, $H_8\beta$), 3.60 (1H, dt, J=7.3, 3.5 Hz, H_6), 3.95 (1H, ddd, J=9.3, 5.4, 3.5 Hz, $H_9\beta$), 4.06 (1H, dt, J=9.3, 3.5 Hz, H_{10}), and 4.14 (1H, ddd, J=9.3, 6.4, 3.5 Hz, H_7).

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