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12-METHYLIDENE-10(Z), 13(Z)-NONADECADIENOIC ACID, A NEW IRREVERSIBLE INHIBITOR OF SOYBEAN LIPOXYGENASE

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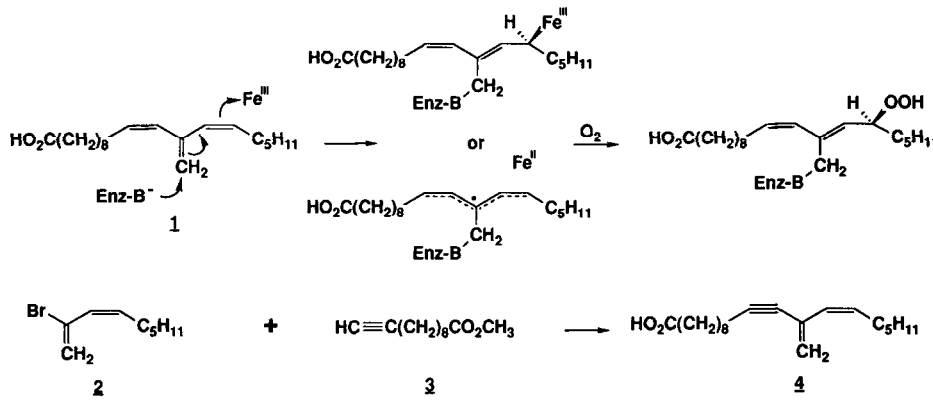
Summary: Aerobic incubation of soybean lipoxygenase with 12-methylidene-10(Z), 13(Z)-nonadecadienoic acid results in irreversible deactivation of the enzyme in a manner consistent with a recently proposed mechanism for lipoxygenation.

The preceding note describes the irreversible inhibition of soybean lipoxygenase (SB-LO) by 13-thiaarachidonic acid and a possible mechanism for that inactivation and for the lipoxygenation of conventional substrates such as linoleic or arachidonic acids.[1] On the basis of this work it seemed a reasonable possibility that analogs of lipoxygenase substrates in which the normal ω subunit, (Z,Z)-CH=CH-CH_2-CH=CH-C_5H_{11}, is replaced by (Z,Z)-CH=CH-C(=CH_2)-CH=CH-C_5H_{11} might function as enzyme activated irreversible inhibitors of SB-LO. We report herein the synthesis of such an analog, 12-methylidene-10 (Z), 13(Z)-nonadecadienoic acid (**1**), and the study of its action on SB-LO. The mechanisms by which **1** might function as an irreversible inactivator of SB-LO are outlined in the following scheme.

The C_{19} trienoic acid **1** was synthesized from commercially available 10-undecynoic acid in a simple way. The bromodiene **2**, prepared in modest yield from 2-bromopropenal and n-pentylidene-triphenylphosphorane in tetrahydrofuran-hexamethylphosphoric triamide at -78°C, was treated with methyl 10-undecynoate (**3**)(1.2 equiv), cuprous iodide (0.3 equiv), tetrakis(triphenylphosphine)palladium(0) (0.03 equiv) and excess n-propylamine in benzene at 45°C for 2 hr to give the coupling product **4** in 75% yield.[2,3] Lindlar reduction of **4** (Lindlar Pd-Pb catalyst, 1 atm H_2, in 10:1 benzene-pyridine) afforded after chromatographic purification on silica gel the methyl ester of **1**, UVmax in ethanol 219 (14,500), 236 (12,400) nm. The trienoic acid **1** was produced from this ester by saponification with 2:1 dimethoxyethane-1M-lithium hydroxide at 22°C for 3.5 hr.



Incubation of **1** with soybean lipoxygenase and air at pH 9.2 and 22°C led to time-dependent inactivation of the enzyme.[4] Activity could not be restored by dialysis of the deactivated enzyme. Kinetic analysis of the deactivation rate was carried out, and from a double reciprocal plot of the apparent rate constant for inactivation vs concentration of **1** (3-17 μM) values of K_i = 35 μM and k_i = 0.82 min^{-1} were obtained. These data indicate that **1** is comparable as an inactivator of SB-LO with 13-thiaarachidonic acid[1] (K_i = 0.8 μM, k_i = 0.41 min^{-1}). These inhibitors both seem to owe their effectiveness to the lack of abstractable hydrogen coupled with susceptibility to activation by SB-LO to form a species capable of attacking the catalytic site of the enzyme. The sequence of events summarized for **1** by the above scheme provides a reasonable explanation of our observations and contributes to the current picture of the normal lipoxygenation process.[5]

REFERENCES AND NOTES

1. E.J. Corey, M. d'Alarcao, and S.P.T. Matsuda, *Tetrahedron Letters*, foregoing paper.
2. K. Sonogashira, Y. Tohda, and N. Hagihara, *Tetrahedron Letters*, 4467 (1975).
3. Satisfactory infrared, ultraviolet, pmr and mass spectroscopic data were obtained for new compounds.
4. Experiments with SB-LO were conducted as previously described.[1] Rates of inactivation of SB-LO by **1** were measured by conducting the enzymic reaction in air at pH 9.2 and 22° for varying periods of time and assaying remaining activity by measuring the rate of oxidation of arachidonic acid to 15-HPETE (followed by UV absorbance at 236 nm).
5. This research was supported by the National Institutes of Health.

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