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IRREVERSIBLE INHIBITION OF THE LEUKOTRIENE PATHWAY BY 4,5-DEHYDROARACHIDONIC ACID

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Summary: The 5-lipoxygenase from rat basophilic leukemic cells (RBL-1) is irreversibly deactivated by 4,5-dehydroarachidonic acid in the presence of oxygen.

The recognition that medically important antiinflammatory agents such as aspirin and indomethacin act by blocking the conversion of arachidonic acid to prostaglandins has generated considerable interest in the inhibition of enzymic lipoxygenase reactions. This has been intensified by the recent discovery of the leukotriene pathway which is initiated by an arachidonate 5-lipoxygenase (5-LO) step. Recent studies in this laboratory have demonstrated that several lipoxygenase reactions of arachidonic acid can be inhibited irreversibly by substrate analogs in which the Z-double bond at the site of lipoxygenation is replaced by an acetylenic linkage.[1,2] The irreversible inactivation requires the presence of oxygen and any cofactors which may be needed for the lipoxygenase to function. 5,6-Dehydroarachidonic acid (5,6-DHA) (1) was found to inhibit irreversibly the 5-lipoxygenase of rat basophilic leukemic (RBL-1) cells,[2,3] and in consequence is of great interest as an agent for blocking leukotriene biosynthesis. The inhibitory action of 1 can be understood in terms of conversion to a vinylic hydroperoxide (2) which undergoes fast O-O bond homolysis to radicals which are capable of inactivating the enzyme. Since it seemed reasonable to expect that the allenic substrate 4,5-dehydroarachidonic acid (4,5-DHA) (3) could function as an irreversible 5-LO inhibitor by the same sort of process, we undertook the study which is described herein.

Synthesis of 4,5-DHA (3). Methyl 5,6-epoxyarachidonate (4) (prepared from arachidonic acid[4]) was dissolved in 10:1 formic acid-acetic anhydride and stirred for 12 hr. at 25°.[5] After removal of solvent, the crude mixture of diformate methyl ester and δ-lactone formate was treated with 1 equiv. of sodium methoxide in methanol for 2 hr. at 25°, then neutralized (HOAc) and concentrated. Chromatography on silica gel afforded the diol methyl ester (5) R_f 0.31[6] (92% overall for two steps). Reaction of 5 with 1.1 equiv. of lead tetraacetate in CH_2Cl_2 at -78° for 20 min., filtration through silica gel, and removal of solvent in vacuo gave a mixture of aldehydes, 6 and 7, which was allowed to react directly with 5 equiv. of lithium 5-lithio-4-pentynoate[7] in THF at -40° for 10 min. and then at 0° for 1 hr. The reaction mixture was quenched with water, partially concentrated, and extracted with hexane to

remove neutral impurities. Acidification (10% aq $NaHSO_4$) of the aqueous layer and extraction into ether gave the crude hydroxy acid. Treatment with etheral diazomethane gave after chromatography on silica gel methyl 6-hydroxy-(8Z,11Z,14Z)-eicosatrien-4-ynoate (8), R_f 0.56 (55% overall for 3 steps). Reaction of 8 with 1.2 equiv. of acetic anhydride in pyridine at 55° for 1.5 hr. yielded after concentration and silica gel chromatography the acetate 9, R_f 0.67, in 95% yield. Treatment of 9 with 5.5 equiv. of ethereal $LiCuMe_2$[8] at -78° for 30 min. followed by warming to ca. -30° over 5 min. to effect homogeneity and quenching with MeOH, afforded after filtration and extractive isolation with ether the methyl ester of 4,5-DHA (3),[9] R_f 0.79, in 83% yield after chromatography on silica gel. This ester was stored under argon in frozen benzene at -20° and hydrolyzed (1:1 1M LiOH:THF, 23°, 12 hr.) and diluted with ethanol (to a final concentration of 1 mg./1 ml.) immediately prior to use.

Inhibition of the 5-Lipoxygenase of RBL-1 Cells by 4,5-DHA (3). The 5-LO enzyme utilized in these studies was derived from rat basophilic leukemic (RBL-1) cells which had been broken using a tissue disrupter, further homogenized in a blender and centrifuged at 10,000g. The 5-LO containing supernatant was kept at -78° until needed. 5-LO activity was assayed as follows. The RBL-1 supernatant was diluted four-fold with pH 7 buffer containing 1mM EDTA, 0.1% gelatin, and 14μM indomethacin to inhibit prostaglandin synthetase. Tritiated arachidonic acid (4.0 μCi., 91.5 nmol., 9.2 μM) was added, followed by $CaCl_2$ (2 μM) and the mixture was aerobically incubated at 23° for 5 min. The mixture was acidified to pH 2 with citric acid and extracted with ether. The organic phase was dried, concentrated, and chromatographed by preparative TLC with 10 : 10 : 5 : 2 water-ethyl acetate-heptane-acetic acid as eluent. The 5-HETE (R_f = 0.45) and LTB (R_f = 0.2) bands were collected and their radioactivity was measured by scintillation counting and compared to the total radioactivity extracted from the reaction mixture. 5-LO activity was calculated as the % of total cpm found in the 5-HETE and LTB bands as compared to the same percentage for a parallel control experiment without inhibitor. Under these conditions, in the absence of inhibitor 3, 15-25% of the arachidonic acid was converted to 5-HETE and LTB.

In order to test inhibition of the RBL-1 5-LO by 4,5-DHA the enzyme was aerobically "preincubated" with 4,5-DHA in the absence of arachidonic acid for varying periods of time at 10° after which remaining 5-LO activity was measured by the addition of tritiated arachidonic acid using the assay described above. A linear relationship was found between the time of

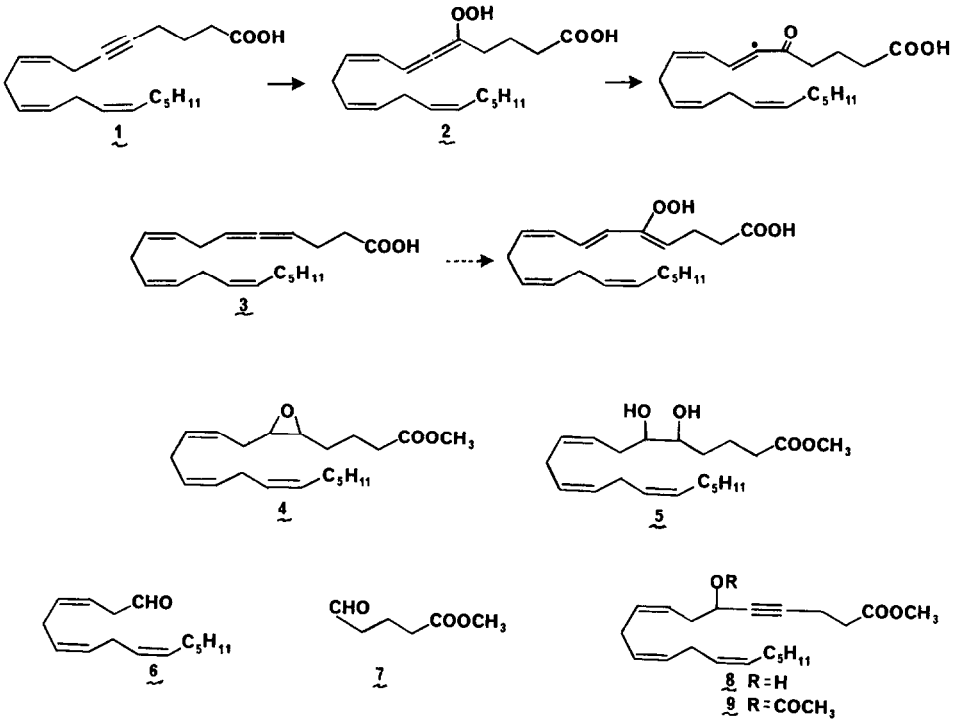

Fig. 1

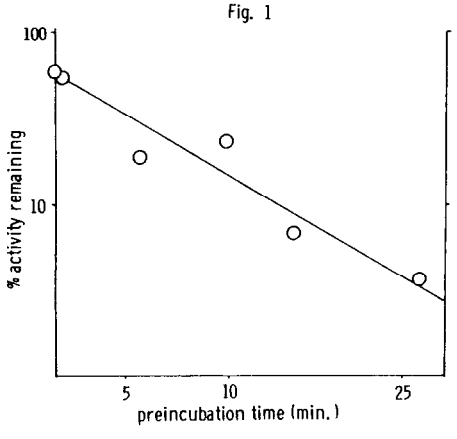

Fig. 2

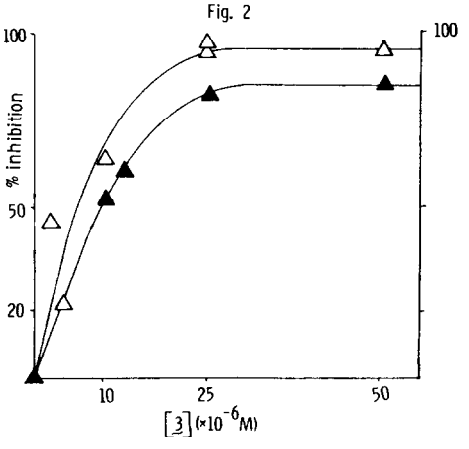

Fig. 1: % 5-LO activity vs. time after preincubation with 3 at 10°C ([AA] = 9.2µM, [3] = 25µM)

Fig. 2: % inhibition vs. concentration of 3 after 10 (▲) and 26 (△) minutes of preincubation

preincubation and the log of the percent of 5-LO activity remaining (fig. 1), indicative of "suicide inhibition."[10] The dependence of % inhibition on the concentration of 4,5-DHA was measured at two different preincubation times (fig. 2). In both cases, saturation kinetics was observed, indicating that the inhibition is active site-directed. The findings with 4,5-DHA and 5,6-DHA are consistent with those obtained with other mono DHA's thus far[1,2] and support the notion that destruction of the enzyme is a consequence of oxidation of the DHA. These results provide an interesting lead for the development of inhibitors of leukotriene biosynthesis which may have therapeutic application.

When 4,5-DHA (3) and substrate were introduced to the RBL-1 cell supernatant simultaneously (preincubation time = 0) significant inhibition (~50%) was observed, whereas 5,6-DHA did not produce appreciable inhibition in a comparable experiment.[3] It appears from this result that unoxidized 4,5-DHA is a competitive inhibitor of the RBL-1 5-LO and is more effective as such than is 5,6-DHA. On the other hand, the rates of oxygen-dependent irreversible deactivation of the 5-LO by 4,5-DHA and 5,6-DHA are nearly the same indicating a similar efficiency in this regard.

Further mechanistic studies of 3 and other 5-LO inhibitors are in progress.[11]

References

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2. E. J. Corey and J. E. Munroe, J. Am. Chem. Soc., 104, 1752 (1982).
3. Extensive studies in this area by Dr. Bennett Lagüzza will be reported elsewhere.
4. E. J. Corey, H. Niwa, and J. R. Falck, J. Am. Chem. Soc., 101, 1586 (1979).
5. All reactions were conducted under an atmosphere of argon. Satisfactory IR, PMR, and mass spectral data were obtained for each intermediate.
6. All R_f values were obtained by thin layer chromatography on silica gel using 1:1 hexane-ethyl acetate unless otherwise indicated.
7. The dianion of 4-pentynoic acid was generated at -40° by adding 2 equiv. ethereal MeLi to a solution of the acid in THF (0.11 M) followed after 2 min. by 1 equiv. HMPA and stirring 15 min.
8. P. Baret, E. Barreiro, A. E. Greene, J.-L. Luche, M.-A. Texeira, and P. Crabbe, Tetrahedron, 35, 2931 (1979).
9. Spectral data for the methyl ester of 3: pmr in $CDCl_3$ (δ): 5.5-5.3 (m, olefin H, 6H), 5.25-5.1 (m, allenic H, 2H), 3.67 (s, OCH_3, 3H), 2.9-2.7 (m, 7, 10, and 13-CH_2, 6H), 2.42 (t, J=7Hz, 2-CH_2, 2H), 2.3 (m, 3-CH_2, 2H), 2.05 (dt, J=7 and 7Hz, 16-CH_2, 2H), 1.4-1.2 (m, 17, 18, and 19-CH_2, 6H), 0.89 (t, J=7Hz, 20-CH_3, 3H); MS: M^+ = 316 (.33), M^+-CH_3 (.85); IR: 1745 $cm.^{-1}$ (ester C=O).
10. C. Walsh, Tetrahedron, 38, 871 (1982).
11. This work was assisted financially by grants from the National Science Foundation and the National Institutes of Health.

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