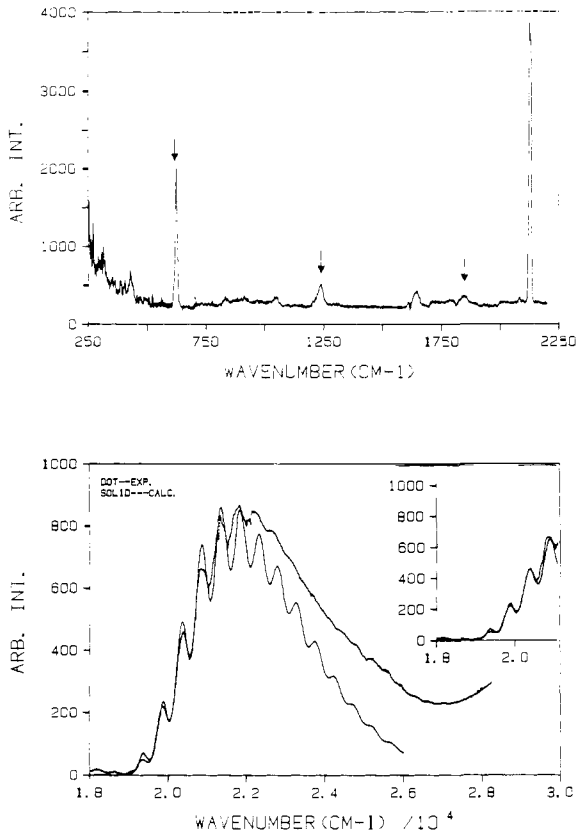

Figure 1. (Top) Raman spectrum of $K_3Cr(CN)_5NO$ excited at 514.5 nm. The arrows indicate the fundamental and first and second overtones of the Cr–N stretching mode. (Bottom) Experimental (dots) and calculated (solid) electronic absorption spectra. Note the presence of a shoulder at 25 000 cm^{-1} and the tail of the next peak at 28 000 cm^{-1}. The spectrum was calculated using $\Gamma = 93$ and the following frequencies and displacements: 400, 1.60; 530, 2.60; 1500, 0.26; 1900 cm^{-1}, 0.80. The insert shows the region near the origin and the spectrum calculated by using *exactly* the Raman-determined displacements, $\Gamma = 105$, and frequencies of 400, 530, 1645, and 2128 cm^{-1}.

Table I. Raman Intensities and Calculated Distortions

ω_k, cm^{-1}	assignment	I_k/I_{624} [b]	Δ_k [c]	Δ_k, [d] Å
428	ν_{CrC} [a]	0.13 ± 0.03	1.37 ± 0.30	0.07
624	ν_{CrN} [a]	1.00 ± 0.20	2.60 ± 0.50	0.10
1645	ν_{NO} [a]	0.07 ± 0.01	0.26 ± 0.05	0.01
2128	ν_{CN} [a]	1.42 ± 0.28	0.90 ± 0.20	0.03
1237	1st overtone	0.18 ± 0.04	2.20 ± 0.40	0.09
1848	2nd overtone	0.09 ± 0.02	N/A	N/A

[a] Assignment from ref 7. [b] Integrated intensity ratios from 514.5-nm excitation. The intensity ratio of the overtone to fundamental is 0.30 when excited at 488 nm. The calculated displacement is 2.0 ± 0.4. The overtone was too weak to be observed under 632.8- and 647.1-nm excitation. [c] Dimensionless normal coordinates. [d] Displacements in Å assuming uncoupled normal modes. More accurate values require a normal coordinate analysis.

signed by Gray et al. to the $^2B_2 \rightarrow {}^2B_2$, metal to NO charge transfer)[7] exhibits resolved vibronic structure. However, the spacing between the vibronic features is not regular. Furthermore, the band is overlapped by additional bands on its high-energy side, which are revealed by polarization studies. The low-energy features of the electronic spectrum, which are unemcumbered by the overlapping bands, can be calculated from the Raman-determined displacements.[4-6] The calculation uses time-dependent theory and the multidimensional excited-state surface obtained from the Raman-determined displacements. An excellent fit to the low-energy features was achieved by using *exactly* the displacements determined from the Raman intensities for the four normal modes. The vibronic features are thus a result of all of the displaced normal modes (i.e., a type of MIME[6,8]) and not the result of a single mode. The calculated electronic spectra provide a further check and verify the accuracy of the Raman-determined displacements.

These results show that overtone intensities can be reliably used to calculate excited-state displacements. These data also shed light on the underlying mechanism giving rise to the overtone intensity. Of the two possible limiting mechanisms derived from time-dependent theory, displaced excited states or vibrational frequency changes in excited states,[3] the former is over 2 orders of magnitude more important than the latter for $Cr(CN)_5NO^{3-}$. Ziegler and Albrecht have discussed seven origins of overtone intensity and have shown that the displaced excited-state mechanism is expected to become important as resonance is approached.[9] The calculation of excited-state displacements from overtone intensities is a new tool in the study of the electronic structure of metal complexes.

Acknowledgment. The support of the U.S. Army Research office is gratefully acknowledged. We also thank Dr. E. J. Heller for helpful discussions.

Registry No. $K_3Cr(CN)_5NO$, 14100-08-4.

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Mechanism of the Irreversible Deactivation of Arachidonate 5-Lipoxygenase by 5,6-Dehydroarachidonate

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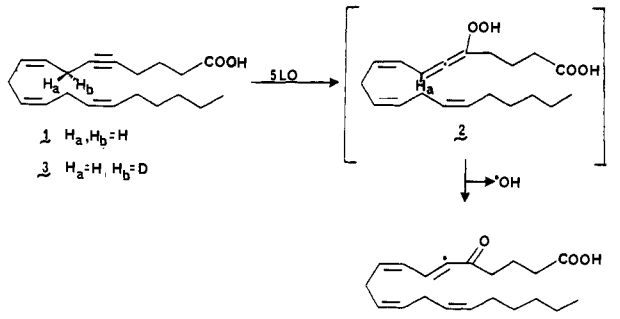
The initial step in leukotriene biosynthesis, conversion of arachidonate to a 5-hydroperoxytetraenoic acid by a 5-lipoxygenation (5-LO) reaction can be inhibited irreversibily in vitro and in whole cells by micromolar concentrations of 5,6-dehydroarachidonate (**1**).[1-5] The time dependence of the degree of inhibition and the requirement of both Ca^{2+} and O_2, each of which is needed for the 5-LO reaction of arachidonate, support the hypothesis that 5-LO inactivation is due to oxidation of **1** to a vinylic hydroperoxide **2** which rapidly decomposes to radicals capable of lethal damage to the catalytic site. The argument is further strengthened



by the finding that 4,5-dehydroarachidonate, an allenic isomer of **1**, causes inactivation of the 5-LO enzyme in the same manner as does **1**.[6] In this communication we show that the rate of 5-LO

(1) Corey, E. J.; Monroe, J. E. *J. Am. Chem. Soc.* **1982**, *104*, 1752.
(2) Corey, E. J.; Park, H. *J. Am. Chem. Soc.* **1982**, *104*, 1750.
(3) Corey, E. J.; Kang, J. *Tetrahedron Lett.* **1982**, *23*, 1651.
(4) Corey, E. J.; *Experientia* **1982**, *38*, 1259.
(5) Razin, E.; Romeo, L. C.; Krillis, S.; Liu, F.-T.; Lewis, R. A.; Corey, E. J.; Austen, K. F. *J. Immunol.*, in press.

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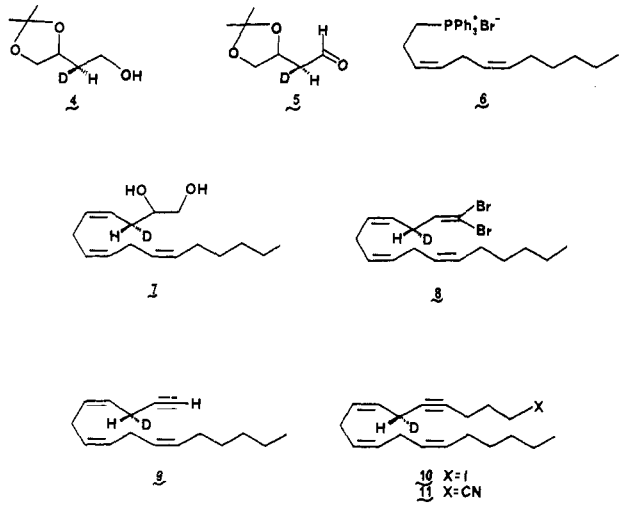
deactivation by **1** is subject to a primary kinetic isotope effect when **1** carries a deuterium 7*R* label at the site of hydrogen removal in the 5-LO reaction of arachidonate.[7] In addition we describe a number of new analogues of 5,6-DHA that are of potential medical value as therapeutic agents for various allergic and inflammatory diseases.

Synthesis of 7(*R*)-Deuterio-5,6-dehydroarachidonate (3). The synthesis of **3** was partly based on the previously described synthesis of chiral 7-deuterioarachidonate.[7] Acetonide alcohol **4**[7] was



oxidized (excess chromium trioxide on zeolite,[9] methylene chloride, 6.5 h, 25 °C) to give aldehyde **5**, which was coupled with the ylide derived from deprotonation (0.97 equiv of sodium hexamethyldisilazide (0.05 M) in 1:1 toluene–tetrahydrofuran (THF), 40 min, 0 °C) of phosphonium salt **6**[7] to afford, after in situ deprotection (1 M hydrochloric acid *in* 10:1 methanol–water, 30 min, 0 °C), diol **7** in 24% yield from **4**. Diol **7** was cleaved with 1.7 equiv of lead tetraacetate (0.02 M **7** in methylene chloride, 2.5 h, −78 °C) to provide the corresponding β,γ-unsaturated aldehyde, which was converted (2.7 equiv of carbon tetrabromide, 5.5 equiv of triphenylphosphine in methylene chloride, 2 h, 0 °C) to dibromoalkene **8** (70%). Treatment of **8** (0.02 M in ether) with 1.96 equiv of *n*-butyllithium[10] (10 min, 0 °C) followed by water gave acetylene **9** (80%) containing 91 ± 3 atom % of deuterium (chemical ionization mass spectrometry using isobutane). Acetylene **9** was converted into the methyl ester of **3** by the sequence (1) lithiation with 1.0 equiv of lithium diisopropylamide (THF, 30 min, 0 °C) and alkylation with 6 equiv of 1,3-diiodopropane (2 h, 25 °C) to form **10**, (2) cyanide displacement of **10** to form **11** using excess sodium cyanide in dimethyl sulfoxide at 25 °C for 20 h, (3) hydration to form amide with excess H_2O_2–0.06 M potassium hydroxide in 2:1 ethanol–water at 25 °C for 15 h, and (4) methanolysis using 1 N sulfuric acid in degassed methanol at 65 °C for 16 h. The ester of **3** was saponified (0.5 M lithium hydroxide in 1:1 water–THF at 25 °C for 4 h) to give **3** quantitatively.[11]

Measurement of 5-Lipoxygenase Deactivation by 1 and 3. The rates of aerobic deactivation of the 5-lipoxygenase from rat basophilic leukemic cells (RBL-1) by 5,6-dehydroarachidonate (**1**) and its 7*R* deuterio form **3** were determined at 22 °C in the concentration range 60–140 μM **1** or **3** by the method used previously[6,12] with the results indicated in Figure 1. A value for

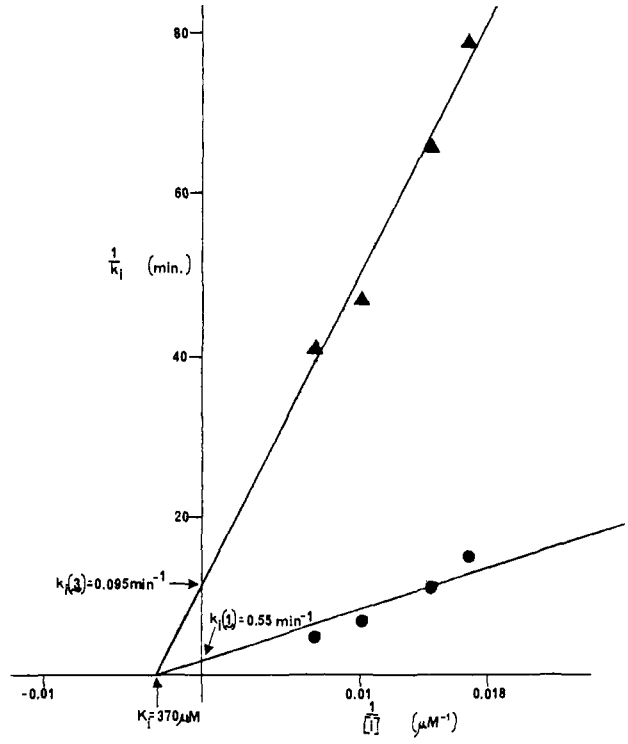


Figure 1. Lineweaver–Burk analysis of 5-LO deactivation by **1** (●) and **3** (▲) at 22 °C.

$k_{inact}(1)/k_{inact}(3)$ of 5.8 ± 1.2 was obtained from a double-reciprocal plot. Calculation of the kinetic deuterium isotope effect from this result using the 91 ± 3 atom % deuterium-labeling value for **3**[13] provides a *lower limit* of 6.0 for k_H/k_D. Our experimental results demonstrate a primary kinetic isotope effect for deactivation of RBL-1 derived 5-lipoxygenase by **3** vs. **1** in the presence of oxygen and Ca^{2+} that is close to that observed[14] with soybean lipoxygenase. We conclude that the inactivation of the enzyme occurs after a 5-lipoxygenase transformation of **1** to an unstable hydroperoxide as originally proposed.[1,2]

Leukotriene Biosynthesis Inhibitors Related to 1. Fatty acids are rapidly incorporated into phospholipid in vivo. For this reason a series of non-carboxylic analogues of **1** were synthesized and tested as irreversible inhibitors of the RBL-1 5-LO enzyme. The rates of aerobic inactivation were obtained for a series of compounds in which the COOH group of **1** is replaced by another functional group. Values for k_{inact}, i.e., rate constant for loss of 5-LO activity (in the presence of inhibitor, O_2 and Ca^{2+}), were determined from the intercept of $1/k_{obsd}$ vs. the reciprocal of inhibitor concentration for each inhibitor at 22 °C over the range 7–100 μM. Each k_{obsd} value was determined from a plot of percent remaining 5-LO activity (using ^3H arachidonate as substrate) vs. time for five–six time points, in duplicate; **1** had a k_{inact} of 0.55 min^{-1}. The rates of inactivation of the RBL-1 5-lipoxygenase by a series of analogues of **1** relative to that for **1** were found to be as follows: $-PO_3H^-$, 0.53; $-SO_3^-$, 0.53; $-P(OCH_3)O_2^-$, 0.67; $-SO_2^-$,

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(7) Corey, E. J.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1983**, *105*, 4093.
(8) Lewis, R. A.; Austen, K. F. *Nature (London)* **1981**, *293*, 103.
(9) Prepared by stirring type L zeolite (K form, 7.1 Å one-dimensional channel) and a solution of $CrO_3 \cdot (py)_2$ in methylene chloride at 20 °C; other uses of this reagent will be described elsewhere.
(10) Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, 3769.
(11) Satisfactory infrared, proton magnetic resonance, and mass spectral data were obtained for each synthetic intermediate from chromatographically purified and homogeneous samples. All temperatures are given in deg Celsius. An argon atmosphere was used for reactions and storage of intermediates.
(12) Corey, E. J.; Shih, C.; Cashman, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3581. For evaluation of time-dependent inhibition the solution of enzyme was preequilibrated with medium in air, the inhibitor **1** or **3** was added at 22 °C and the deactivation was allowed to proceed with gentle agitation for varying periods of time. At the appropriate time tritiated arachidonate was added (34–74 mCi/mmol, final concentration 60 μM) to the incubation vessel. After 7 min of incubation at 35 °C the 5-LO reaction was quenched with cold methanolic trimethyl phosphite and worked up as described above. For calculation of isotope effect it was assumed that **1** and **3** have identical K_i's.
(13) The calculation was made by using the relationship

$$k_{inact}^{obsd}(3) = \%H(k_{inact}^{obsd}(1)) + \%D(k_{inact}^{D});\ k_{inact}^{D}$$

refers to 100% deuterated **3**.
(14) Vliegenthart, J. F. G.; Egmond, M. R.; Veldink, G. A.; Boldingh, J. *Biochem. Biophys. Res. Commun.* **1973**, *54*, 1178.

1.14; $-CONH_2$, 1.26; $-P(=O)(OCH_3)_2$, 1.68; $-S(=O)CH_3$, 2.26; $-S(=O)_2CH_3$, 3.2.[15]

Several of the members of the series are good candidates for in vivo inhibition of leukotriene biosynthesis, including the sulfoxide, sulfone, and amide derivatives indicated. These compounds obviously are not susceptible to incorporation into phospholipid. Clearly, the RBL-1 5-lipoxygenase does not demand that the carboxyl surrogate be an anionic group.[16]

(15) The amide of **1** was prepared by ammonolysis of the methyl ester; all other carboxyl replacement analogues were synthesized from bromoacetylene **10** (undeuterated) by standard methods. Each of the analogues in the series listed here has the structure in which the group indicated replaces the COOH group of **1**.

(16) This research was assisted financially by grants from the National Science Foundation and the National Institutes of Health.

Rationally Designed, Potent Competitive Inhibitors of Leukotriene Biosynthesis

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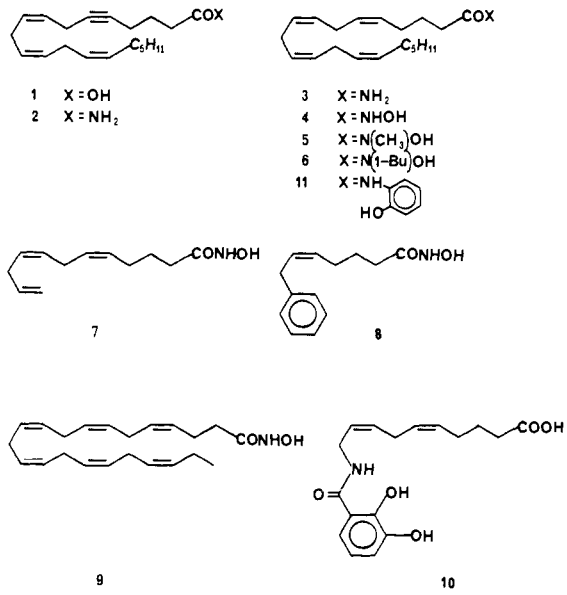
Oxidation of arachidonic acid by various lipoxygenase (LO) enzymes is inhibited by dehydroarachidonic acids having a triple[1] or allenic double[2] bond at the normal site of oxygenation in a time- and oxygen-dependent irreversible process. The preceding paper[3] describes compelling evidence that the inhibition of leukotriene biosynthesis by 5,6-dehydroarachidonate (**1**) is caused by its



conversion to an oxidation product that inactivates the 5-lipoxygenase involved in the initial step. Since a number of analogues of **1** in which the carboxyl group is replaced by various nonanionic groups, e.g., carboxamide **2**, are also potent inactivators of the 5-LO from rat basophilic leukemic (RBL-1) cells, it was apparent that neutral derivatives of arachidonic acid might serve as substrates for, or competitive inhibitors of, this enzyme. Indeed, arachidonamide (**3**) was found to be transformed into the amide of 5(*S*)-hydroxy-6-*trans*,8,11,14-*cis*-eicosatetraenoic acid (5-HETE amide) by aerobic incubation with the RBL-1 enzyme followed by reduction with sodium borohydride. The rate of the 5-LO reaction of arachidonamide was ca. 7% of that of arachidonate. The K_m value for conversion of arachidonate to 5-HPETE is 10 μM, but in the presence of 90 μM arachidonamide the K_m(app) becomes 50 μM with essentially identical V_{max} values, indicative of competitive inhibition. In view of these results and the key role of iron in catalysis by other lipoxygenases,[4] it was decided to study amide analogues of arachidonate in which strong coordination to iron is possible. This working hypothesis has led to the discovery of a family of powerful, competitive inhibitors of the RBL-1 5-LO enzyme.

Table I. Inhibition of the Oxidation of Arachidonate by the 5-Lipoxygenase from RBL-1 Cells

inhibitor	K_m,[a] μM	K_m(app), μM	K_i, μM	EC_{50},[g] μM
4	13.1	28.6[b]	0.13	0.1
5	11.6	24.0[c]	0.04	0.03
6	10	66.0[d]	0.11	0.2
9	10	31.0[e]	0.43	1.2
11	13	48[f]	5.5	12.0

[a] K_m determined by Lineweaver–Burk analysis of the arachidonate → 5-HPETE transformation by RBL-1 5-LO in the absence of inhibitor. K_m(app) determined for the conversion of arachidonate to 5-HPETE in the presence of [b] 2 μM, [c] 0.5 μM, [d] 0.34 μM, [e] 1.05 μM, or [f] 5 μM inhibitor. [g] Effective inhibitor concentration for 50% enzyme inhibition in the presence of 6 μM arachidonate.

The first chelating analogues studied were *N*-hydroxyarachidonamides since *N*-hydroxy amides (pK_a's ca. 7.5) are known to be excellent ligands for Fe(III) (K_{assoc} ca. 10^{12}).[5] The *N*-hydroxy amides **4**, **5**, and **6** were prepared from arachidonic acid via the acid chloride.[6] Inhibition of the oxidation of arachidonate by **4**, **5**, and **6** with the RBL-1 5-LO enzyme was studied kinetically at 35 °C by the method described previously.[7] Values of K_m(app) were determined from a double-reciprocal plot, $1/V$ vs. $1/S$ at different concentrations of inhibitor, to demonstrate the competitive nature of inhibition. Neither **4**, **5**, nor **6** was found to be a substrate for the RBL 5-LO enzyme. Values of K_i were obtained from Dixon plots.[8] EC_{50} determinations were made for 6 μM arachidonate by varying the inhibitor concentration over a range leading to 10–90% inhibition. All three hydroxamates **4**, **5**, and **6** are powerful inhibitors of the 5-LO reaction of arachidonate as is indicated by the data in Table I. The inhibition of the 5-LO enzyme by **4**, **5**, and **6** was time independent. To our knowledge no leukotriene biosynthesis inhibitors of comparable potency have been reported. On a molar basis **4**, **5**, and **6** are roughly 100 000 times more potent as inhibitors of leukotriene biosynthesis than is aspirin as an inhibitor of prostaglandin biosynthesis.

It seems reasonable that the hydroxamate function in **4**, **5**, and **6** might be well positioned to coordinate with a catalytically crucial metal ion. The full eicosanoid chain is not required for strong inhibition, as shown by the fact that the synthetic hydroxamates **7** and **8** were found to be very good inhibitors of the 5-LO reaction

(1) (a) Corey, E. J.; Monroe, J. E. *J. Am. Chem. Soc.* **1982** *104*, 1752. (b) Corey, E. J.; Park, H. *Ibid.* **1982**, *104*, 1750. (c) Corey, E. J. *Experientia* **1982**, *38*, 1259.

(2) Corey, E. J.; Kantner, S. S.; Lansbury, P. T., Jr. *Tetrahedron Lett.* **1983**, *24*, 265.

(3) Corey, E. J.; Lansbury, P. T., Jr.; Cashman, J. R.; Kantner, S. S. *J. Am. Chem. Soc.*, preceding paper in this issue.

(4) See: Pistorius, E. K.; Axelrod, B. *J. Biol. Chem.* **1974**, *249*, 3183.

(5) (a) Neilands, J. B. In "Inorganic Biochemistry"; Eichorn, G., Ed.; Elsevier: New York, 1973; p 167. (b) Chatterjee, B. *Coord. Chem. Rev.* **1978**, *26*, 281.

(6) The acid chloride was synthesized by reaction of arachidonic acid in benzene with 2 equiv of oxalyl chloride in the presence of 1 equiv of dimethylformamide and converted to **4** and **5** by reaction at 0 °C with the appropriate hydroxylamine in 2:1 tetrahydrofuran–water (90% yield). The hydroxylamine **6** was synthesized by reaction of arachidonyl chloride with *N*-*tert*-butylacetoxyamine and subsequent alkaline deacetylation (see: Alewood, P. F.; Hussain, S. A.; Jenkins, T. C.; Perkins, M. J.; Sharma, A. H.; Slew, N. P. Y.; Ward, P. *J. Chem. Soc., Perkin Trans. 1* **1978**, 1066. We are indebted to Alan Barton for the preparation of **6**. Satisfactory spectroscopic data were obtained for all new compounds.

(7) Corey, E. J.; Shih, C.; Cashman, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3581.

(8) Dixon, M. *Biochem. J.* **1953**, *55*, 170.

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