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**.

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Rationally Designed, Potent Competitive Inhibitors of Leukotriene Biosynthesis

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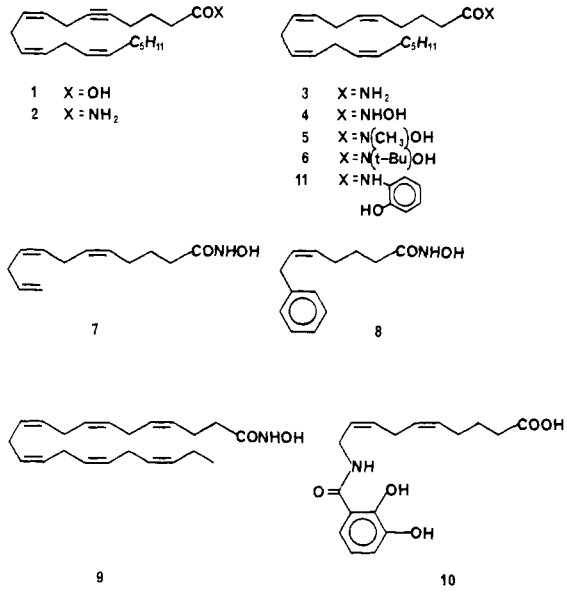
Oxidation of arachidonic acid by various lipoxygenase (LO) enzymes is inhibited by dehydroarachidonic acids having a triple[1] or allenic double[2] bond at the normal site of oxygenation in a time- and oxygen-dependent irreversible process. The preceding paper[3] describes compelling evidence that the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

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(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.

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of arachidonate, displaying EC_{50} values of 1.9 and 15 μM, respectively, at 6 μM arachidonate concentration (35 °C).[9]

Kinetic studies with **9** showed that it inhibits the 5-LO enzyme (Table I) less well than **4** as expected from previous work.[7]

The 5-LO inhibitory action of **7** and **8** prompted the study of an entirely different metal-chelating analogue of arachidonate, the 2,3-dihydroxybenzamide derivative **10**. The 2,3-dihydroxybenzoyl group is known to possess high affinity for Fe(III). In fact, **10** was found to be a potent 5-LO inhibitor, with EC_{50} of 10 μM.[10]

Since a number of antioxidants that serve as hydrogen atom donors can function as lipoxygenase inhibitors,[11] the phenolic arachidonamide **11** was synthesized and tested as a 5-LO inhibitor. It proved to have activity (Table I), but it was considerably less effective than the hydroxamates **4-6**.

The studies reported herein provide important new guidance for the design of potent leukotriene biosynthesis inhibitors.[12,13]

(9) The synthesis of **7** was accomplished from 3(*Z*),6-heptadien-1-ol via the corresponding triphenylphosphonium bromide through a Wittig coupling with methyl 4-formylbutyrate (6:1 tetrahydrofuran–hexamethylphosphoric triamide at −78 °C) and subsequent conversion of the resultant methyl ester to **7** using hydroxylamine–sodium methoxide in methanol at 25 °C. The synthesis of **8** was conducted in a similar way starting with (2-phenylethyl)-triphenylphosphonium bromide and methyl 4-formylbutyrate.

(10) The synthesis of **10** was carried out from methyl 10-aminodeca-5-(*Z*),8(*Z*)-dienoate by (1) reaction with the cyclic sulfite of 2,3-dihydroxybenzoyl chloride (from the dihydroxybenzoic acid and thionyl chloride) and (2) hydrolysis with lithium hydroxide in tetrahydrofuran–water at 20 °C. The aminodecadienoate ester was synthesized from $THPOCH_2C{\equiv}CCH_2CH_2OH$ by the sequence (1) $CH_2OH \rightarrow CH_2I \rightarrow CH_2P^+Ph_3I^-$, (2) Wittig coupling of the phosphonium iodide with methyl 4-formylbutyrate, (3) CH_2–OTHP $\rightarrow CH_2OH \rightarrow CH_2OSO_2CH_3 \rightarrow CH_2N_3 \rightarrow CH_2NH_2$, and (4) hydrogenation of C≡C to *cis*-CH=CH using Lindlar catalyst.

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(13) This research was supported in part by the National Institutes of Health and the National Science Foundation.

Table I[a]

% RuO_2	$\chi_m(RuO_2) \times 10^6$	$\mu(RuO_2)$	*T*, K
10.0	223	0.52	150.8
	200	0.56	199.0
	185	0.60	247.1
	178	0.65	295.1
7.5	337	0.64	151.1
	296	0.69	199.3
	273	0.73	247.5
	260	0.78	295.4
5.0	488	0.77	150.6
	425	0.82	198.7
	385	0.87	246.9
	365	0.93	295.1
3.0	332	0.63	149.8
	286	0.67	198.2
	256	0.71	246.5
	244	0.76	294.6
2.0	378	0.67	150.6
	315	0.71	198.8
	276	0.74	247.0
	264	0.79	294.9
1.0	450	0.74	150.8
	372	0.77	198.9
	317	0.79	247.0
	378	0.94	295.0
pure RuO_2, ref 9, p 341	165		149.0
	164		163.0
	165		190.0
	164		236.0
	165		255.0
	168		297.0

[a] $\chi_g(TiO_2) = 0.043 \times 10^{-6}$ cgsu/g (Figure 1). $\chi_g^{dia}(RuO_2) = -0.323 \times 10^{-6}$ cgsu/g (ref 9).

Quantitative Studies on the Paramagnetic Behavior of RuO_2–TiO_2 (Anatase) Powders Catalytically Active in Water Oxidation

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Transition-metal ion such as Ce^{4+} and Fe^{3+} in $HClO_4$ media oxidize water[1] to oxygen upon photolysis in a photoassisted reaction. Ce^{4+} in the presence of a macrodisperse RuO_2 redox catalyst[2-4] (in the dark) has been shown to be capable of oxidizing water as shown by eq 1. Recently, studies on RuO_2 systems

$$4Ce^{4+} + 2H_2O \xrightarrow{RuO_2\ catalyst} 4Ce^{3+} + 4H^+ + O_2 \qquad (1)$$

stabilized by TiO_2[5] have shown that TiO_2 is a suitable host to stabilize RuO_2. The purpose of this communication is to report in detail the paramagnetic properties of RuO_2–TiO_2 powders which are catalytically active in mediating water oxidation. The necessity of examining more closely the nature of these surfaces arises from the fact that the exact nature of the material intervening in the reaction determines the observed efficiency in water cleavage.[2,4,5]

Magnetic susceptibility of the samples was measured with a Faraday balance from Oxford Instruments. All powder samples were mechanically pressed to avoid alignment along a principal magnetization axis of the crystallites in the powder parallel to the applied magnetic field.[6] Thirty-milligram samples were sufficient to obtain sizeable effects, and all measurements were carried out in a field of 5.41 T with gradients of 0.3, 0.5, and 0.7 T/cm. The magnetic field was calibrated with $HgCo(NCS)_4$ at 273 K.[7] In the present study, chemical hydrolysis of $RuCl_3$ under controlled conditions has been used to produce islands of ruthenium dioxide. Details of this preparation have been previously reported.[5e]

Figure 1 shows the magnetic susceptibility (χ_g') per gram of RuO_2/TiO_2 sample vs. the temperature employed during the runs. From this figure, it is readily seen that, as usual for paramagnetic species,[8,9] the magnetic susceptibility per gram (χ_g') decreases as the temperature increases.

Table I shows the values for some of the runs shown in Figure 1, for the magnetic susceptibility and magnetic moment of RuO_2.

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[‡] The City University.
[§] Ecole Polytechnique Fédérale.

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