that the total concentration of the α-cyanonicotines was 50 μM. The deuterium composition of the unmetabolized substrate was determined by the same GC-EIMS procedure. (*S*)-Cotinine analyses were performed on 1-mL aliquots of the incubation mixtures according to the procedure described by Jacob et al.[16]

Acknowledgment. We thank Drs. J. Trudell (Stanford University, Stanford, CA) and L. Waskell (Veteran's Administration Hospital, San Francisco, CA) for human liver tissue; Dr. F. Kadlubar (National Center for Toxicological Research, Jefferson, AR) for human liver microsomal preparations; Dr. N. L. Benowitz, Dr. P. Jacob, III, and C. Savanapridi (Clinical Pharmacology Unit, San Francisco General Hospital, San Francisco, CA) for cotinine analyses; and E. Roitman for technical assistance. We also acknowledge the Bio-organic, Biomedical Mass Spectrometry Resource at the University of California, San Francisco (A. L. Burlingame, Director) supported by NIH Division of Research Resources Grant RR01614. This work was supported by NIH Training Grant GMO-7175 and NCI Grant CA-35678.

Registry No. **1**, 54-11-5; **2**, 105090-00-4; **2** (bisperchlorate), 71014-67-0; **3**, 486-56-6; **7**-d_1, 105089-99-4; **8**-d_1, 105120-31-8; **9**, 105120-32-9; **10**, 105089-96-1; **10** (bis-*l*-tartrate), 105089-97-2; **12**, 105089-98-3; cytochrome P-450, 9035-51-2.

Structural Requirements for the Inhibition of 5-Lipoxygenase by 15-Hydroxyeicosa-5,8,11,13-tetraenoic Acid Analogues

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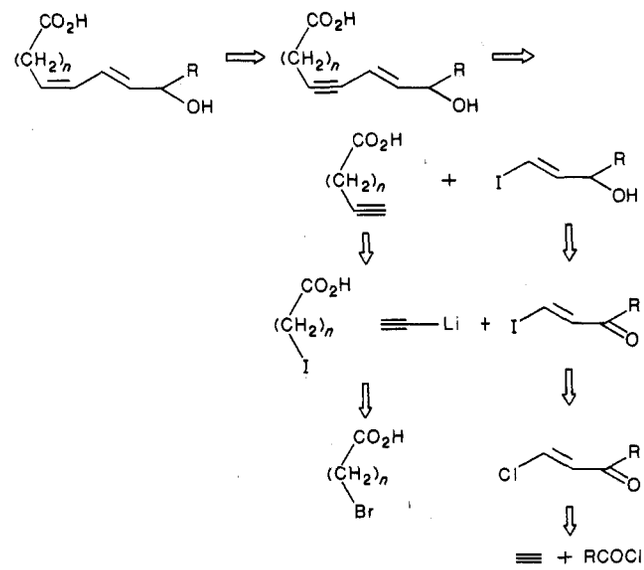
The structural requirements for inhibition of RBL-1 (rat basophilic leukemia) 5-lipoxygenase by 15-hydroxyeicosa-5,8,11,13-tetraenoic acid (15-HETE, **1**) were studied by systematic chemical modifications of the molecule at the hydroxyl and carboxyl groups, the double bonds, and the carboxylate and ω side chains. The most potent inhibitors were analogues that contained a 5,8-*cis*,*cis*-diene system and acted as alternate substrates for the enzyme. However, several analogues in which the 5,8-diene had been reduced were also found to inhibit the enzyme. Inhibition of 5-lipoxygenase by 15-hydroxyeicosa-11,13-dienoic acid (15-HEDE) analogues was optimal in compounds that generally contained a free carboxyl group, a carboxylate side chain of nine carbons, an ω side chain of five or six carbons, a *cis*,*trans*- or *trans*,*cis*-11,13-diene or 11,13-diyne system, and a 15-hydroxyl group. Conversion of 15-HEDE to its 16-membered lactone reduced but did not eliminate 5-lipoxygenase inhibitory activity. In contrast, a 3- to 10-fold enhancement of activity occurred when 5,15-diHETE (**58**) or 5-HETE (**56**) were cyclized to their respective δ-lactones. Molecular modeling of 15-HEDE analogues, modified in the C_{11}–C_{15} region, showed that inactive analogues protrude into regions in space not occupied by active analogues. These structural studies indicate that multiple regions are important for 5-lipoxygenase inhibition by both 15-HETE and 15-HEDE analogues and that no single region plays a predominant role in inhibition.

Several laboratories have reported that 15-hydroxyeicosa-5,8,11,13-tetraenoic acid (15-HETE) is a potent inhibitor of platelet 12-lipoxygenase[1] and neutrophil 5-lipoxygenase.[2-5] Since neutrophils generate both 15-HETE and 5-HETE,[6] a possible regulatory role for 15-HETE in the control of cellular 5-lipoxygenase activity has been suggested.[1,5] Additionally, the formation of 5,15-diHETE, when 15-HPETE was incubated with human neutrophils,[4] led to the suggestion that 15-HPETE and possibly 15-HETE could serve as alternate substrates for 5-lipoxygenase. These interesting findings prompted us to investigate the structural features of 15-HETE required for 5-lipoxygenase inhibitory activity as departure point for the design of therapeutically useful 5-lipoxygenase inhibitors.

Scheme I



Synthesis

Gram quantities of 15-HETE (**1**) and analogues **7**, **8**, **10**, **12**, and **14** were prepared from the corresponding ω6 fatty

(1) Vanderhoek, J. Y.; Bryant, R. W.; Bailey, J. M. *J. Biol. Chem.* **1980**, *255*, 5996.
(2) Vanderhoek, J. Y.; Bryant, R. W.; Bailey, J. M. *J. Biol. Chem.* **1980**, *255*, 10064.
(3) Vanderhoek, J. Y.; Bryant, R. W.; Bailey, J. M. *Biochem. Pharmacol.* **1982**, *31*, 3463.
(4) Sok, D. E.; Han, C. Q.; Shieh, W. R.; Zhou, B. N.; Sih, C. J. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 1363.
(5) Sok, D. E.; Han, C. Q.; Pai, J. K.; Sih, C. J. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 101.
(6) Abbreviations: 5-HETE, 5-hydroxy-6,8,11,14-eicosatetraenoic acid; 15-HPETE, 15-hydroperoxyeicosatetraenoic acid; 5,15-diHETE, 5,15-dihydroxy-6,8,11,13-eicosatetraenoic acid; 15-HEDE, 15-hydroxy-11,13-eicosadienoic acid.

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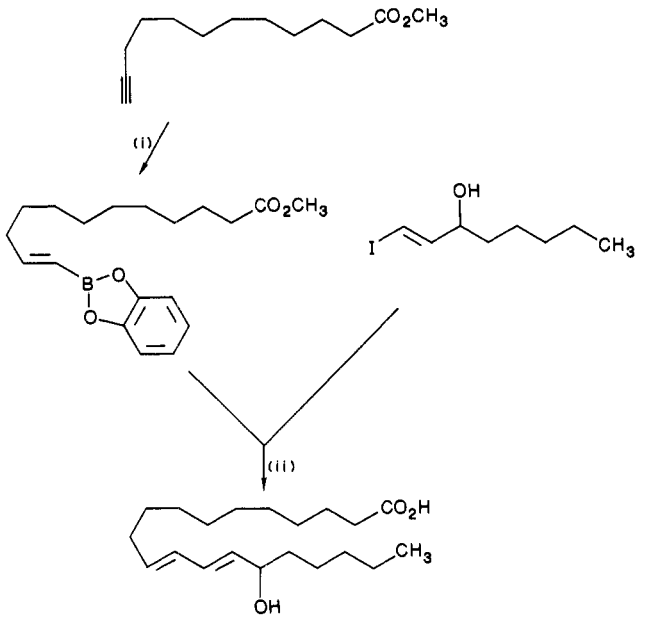
Scheme II[a]



[a] (i) Catecholborane, THF; (ii) $Pd(PPh_3)_4$, THF, 1 N NaOH.

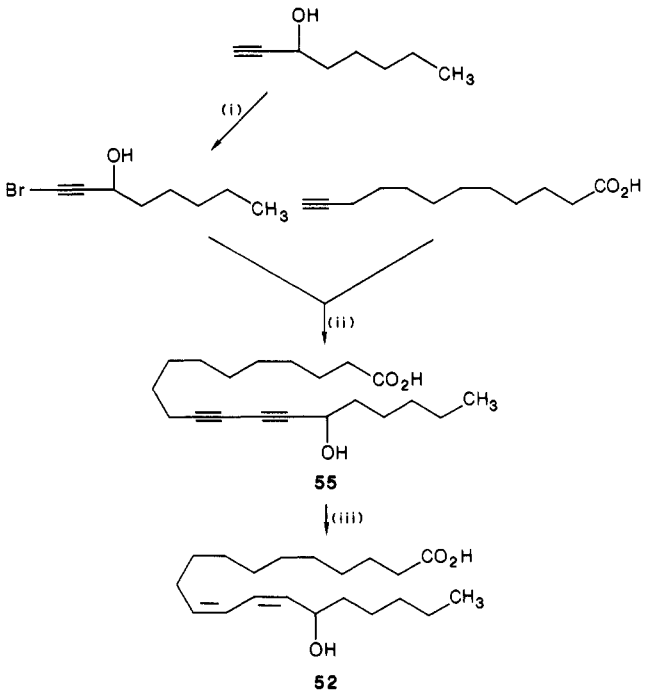
acids with soybean lipoxygenase, followed by sodium borohydride reduction and chromatographic purification.[7] 5-HETE (**56**) was similarly converted to 5,15-diHETE (**58**). The 15-HETE derivatives **4–6** were prepd. by using conventional methods. 5,15-DiHETE easily cyclized to the δ-lactone **59** upon heating under reflux in benzene in the presence of *p*-toluenesulfonic acid.

Although the soybean lipoxygenase catalyzed reaction was a convenient method to obtain several 15-HETE analogues, the synthesis of the many desired analogues by this method was not feasible because of the limited commercial availability of fatty acid substrates containing a *cis,cis*-1,4-pentadiene system at the ω 6-position, as required by the soybean enzyme. Therefore, a versatile procedure for the total chemical synthesis of these compounds was devised.

A retrosynthetic analysis of the 15-hydroxyeicosa-11,13-dienoic acid (**8**) molecule revealed that a feasible method to construct the system was through the coupling of fatty acids containing a terminal acetylene with 2-hydroxyvinyl halides to give the hydroxy enyne intermediates (Scheme I). The hydroxy enynes could subsequently be reduced to the desired hydroxy *trans,cis*-dienes. The preferred method to effect this coupling was use of tetrakis(triphenylphosphine)palladium(0) and copper(I) iodide in diethylamine at room temperature.[8] This method produced high and reproducible yields under mild conditions and did not require protecting the hydroxyl or carboxyl group. Nicolaou and co-workers have reported similar findings.[9]

The palladium-complex-assisted coupling reaction also worked well for the synthesis of **50**, wherein the geometry of the 11,13-diene is trans,cis. For the synthesis of the trans,trans isomer **51**, the coupling reaction conditions were modified.[10] (1*E*)-11-Carbomethoxy-1-undecenyl-

Scheme III[a]



[a] (i) NaBrO, H_2O, 0 °C; (ii) CuCl, NH_2OH·HCl, $EtNH_2$–H_2O, CH_3OH; (iii) three steps: esterification with CH_2N_2, reduction with Fe powder in 1-propanol–water, and hydrolysis with LiOH.

catecholborane was coupled with 3-hydroxy-1-octenyl iodide in the presence of tetrakis(triphenylphosphine)-palladium(0) and 1 N sodium hydroxide in THF at 65 °C to give **51** (Scheme II).

The palladium-complex-assisted reaction gave diyne **55** in a poor yield when 1-bromo-1-octyn-3-ol was reacted with 11-dodecynoic acid. A higher yield was obtained when the reaction was carried out in methanol–water in the presence of Copper(I) chloride, hydroxylamine hydrochloride, and ethylamine[11] (no palladium complex was used). The catalytic hydrogenation of diyne **55** using Lindlar catalyst or nickel boride failed to give the desired diene **52**. However, when the methyl ester of **55** was treated with iron powder in *n*-propyl alcohol and water and heated under reflux, according to Morris et al.,[12] a moderate yield of the methyl ester of **52** was obtained (Scheme III).

Compound **62** was prepared from 3-hydroxybenzaldehyde through a Grignard reaction of iodopentane with 3-hydroxybenzaldehyde followed by alkylation of the resulting phenol with bromooctanoic acid. Compounds **16** and **34** easily cyclized to give lactones **60** and **61**, respectively, upon mild treatment with diethyl azodicarboxylate and triphenylphosphine in benzene at room temperature.[13]

Biological Results and Discussion

Inhibitory Activities of 15-HETE Analogues. 15-HETE (**1**) inhibited RBL-1 5-lipoxygenase with an IC_{50} value of 7.3 μM. Substituting hydroperoxy for 15-hydroxy increased activity 2-fold (**3**), whereas other modifications at position 15 (i.e., 15-keto **4** and 15-acetoxy **5**) reduced activity. Since methyl ester **2** was hydrolyzed to 15-HETE in the RBL-1 5-lipoxygenase enzyme preparation, no conclusion could be made about the inhibitory activity of the ester compared to the acid. Lengthening the carboxylate side chains by two carbons (**10**) decreased activity,

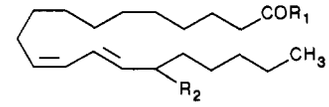
(7) Baldwin, J. E.; Daires, D. I.; Hughes, L.; Gutteridge, N. J. A. *J. Chem. Soc., Perkin. Trans. 1* **1979**, 115.
(8) Jeffrey-Luong, T.; Linstrumelle, G. *Synthesis* **1983**, 32.
(9) Nicolaou, K. C.; Zipkin, R. E.; Dolle, R. E.; Harris, B. D. *J. Am. Chem. Soc.* **1984**, *106*, 3548.
(10) Cassani, G.; Massardo, P.; Piccardi, P. *Tetrahedron Lett.* **1983**, *24*, 2513.
(11) Villemin, D.; Cadiot, P.; Kuetegan, M. *Synthesis* **1984**, 230.
(12) Morris, S. G.; Magidman, P.; Herb, S. F. *J. Amer. Oil Chem. Soc.* **1972**, *49*, 505.
(13) Trost, B. M.; McDougal, P. G. *J. Org. Chem.* **1984**, *49*, 458.

Table I. Inhibitory Activites of (5*Z*,8*Z*,11*Z*,13*E*)-15(*S*)-Hydroxyeicosa-5,8,11,13-tetraenoic Acid (15-HETE) Analogues on RBL-1 5-Lipoxygenase

compd	abbreviated structure	double bond positions	yield, %	formula	IC_{50} (95% CL),[a] μM
1	15(*S*)-OH-20:4	5,8,11,13	80	$C_{20}H_{32}O_3$	7.3 (6.8–7.8)
2	15(*S*)-OH-20:4,Me	5,8,11,13	95	$C_{21}H_{34}O_3$	64 (57–70)
3	15(*S*)-OOH-20:4	5,8,11,13	92	$C_{20}H_{32}O_4$	3.4 (3.0–3.7)
4	15-keto-20:4	5,8,11,13	40	$C_{20}H_{30}O_3$	26 (3.0–3.7)
5	15(*S*)-OAc-20:4	5,8,11,13	77	$C_{22}H_{34}O_4$	17 (15–19)
6	15(*S*)-OAc-20:4,Me	5,8,11,13	70	$C_{23}H_{36}O_4$	7.2 (6.3–8.2)
7	15(*S*)-OH-20:3	8,11,13	35	$C_{20}H_{34}O_3$	77 (68–89)
8	15(*S*)-OH-20:2	11,13	95	$C_{20}H_{36}O_3$	26 (24–27)
9	15(*S*)-OH-20:0		60	$C_{20}H_{40}O_3$[b]	NS @ 100 μM
10	17(*S*)-OH-22:4	7,10,13,15	70	$C_{22}H_{36}O_3$	62 (51–79)
11	17(*S*)-OH-22:4,Me	7,10,13,15	90	$C_{23}H_{38}O_3$	6.5 (5.5–8.6)
12	13(*S*)-OH-18:2	9,11	60	$C_{18}H_{32}O_3$	82 (76–87)
13	13(*S*)-OH-18:2,Me	9,11	98	$C_{19}H_{34}O_3$	34% @ 300 μM
14	13(*S*)-OH-18:3	6,9,11	85	$C_{18}H_{30}O_3$	29 (25–32)
15	13(*S*)-OH-18:3,Me	6,9,11	90	$C_{19}H_{32}O_3$	94 (69–350)
phenidone (1-phenyl-3-pyrazolidinone)					5.9 (5.6–6.2)

[a] The concentration estimated to produce 50% inhibition with 95% confidence limits in parentheses. Compouds that failed to cause 50% inhibition are listed as the percent inhibition obtained at the highest soluble tested concentration. NS = no significant activity. [b] Mp 68–72 °C.

Table II. Effects of Modifications of the Hydroxyl and Carboxyl Groups on the Inhibitory Activity of (11*Z*,13*E*)-15(*S*)-Hydroxyeicosa-11,13-dienoic Acid (15-HEDE) on RBL-1 5-Lipoxygenase



compd	R_1	R_2	yield, %	formula	IC_{50} (95% Cl),[a] μM
8 (15(*S*)-HEDE)	OH	OH	Table I	Table I	26 (24–27)
16 (15-HEDE)	OH	OH	30	$C_{20}H_{36}O_3$	35 (33–38)
17 (15(*S*)-HEDE-Me)	OCH_3	OH	95	$C_{21}H_{38}O_3$	34% @ 300 μM
18	NEt_2	OH	60	$C_{24}H_{45}NO_2$	39% @ 30 μM
19 (*S* isomer)	OH	$OCOCH_3$	60	$C_{22}H_{38}O_4$	76 (67–93)
20	OH	=O	50	$C_{20}H_{34}O_3$	55 (47–71)
21	OH	H	73	$C_{20}H_{36}O_2$	16% @ 100 μM

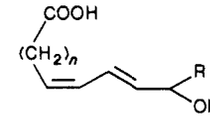
[a] The concentration estimated to produce 50% inhibition with the 95% confidence limits in parentheses. Compounds that failed to cause 50% inhibition are listed as the percent inhibition obtained at the highest soluble tested concentration.

as did selective saturation of the 5 or 5 and 8 double bonds (**7** and **8**). 5,15-DiHETE (**58**) was much less active than 15-HETE (**1**) (IC_{50} = 270 μM).

Inhibitory Activities of 15-HEDE Analogues. Our early investigations of 15-HETE analogues showed that compounds that contained a *cis,cis*-5,8-diene system inhibited RBL-1 5-lipoxygenase by serving as alternate substrates.[14] Subsequent chemical efforts were directed toward the synthesis of 15-HETE analogues that lack the *cis,cis*-5,8-diene system.

The prototype of this series, 15-hydroxy-11,13-eicosadienoic acid (15-HEDE,[6] **8**), inhibited the enzyme with an IC_{50} value of 26 μM. The inhibitory activity of racemic 15-HEDE (**16**) and its *S* isomer (**8**) were not significantly different (Table II), suggesting there is no stereospecific requirement of the 15-hydroxyl group for enzyme inhibition. Acetylation (**19**) or oxidation (**20**) of the hydroxyl group decreased activity 2- to 3-fold, whereas deoxygenation (**21**) destroyed activity (Table II). Esterification of the carboxyl group (**17**) substantially reduced activity, whereas amidation (**18**) had less effect. The optimal length of the carboxylate chain is between seven and 10 carbons, shortening beyond this length caused almost complete loss of activity (Table III). The ω chain can be between five or six carbons long (**16** and **29**); conformational restriction into a cyclopentyl ring (**28**) reduced but did not eliminate activity. The ω chain apparently interacts with a hyrophobic site, since analogues containing hydrophilic groups

Table III. Effects of Variations of the Length of the Carboxylate Side Chain and Modifications of the ω Side Chain on the Inhibitory Activity of (11*Z*,13*E*)-15-Hydroxyeicosa-11,13-dienoic Acid (15-HEDE) on RBL-1 5-Lipoxygenase



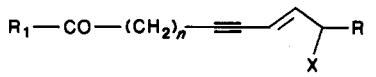
compd	*n*	R	yield, %	formula	IC_{50} (95% CL),[a] μM
22	10	C_5H_{11}	16	$C_{21}H_{38}O_3$	41% @ 30 μM
16	9	C_5H_{11}	Table II	Table II	35 (33-38)
23	8	C_5H_{11}	30	$C_{19}H_{34}O_3$	39 @ 100 μM
12	7	C_5H_{11}	Table I	Table I	82 (76-87)
24	6	C_5H_{11}	35	$C_{17}H_{30}O_3$	24% @ 10 μM
25	5	C_5H_{11}	28	$C_{16}H_{28}O_3$	17% @ 100 μM
26	4	C_5H_{11}	37	$C_{15}H_{26}O_3$	NS @ 100 μM
27	9	C_3H_7	31	$C_{18}H_{32}O_3$	160 (130-190)
28	9	CH_2-c-C_5H_9	41	$C_{21}H_{36}O_3$	61 (56-66)
29	9	C_6H_{13}	33	$C_{21}H_{38}O_3$	49% @ 40 μM
30	9	$C_4H_8CO_2CH_3$	42	$C_{21}H_{36}O_5$	31% @ 100 μM
32	9	$C_4H_8CH_2OH$	28	$C_{20}H_{36}O_4$	46% @ 100 μM

[a] The concentration estimated to produce 50% inhibition with 95% confidence limits in parentheses. Compounds that failed to cause 50% inhibition are listed as the percent inhibition obtained at the highest soluble tested concentration. NS = no significant activity. [b] Mp 58–60 °C.

such as carboxyl, carbomethoxy, and hydroxylmethyl (**31**, **30**, and **32**) were substantially less active than 15-HEDE (Table III). Inverting the geometry of the double bonds in 15-HEDE from a cis,trans to trans,cis (**50**) or trans,trans

(14) Dyer, R. D.; Haviv, F.; Hanel, A. M.; Bornemeier, D. A.; Carter, G. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1984**, *43*, 1462.

Table IV. Inhibitory Activities of (11*Z*,13*E*)-15-Hydroxyeicosa-13-en-11-ynoic Acid Analogues on RBL-1 5-Lipoxygenase



compd	*n*	R_1	R	X	yield, %	formula	IC_{50} (95% CL),[a] μM
33	10	OH	C_5H_{11}	OH	88	$C_{21}H_{36}O_3$	24% @ 30 μM
34	9	OH	C_5H_{11}	OH	91	$C_{20}H_{34}O_3$	66 (60–74)
35	8	OH	C_5H_{11}	OH	88	$C_{19}H_{32}O_3$	73 (63–90)
36	7	OH	C_5H_{11}	OH	85	$C_{18}H_{30}O_3$	24% @ 100 μM
37	6	OH	C_5H_{11}	OH	86	$C_{17}H_{28}O_3$	18% @ 100 μM
38	5	OH	C_5H_{11}	OH	88	$C_{16}H_{26}O_3$	NS @ 100 μM
39	4	OH	C_5H_{11}	OH	93	$C_{15}H_{24}O_3$	NS @ 100 μM
40	8	OH	C_3H_7	OH	97	$C_{17}H_{28}O_3$	46% @ 300 μM
41	9	OH	C_3H_7	OH	91	$C_{18}H_{30}O_3$	260 (220–320)
42	9	OH	CH_2-c-C_5H_9	OH	85	$C_{21}H_{34}O_3$	82 (71–102)
43	9	OH	C_6H_{13}	OH	88	$C_{21}H_{36}O_3$	32 (28–39)
44	9	OH	$C_4H_8CO_2CH_3$	OH	65	$C_{21}H_{34}O_5$	22% @ 100 μM
45	9	OH	$C_4H_8CO_2H$	OH	78	$C_{20}H_{32}O_5$	NS @ 100 μM
46	8	OH	C_5H_{11}	H	66	$C_{19}H_{32}O_2$	33% @ 30 μM
47	9	OH	C_5H_{11}	H	35	$C_{20}H_{34}O_2$	48% @ 100 μM
48	9	OH	C_5H_{11}	$OCOCH_3$	75	$C_{22}H_{36}O_4$	40% @ 100 μM
49	9	NEt_2	C_5H_{11}	OH	40	$C_{24}H_{43}NO_2$	49% @ 30 μM

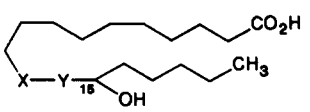
[a] The concentration estimated to produce 50% inhibition with 95% confidence limits in parentheses. Compounds that failed to cause 50% inhibition are listed as the percent inhibition obtained at the highest soluble tested concentration. NS = no significant activity.

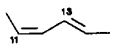
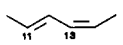
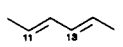
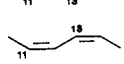
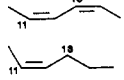
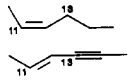
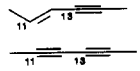
system (**51**) did not significantly affect activity, whereas the cis,cis system (**52**) had reduced activity (Table V). Enyne **43** and diyne **55** had activity in the range of 15-HEDE (Tables IV and V). Compound **62**, a conformationally constrained analogue of 15-HEDE in which double bonds 11 and 13 had been incorporated into a phenyl ring, was inactive (Table VI).

Cyclization of 15-HEDE to the macrocyclic lactone **60** caused partial loss of activity. Similar results were obtained upon lactonization of enyne **34**. On the contrary, cyclization of 5,15-diHETE (**58**) or 5-HETE (**56**) to their respective δ-lactones gave compounds with 3- to 10-fold greater potency[15] (Table VI).

The subtlety of the structure–activity relationships suggested to us that three-dimensional modeling of the compounds might provide insight into the features responsible for the observed differences in potency. Compound **60**, the macrocyclic lactone of 15-HEDE, is more potent than the corresponding methyl ester **17** and roughly one-third as potent as the open-chain compound **16**. We assume that **60** overcomes the loss of binding energy of the carboxylate by having the binding conformation held in place by the macrocycle. Therefore, we performed a thorough conformational analysis[16] and energy minimization[17] on **60**. Similar considerations led us to also examine **59**, the δ-lactone of 5,15-diHETE (**58**). For both **59** and **60** we found several conformations within 2 kcal of the global minimum. However, since the low-energy conformation of each of these two compounds is similar to the other (Figure 1), and the compounds compared below all have the same flexibility in the C_1–C_8 region, for further modeling we used the low-energy conformations as reference points. We then modeled **16** and its C_{11}–C_{15} region analogues **9**, **12**, **20**, **34**, **50–56**, **61**, and **62**. These compounds were superimposed to overlap C_{16}, O_{15}, and the C_{11}–C_{14} region of **55**, the most potent analogue. Figure 1 shows the superposition of the two inhibitory lactones **59** and **60** and the two most potent open-chain diunsaturated inhibitors, 15-HEDE (**16**) and its 11,13-diyne analogue **55**.

Table V. Effects of the Double-Bond Geometry and Saturation on the Inhibitory Activity of (11*Z*,13*E*)-15-Hydroxyeicosa-11,13-dienoic Acid (15-HEDE) on RBL-1 5-Lipoxygenase



compd	X–Y	yield, %	formula	IC_{50} (95% CL),[a] μM
16		Table II	Table II	35 (33–38)
50		90	$C_{20}H_{36}O_3$	58 (54–62)
51		15	$C_{20}H_{36}O_3$	33% @ 30 μM
52		7	$C_{20}H_{36}O_3$	44% @ 100 μM
53		22	$C_{20}H_{38}O_3$	36% @ 75 μM
54		80	$C_{20}H_{34}O_3$	30% @ 100 μM
55		82	$C_{20}H_{32}O_3$	27 (21–31)

[a] The concentration estimated to produce 50% inhibition with 95% confidence limits in parentheses. Compounds that failed to cause 50% inhibition are listed as the percent inhibition obtained at the highest soluble tested concentration.

Notice that the C_{10}–C_{16} region is remarkably similar in shape for these compounds and that the C_1–C_9 region is more dissimilar. Indeed, the most potent diunsubstituted analogue **55** extends into new regions in space in the region of C_9, to the left in Figure 1. When the less potent C_{11}–C_{15} region analogues **20**, **34**, **50–54**, and **62** and the inactive analogue **9** are added to the comparison, much more variation in the shape of the C_{10}–C_{16} region is seen (Figures 2 and 3). Each less potent analogue penetrates into space not occupied by **16** or **66**. According to the concepts of receptor mapping formulated by others,[18,19] we interpret these modeling results to suggest that there is a specific binding site on 5-lipoxygenase for the C_{10}–C_{15} region of 15-HETE and arachidonic acid, which is shown at the bottom of the figures.

(15) Dyer, R. D.; Bornemeier, D. A.; Haviv, F.; Carter, G. W. *Fed. Proc., Fed. Am. Exp. Biol.* **1985**, *44*, 904.
(16) The Chemical Modeling Laboratory, Version 8.0, Molecular Design Limited, Hayward, CA.
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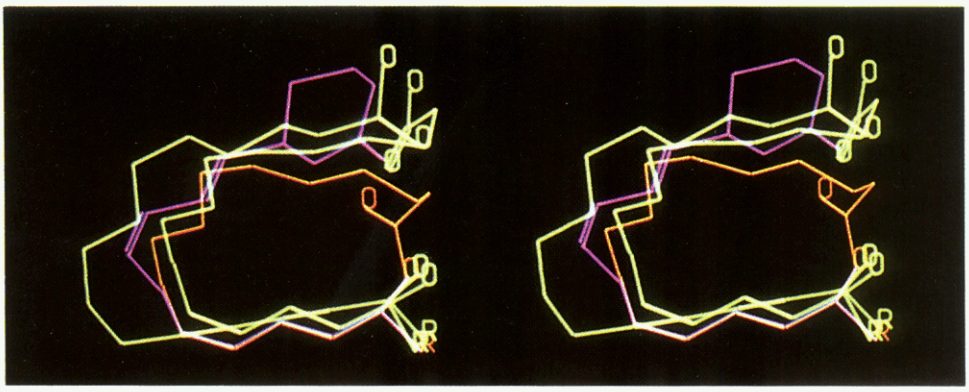


Figure 1. A stereoview of the superposition of the low-energy conformation of **16** (green), **55** (green), **59** (orange), and **60** (purple).

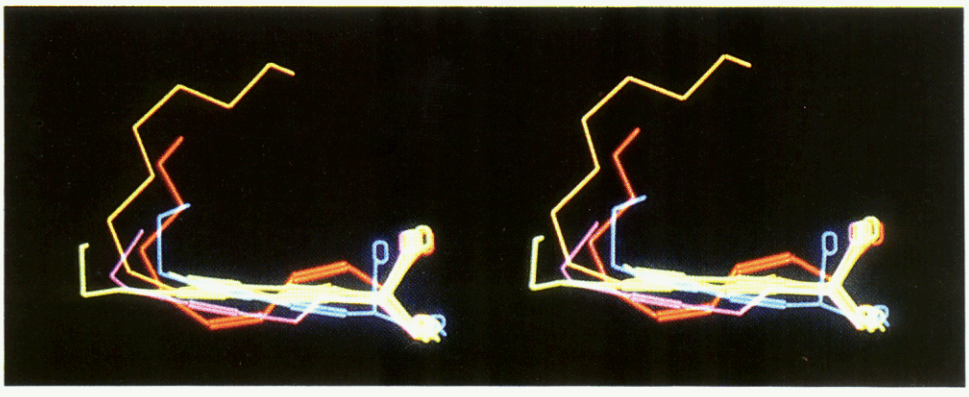


Figure 2. A stereoview of the C_8–C_{16} region of the most potent inhibitors **16** and **55** in green and the less potent inhibitors **34** (pink), **50** (blue), **51** (orange), and **52** (red).

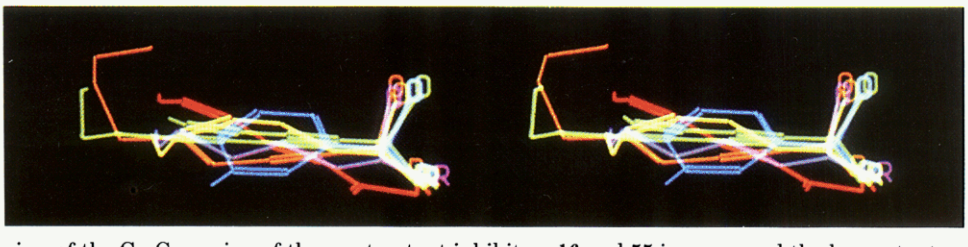


Figure 3. A stereoview of the C_8–C_{16} region of the most potent inhibitors **16** and **55** in green and the less potent or inactive inhibitors **9** (purple), **20** (red), **53** (yellow), **54** (orange), and **62** (blue).

Conclusions

Although 15-HETE analogues containing a *cis,cis*-5,8-diene system inhibit RBL-1 5-lipoxygenase by acting as alternate substrates, analogues such as 15-HEDE (**8**), which lack a *cis,cis*-5,8-diene system, retain inhibitory activity without being substrates.

A number of structural features in both 15-HEDE and 15-HETE analogues were identified to be important for 5-lipoxygenase inhibition. However, no single region appears to play a dominant role in determining inhibitory activity. These studies support the view that an interaction of essentially the entire molecule is required for optimal 5-lipoxygenase inhibition and suggest the presence of multiple binding sites on the enzyme for these inhibitors.

Structure–activity relationship studies of 15-HEDE analogues revealed that inhibition of the enzyme was optimal in compounds that generally contained a free carboxyl group, a carboxylate side chain of nine carbons, a hydrophobic ω side chain of five or six carbons, a *cis,trans*- or *trans,cis*-11,13-diene or 11,13-diyne system, and a 15-hydroxyl group. Conformational restriction of the ω chain reduced activity by 2-fold. The δ-lactones of 5-HETE and 5,15-diHETE, **57** and **59**, were 3- to 10-fold more potent than their precursors **56** and **58**, respectively.

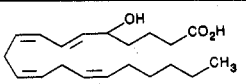
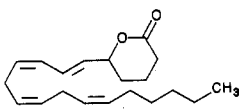
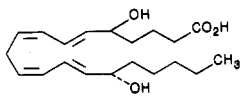
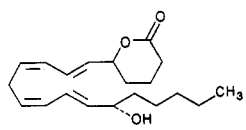
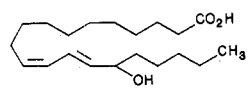
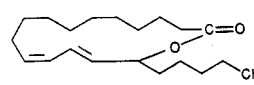
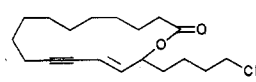
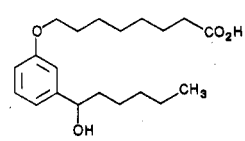
Since the structure–activity analysis suggests hydrophobic binding of the ω chain and the reaction site is at C_5, the enzyme appears to interact with C_5–C_{20}. Any variation of shape or flexibility in this portion of the substrate or inhibitor leads to variation in potency.

Experimental Section

All melting points were taken on a Thomas-Hoover apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 521 spectrophotometer, ^{1}H NMR spectra on a QE-300 (General Electric) spectrometer, and mass spectra on a Varian CH-7 spectrometer. All new compounds had NMR and IR spectra consistent with their structures and also gave satisfactory C and H analyses in the range of ±0.4% from the calculated values. All the organic phases were dried over anhydrous Na_2SO_4. Fatty acids were purchased from NuCheck Prep., Inc., Elysian, MN.

Enzymatic Synthesis of (5*Z*,8*Z*,11*Z*,13*E*)-15(*S*)-Hydroxyeicosa-5,8,11,13-tetraenoic Acid (1), (5*Z*,8*Z*,11*Z*,13*E*)-15(*S*)-Hydroperoxyeicosa-5,8,11,13-tetraenoic Acid (3), (8*Z*,11*Z*,13*E*)-15(*S*)-Hydroxyeicosa-8,11,13-trienoic Acid (7), (11*Z*,13*E*)-15(*S*)-Hydroxyeicosa-11,13-dienoic Acid (8), (7*Z*,10*Z*,13*Z*,15*E*)-17(*S*)-Hydroxydocosa-7,10,13,15-tetraenoic Acid (10), (9*Z*,11*E*)-13(*S*)-Hydroxyoctadeca-9,11-dienoic Acid (12), and

Table VI. Effects of Lactonization and Conformation Restriction on the Inhibitory Activity of 5-HETE, 5,15-diHETE, 15-HEDE, and 15-HEDE Enyne (**34**) on RBL-1 5-Lipoxygenase

structure	compd	yield, %	formula	IC_{50} (95% CL),[a] μM
	**56**	ref 24	$C_{20}H_{32}O_3$	77 (70–85)
	**57**	ref 24	$C_{20}H_{30}O_2$	23 (21–25)
	**58**	35	$C_{20}H_{32}O_4$	270 (220–390)
	**59**	48	$C_{20}H_{30}O_3$	25 (23–27)
	**16**	Table II	Table II	35 (33–38)
	**60**	25	$C_{20}H_{34}O_2$	42% @ 100 μM
	**61**	25	$C_{20}H_{32}O_2$	16% @ 33 μM
	**62**	11	$C_{20}H_{32}O_4$	23% @ 100 μM

[a] The concentration estimated to produce 50% inhibition with 95% confidence limits in parentheses. Compounds that failed to cause 50% inhibition are listed as the percent inhibition obtained at the highest soluble tested concentration.

(6*Z*,9*Z*,11*E*)-13(*S*)-Hydroxyoctadeca-6,9,11-trienoic Acid (14). The hydroxylated compounds **1**, **3**, **7**, **8**, **10**, **12**, and **14** were prepared from the appropriate fatty acids by using a method similar to that described by Baldwin et al.,[7] the only difference being that 5×10^6 units of soybean lipoxygenase (type IV, Sigma) in 1.2 mL of a 50% $(NH_4)_2SO_4$ solution was used. The products were purified by flash column chromatography (silica gel, hexane–ether–acetic acid (2.5:10.0:0.25, v/v/v)). The 15-hydroperoxyeicosatetranoic acid **3** was purified at 0 °C by using the same chromatographic technique. Ester **11**, **13**, and **15** were prepared from **10**, **12**, and **14**, respectively, upon treatment with diazomethane.

Methyl (5*Z*,8*Z*,11*Z*,13*E*)-15(*S*)-Hydroxyeicosa-5,8,11,13-tetraenoate (2),[7] (5*Z*,8*Z*,11*Z*,13*E*)-15-Ketoeicosa-5,8,11,13-tetraenoic Acid (4),[20] (5*Z*,8*Z*,11*Z*,13*E*)-15(*S*)-Acetoxyeicosa-5,8,11,13-tetraenoic Acid (5),[21] Methyl (5*Z*,8*Z*,11*Z*,13*E*)-15(*S*)-Acetoxyeicosa-5,8,11,13-tetraenoate (6),[3] Eicosanoic Acid (9),[22] Methyl (11*Z*,13*E*)-15(*S*)-Hydroxyeicosa-11,13-dienoate (17),[7] (11*Z*,13*E*)-15(*S*)-Acetoxyeicosa-11,13-dienoic Acid (19),[20] and (11*Z*,13*E*)-15(*S*)-Ketoeicosa-11,13-dienoic Acid (20).[20] These compounds were prepared from the appropriate hydroxy fatty acid by using conventional methods.[7,20-22]

(11*Z*,13*E*)-15-Hydroxyeicosa-11,13-dienoic Acid (16). 10-Bromodecanoic acid was converted to 10-iododecanoic acid upon treatment with sodium iodide as described by Patterson et al.[23] 10-Iododecanoic acid was reacted with lithium acetylide in HMPA to give 11-dodecynoic acid.[24]

Hexanoyl chloride was reacted with acetylene in carbon tetrachloride and in the presence of $AlCl_3$[25] to give 1-chloro-3-keto-1-octene. This was converted to 1-iodo-3-hydroxy-1-octene upon treatment first with sodium iodide followed by sodium borohydride.[26]

Copper(I) iodide (0.03 g, 0.157 mmol) was added to a stirred solution of 11-dodecynoic acid (1.37 g, 10 mmol), 1-iodo-3-hydroxy-1-octene (1.91 g, 7.5 mmol), and tetrakis(triphenylphosphine)palladium (0.091 g, 0.078 mmol) in diethylamine (10 mL) under argon and at room temperature. Stirring was continued for 3 h. The reaction mixture was diluted with water (5 mL) and concentrated in vacuo to remove volatiles. The residue, after adjustment to pH 2 with dilute HCl, was extracted with ether (3 × 100 mL). The extracts were washed with saturated NaCl solution (5 × 15 mL), dried, and concentrated in vacuo. The oily residue was purified by flash column chromatography (silica gel, hexane–ether–acetic acid (2.5:1.0:0.05, v/v/v)) to give (13*E*)-15-hydroxyeicosa-13-en-11-ynoic acid (**34**) 2.05 g) as a semisolid oil: ^1H NMR ($CDCl_3$) δ 6.04 (dd, 1 H, H-14, $J_{14,13}$ = 15 Hz, $J_{14,15}$ = 6 Hz), 5.67 (d, 1 H, H-13, $J_{13,14}$ = 15 Hz), 4.12 (dd, 1 H, H-15, $J_{15,14}$ = 6 Hz, $J_{15,16}$ = 15 Hz), 2.45–2.20 (m, 4 H, H-10, H-2), 1.75–1.15 (m, 22 H, H-3 to H-9, H-16 to H-19), 0.88 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3400 (m), 3040 (m), 2945 (s), 2850 (s), 1720 (s); MS, $M^{\bullet+}$ 322.

A solution of **34** (1.0 g) in ethyl acetate (15 mL) and quinoline (0.6 mL) was hydrogenated at room temperature and atmospheric pressure in the presence of modified Lindlar catalyst (10%

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(24) DeJarlais, W. J.; Emken, E. A. *Synth. Commun.* **1980**, *10*, 653.
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$Pd/BaSO_4$) (0.03 g). The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was dissolved in ether and washed first with 3 N HCl and then with water. The extracts were dried and concentrated again. The residue was purified by flash column chromatography (silica gel, hexane–acetic acid (10:1, v/v)) to give **16** (0.27 g) as a colorless oil: ^1H NMR ($CDCl_3$) δ 6.50 (dd, 1 H, H-14, $J_{14,13}$ = 15 Hz, $J_{14,15}$ = 10.5), 5.95 (br t, 1 H, H-12, $J_{12,11}$ = 10.5 Hz), 5.65 (dd, 1 H, H-13), $J_{13,14}$ = 15 Hz, $J_{13,12}$ = 7.5 Hz), 5.50–5.40 (m, 1 H, H-11, $J_{11,12}$ = 10.5 Hz), 4.25–4.12 (m, 1 H, H-15), 2.45–2.25 (t, 2 H, H-2), 2.25–2.10 (q, 2 H, H-10), 1.8–1.15 (m, 22 H, H-3 to H-9, H-16 to H-19), 0.88 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}), 3400 (m), 3040 (m), 2945 (s), 2860 (s), 1710 (s); MS, $M^{\bullet+}$ 324. **(11*Z*)-15-Hydroxyeicosa-11-enoic acid 53** (0.22 g) was isolated as a byproduct: ^1H NMR ($CDCl_3$) δ 5.60–5.30 (m, 2 H, H-11 and H-12), 3.70–3.50 (m, 1 H, H-15), 2.45–1.95 (m, 6 H, H-2, H-10, and H-12), 1.75–1.17 (m, 22 H, H-3 to H-9, H-16 to H-19), 0.9 (t, 3 H, CH_3). Decoupling experiments confirmed the structure; IR ($CHCl_3$), ν_{max} (cm^{-1}) 3600 (m), 2940 (s), 2860 (s), 1715 (s); MS, $M^{\bullet+}$ 324. Anal. ($C_{20}H_{38}O_3$) C, H.

(12*Z*,14*E*)-16-Hydroxyheneicosa-12,14-dienoic Acid (22), (10*Z*,12*E*)-14-Hydroxynonadeca-10,12-dienoic Acid (23), (8*Z*,10*E*)-12-Hydroxyheptadeca-8,10-dienoic Acid (24), (7*Z*,9*E*)-11-Hydroxyhexadeca-7,9-dienoic Acid (25), (6*Z*,8*E*)-10-Hydroxypentadeca-6,8-dienoic Acid (26), (11*Z*,13*E*)-15-Hydroxyoctadeca-11,13-dienoic Acid (27), (11*Z*,13*E*)-15-Hydroxy-16-cyclopentylhexadeca-11,13-dienoic Acid (28), (11*Z*,13*E*)-15-Hydroxyheneicosa-11,13-dienoic Acid (29), and (11*Z*,13*E*)-15-Hydroxy-19-carbomethoxynonadeca-11,13-dienoic Acid (30). These compounds were prepared by using synthetic procedures similar to that described above for **16**. The intermediates of **33**, **35–40**, and **42–44** have been fully characterized. The enyne intermediates were hydrogenated to the dienes **22–30** in the presence of nickel boride.[26] All of the spectral data for the compounds were compatible with the structures.

(13*E*)-15-Hydroxy-19-carboxynonadec-13-en-11-ynoic Acid (45). A solution of lithium hydroxide (0.382 g, 9 mmol) in water (15 mL) was added to a solution of methyl ester **48** (0.748 g, 2 mmol) in isopropyl alcohol (30 mL) under nitrogen. The solution was stirred for 2 h at room temperature and then concentrated in vacuo. The residue was treated with ice water and acidified with dilute HCl. A white solid precipitated. This was filtered and dried to give acid **45** (0.660 g): mp 65–69 °C; ^1H NMR ($CDCl_3$) δ 6.05 (dd, 1 H, H-14, $J_{14,13}$ = 15 Hz, $J_{14,15}$ = 6 Hz), 5.68 (d, 1 H, H-13, $J_{13,14}$ = 15 Hz), 4.15 (dd, 1 H, H-15, $J_{15,16}$ = 15 Hz, $J_{15,14}$ = 6 Hz), 2.45–2.25 (m, 6 H, H-2, H-10, H-19), 1.75–1.25 (m, 20 H, H-3 to H-9, H-16 to H-18); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3600 (m), 3500–3000 (br), 2940 (s), 2860 (s), 1710 (s), 1630 (w); MS $M^{\bullet+}$ 352.

(11*Z*,13*E*)-15-Hydroxy-19-carboxynonadeca-11,13-dienoic Acid (31). Methyl ester **30** was hydrolyzed to acid **31** by using a synthetic procedure similar to that described above for **45**.

(11*Z*,13*E*)-15,20-Dihydroxyeicosa-11,13-dienoic Acid (32). A toluene solution of 1 M diisobutylaluminum hydride (1.7 mL) was added to a solution of **30** (0.204 g, 0.55 mmol) in dry methylene chloride (15 mL) under nitrogen at −78 °C. The reaction mixture was stirred for 1 h at −78 °C, for 2 h at 0 °C, and then for overnight at room temperature. The mixture was cooled again to −60 °C and a saturated ammonium chloride solution was added. Stirring was continued for an additional 20 min and then the mixture was acidified with 5% H_2SO_4 and extracted three times with methylene chloride. The extracts were dried and concentrated in vacuo to give an oily residue (0.2 g). This residue was purified by flash chromatography (silica gel, ether–acetic acid (40:0.1, v/v)) to give compound **32** (0.0525 g): ^1H NMR ($CDCl_3$) δ 6.50 (dd, 1 H, H-14, $J_{14,13}$ = 15 Hz, $J_{14,15}$ = 10.5 Hz), 6.05–5.92 (m, 1 H, H-12, $J_{12,11}$ = 10.5 Hz), 5.70–5.60 (m, 1 H, H-13, $J_{13,14}$ = 15 Hz), 5.50–5.40 (m, 1 H, H-11, $J_{11,12}$ = 10.5 Hz), 4.25–4.10 (m, 1 H, H-15), 3.75–3.60 (m, 2 H, H-20), 2.40–2.05 (m, 2 H, H-10), 1.75–1.10 (m, H-22, H-3 to H-9, H-16 to H-19); IR ($CHcl_3$) ν_{max} (cm^{-1}) 3610 (m), 2920 (s), 2860 (s), 1710 (s); MS, $M^{\bullet+}$ 340. (11*Z*,13*E*)-15-Keto-20-hydroxyeicosa-11,13-dienoic acid (0.066 g) was isolated as a byproduct: ^1H NMR ($CDCl_3$) δ 7.55–7.45 (m, 1 H, H-14, $J_{14,13}$ = 15 Hz), 6.22–6.05 (m, 2 H, H-13 and H-12), 6.00–5.85 (m, 1 H, H-11), 3.75–3.62 (m, 2 H, H-20), 2.65–2.53 (m, 2 H, H-10), 2.45–2.25 (m, 4 H, H-2, H-16), 1.75–1.15 (m, 20 H, H-3 to H-9, H-17 to H-19); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3610 (m), 2920 (s), 2860 (s), 1710 (s); MS, $M^{\bullet+}$ 338. Anal. ($C_{20}H_{34}O_4$) C, H.

Methyl (11*Z*,13*E*)-15-hydroxyeicosa-11,13-dienoate (17), (11*Z*,13*E*)-15-acetoxyeicosa-11,13-dienoic acid (19), (11*Z*,13*E*)-15-ketoeicosa-11,13-dienoic acid (20), and (13*E*)-15-acetoxyeicosa-13-en-11-ynoic acid (48) were prepared from the appropriate hydroxy fatty acids by using conventional methods.[10,19–21]

(13*E*)-15-Hydroxyeicosa-13-en-11-ynoic Acid Diethylamide (49). A mixture of 11-dodecynoic acid (5 g, 25.7 mmol) and thionyl chloride (6.13 g, 51.54 mmol) was stirred at room temperature overnight and then heated under reflux for 1 h. The reagent was removed in vacuo and the residue was dissolved in hexane. The solution was treated with charcoal and filtered, and the filtrate was concentrated again in vacuo. The residue was dissolved in dry ether (5 mL) and to the solution diethylamine (3.168 g, 43.4 mmol) in ether (10 mL) was added. The reaction mixture was stirred at room temperature overnight. The precipitate was filtered and the filtrate was concentrated. The residue was purified by flash column chromatography (silica gel, hexane–ether (1:1, v/v)) to give 11-dodecynoic acid diethylamide (4.16 g); mp 29–30 °C.

11-Dodecynoic acid diethylamide (2.51 g, 10 mmol) was coupled with 1-iodo-3-hydroxy-1-octene (2.49 g, 10.5 mmol), by using the same synthetic procedure described above for enyne **34**, to give **49** (1.49 g): ^1H NMR ($CDCl_3$) δ 6.05 (dd, 1 H, H-14, $J_{14,13}$ = 15 Hz, $J_{14,15}$ = 6 Hz), 5.65 (d, 1 H, H-13, $J_{13,14}$ = 15 Hz), 4.12 (dd, 1 H, H-15, $J_{15,16}$ = 15 Hz, $J_{15,14}$ = 6 Hz), 3.45–3.20 (m, 4 H, 2 NCH_2), 2.40–2.20 (m, 4 H, H-2 and H-10), 1.75–1.25 (m, H-22, H-3 to H-9, H-16 to H-19), 1.25–1.05 (2 t, H-6, 2 CH_3), 0.90 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3600 (m), 2940 (s), 2860 (s), 1710 (m), 1620 (s); MS, $M^{\bullet+}$ 377.

(11*Z*,13*E*)-15-Hydroxyeicosa-11,13-dienoic Acid Diethylamide (18). Enyne **34** (0.4 g) was hydrogenated in the presence of nickel boride[27] to give diene **18** (0.240 g): ^1H NMR ($CDCl_3$) δ 6.55–6.42 (m, 1 H, H-14, $J_{14,13}$ = 15 Hz), 6.05–5.92 (m, 1 H, H-12, $J_{12,11}$ = 10.5 Hz), 5.75–5.60 (m, 1 H, H-13, $J_{13,14}$ = 15 Hz), 5.50 (m, 1 H, H-11, $J_{11,12}$ = 10.5 Hz), 4.20–4.10 (dd, 1 H, H-15, $J_{15,16}$ = 15 Hz, $J_{15,14}$ = Hz), 3.45–3.20 (m, 4 H, 2 NCH_2), 2.35–2.10 (m, 4 H, H-2 and H-10), 1.80–1.20 (m, H-22, H-3 to H-9, H-16 to H-19), 1.20–1.05 (2 t, H-6, 2 CH_3), 0.90 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3600 (m), 2910 (s), 2830 (s), 1620 (s); MS, $M^{\bullet+}$ 379.

(13*E*)-Eicosa-13-en-11-ynoic Acid (47). 1-Octyne (21.8 g, 198 mmol) was added dropwise under nitrogen and at room temperature to cathecolborane (25 g, 198 mmol). The mixture was stirred for 2 h at 70–80 °C, then cooled to 27 °C, treated with water (200 mL), and stirred overnight at 5 °C. The cream-colored solid was collected, washed with cold water, and dried to give the *trans*-1-octenylboronic acid (20.33 g). This acid was dissolved in ether (200 mL) and the resulting solution was cooled to 0 °C and treated with 3 N sodium hydroxide solution (200 mL). The two-phase stirred mixture was further treated at the same temperature with an iodine (30.4 g, 240 mmol) solution in ether (600 mL). Stirring was continued for 20 min after the addition was complete. Sodium thiosulfate was added until the ether layer was clear yellow. The ether layer was separated, washed with water, dried, and concentrated in vacuo. The oily residue was distilled to give *trans*-1-octenyl iodide (12.3 g); bp 51.5–53 °C (0.1 mmHg).

11-Dodecynoic acid (6.86 g, 35 mmol) was coupled with *trans*-1-octenyl iodide (9.29 g, 39 mmol), by using the same synthetic procedure described above for enyne **34**, to give **47**: mp 46–47 °C; ^1H NMR ($CDCl_3$) δ 6.15–5.95 (m, 1 H, H-14, $J_{14,13}$ = 16.5 Hz), 5.50–5.35 (d, 1 H, H-13, $J_{13,14}$ = 16.5 Hz), 2.45–2.20 (m, 4 H, H-2 and H-15), 2.15–2.00 (q, 2 H, H-10), 1.80–1.10 (m, 22 H, H-3 to H-9, H-16 to H-19), 0.87 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3520 (w), 3020 (m), 2940 (s), 2860 (s), 1710 (s); MS, $M^{\bullet+}$ 306.

(11*Z*,13*E*)-Eicosa-11,13-dienoic Acid (21). Enynoic acid **47** (2.145 g, 7.0 mmol) was hydrogenated in the presence of nickel boride[27] to give dienoic acid **21** (1.57 g) as a colorless oil: ^1H NMR ($CDCl_3$) δ 6.35–6.25 (m, 1 H, H-13 of H-12), 6.00–5.90 (m, 1 H,

(27) Brown, C. A.; Ahuja, V. K. *J. Chem. Soc., Chem. Commun.* **1973**, 553.

H-12 or H-13), 5.75–5.60 (1 H, m, H-11 or H-14), 5.40–5.25 (m, 1 H, H-14 or H-11), 2.40–2.30 (t, 2 H, H-2), 2.20–1.90 (m, 4 H, H-10 and H-15), 1.70–1.20 (m, 22-H, H-3 to H-9, H-16 to H-19), 0.90 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3020 (m), 2040 (s), 2860 (s), 1720 (s); MS, $M^{\bullet+}$ 308.

(12*E*)-Nonadec-12-en-10-ynoic Acid (46). Enynoic acid **46** was synthesized by using a procedure similar to that described for **21**.

(11*E*,13*Z*)-15-Hydroxyeicosa-11,13-dienoic Acid (50). A solution of 1 M catecholborane in THF (35 mL) was added dropwise at room temperature, under argon, to a stirred solution of methyl 11-dodecynoate (6.5 g, 30.9 mmol) in dry THF (5 mL). The reaction mixture was refluxed for 3 h, cooled, treated with water (175 mL), and stirred at room temperature overnight. The milky mixture was concentrated in vacuo to remove the THF and then cooled with ice. The solid precipitate was filtered, washed with water, and dried by suction to give crude ((1*E*)-11-carbomethoxy-1-undecenyl)boronic acid (7.9 g).[28]

A solution of ((1*E*)-11-carbomethoxy-1-undecenyl)boronic acid (7.9 g, 30.9 mmol) in ether (60 mL) was chilled in ice to 0 °C and while being stirred was treated with a cold solution of 3 N NaOH (22 mL, 66 mmol). A solution of iodine (3.1 g, 25 mmol) in ether (100 mL) was added dropwise to the mixture, which was stirred for an additional 15 min at 0 °C. The organic phase was separated and the aqueous phase was extracted three times with ether. The extracts were combined, washed with water, dried, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, pentane–ether (20:1, v/v)) to give (1*E*)-11-carbomethoxy-1-undecenyl iodide (1.49 g).

A solution of (1*E*)-11-carbomethoxy-1-undecenyl iodide (1.49 g, 4.1 mmol), 1-octyn-3-ol (0.51 g, 4.0 mmol), and tetrakis(triphenylphosphine)palladium (0.046 g, 0.04 mmol) in diethylamine (15 mL) was treated with copper(I) iodide (0.016 g, 0.08 mmol) at room temperature for 4 h. The reaction was worked up as described previously for **34**. The residue was purified by flash column chromatography (silica gel, hexane–ether (3:1, v/v)) to give methyl (11*E*)-15-hydroxyeicosa-11-en-13-ynoate (1.02 g).

A solution of methyl (11*E*)-15-hydroxyeicosa-11-en-13-ynoate (2.02 g, 6.0 mmol) in isopropyl alcohol (75 mL) was treated with a solution of lithium hydroxide monohydrate (0.76 g, 18 mmol) in water (36 mL). The mixture was stirred at room temperature under argon for 2 h and then concentrated in vacuo. The residue was treated with water, the solution was then acidified with 3 N HCl to pH 2, and extracted twice with ether. The extracts were washed with NaCl solution, dried, and concentrated in vacuo to give (11*E*)-15-hydroxyeicosa-11-en-13-ynoic acid (**54**) (1.92 g): ^1H NMR ($CDCl_3$) δ 6.20–6.05 (m, 1 H, H-11, $J_{11,12}$ = 15 Hz), 5.48 (d, H-12, $J_{12,11}$ = 15 Hz), 4.47 (t, 1 H, H-15, $J_{15,16}$ = 7.5 Hz), 2.40–2.30 (m, 2 H, H-2), 2.15–2.05 (m, 2 H, H-10), 1.80–1.20 (22 H, H-3 to H-9, H-16 to H-19), 0.95–0.80 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3600 (m), 2940 (s), 2860 (s), 1710 (s); MS, $M^{\bullet+}$ 322.

A solution of enyne **54** (1.0 g, 3.1 mmol) was hydrogenated in the presence of nickel boride[26] to give diene **50** (0.9 g) after two flash column chromatographies (silica gel, hexane–ether–acetic acid (4:1:0.10, v/v/v)): ^1H NMR ($CDCl_3$) δ 6.40–6.25 (m, 1 H, H-12, $J_{12,11}$ = 15 Hz), 6.08–5.95 (m, 1 H, H-14, $J_{14,13}$ = 10.5 Hz), 5.85–5.65 (m, 1 H, H-11, $J_{11,12}$ = 15 Hz), 5.35–5.20 (m, 1 H, H-13, $J_{13,14}$ = 10.5 Hz), 4.65–4.52 (m, 1 H, H-15), 2.40–2.25 (t, 1 H, H-2), 2.20–2.00 (q, 1 H, H-10), 1.75–1.10 (m, 22 H, H-3 to H-9, H-16 to H-19), 1.0–0.81 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3560 (m), 2040 (s), 2860 (s), 1710 (s); MS, $M^{\bullet+}$ 324.

(11*E*,13*E*)-15-Hydroxyeicosa-11,13-dienoic Acid (51). A solution of methyl 11-dodecynoate (1.05 g, 5 mmol) in anhydrous THF (3 mL) was treated with 1 M catecholborane in THF (5 mL) under argon and with stirring. The solution was heated under reflux for 3 h, then cooled, and concentrated in vacuo to give crude ((1*E*)-11-carbomethoxy-1-undecenyl)catecholborane (1.7 g). The residue was redissolved in THF (10 mL) and treated with 1 N sodium hydroxide solution (10 mL) followed by the addition of 3-hydroxy-1-octenyl iodide (1.27 g, 5 mmol) and tetrakis(triphenylphosphine)palladium (0.140 g, 0.1 mmol). The mixture was stirred under argon at 65 °C for 2 h and then poured into ice water (60 mL). The resulting mixture was extracted with ether, and the extracts were washed with saturated sodium chloride solution, dried, and concentrated in vacuo to give crude methyl (11*E*,13*E*)-15-hydroxyeicosa-11,13-dienoate (1.34 g). The aqueous phase was adjusted to pH 1.0 with dilute HCl and extracted with ether. The ether extracts were dried and concentrated in vacuo to give crude (11*E*,13*E*)-15-hydroxyeicosa-11,13-dienoic acid (0.603 g).

The crude methyl (11*E*,13*E*)-15-hydroxyeicosa-11,13-dienoate (1.34 g) was dissolved in isopropyl alcohol (50 mL) and treated with a solution of lithium hydroxide (0.42 g, 10 mmol) in water (25 mL). The mixture was stirred at room temperature under argon for 3 h and then concentrated in vacuo. The residue was dissolved in water, and the solution was first acidified with dilute HCl to pH 1.0 and then extracted with ether three times. The extracts were dried and concentrated in vacuo to give crude (11*E*,13*E*)-15-hydroxyeicosa-11,13-dienoic acid (1.11 g). This was combined with the crude acidic product previously obtained and purified twice by flash column chromatography (silica gel, hexane–ether–acetic acid (3:1:0.1, v/v/v)) to give (11*E*,13*E*)-15-hydroxyeicosa-11,13-dienoic acid (0.250 g): mp 64–69 °C; ^1H NMR ($CDCl_3$) δ 6.25–6.12 (m, 1 H, H-14), 6.08–5.95 (m, 1 H, H-12), 5.75–5.63 (m, 1 H, H-11), 5.62–5.55 (m, 1 H, H-13), 4.20–4.07 (m, 1 H, H-15), 2.40–2.25 (t, 2 H, H-2), 2.12–2.02 (m, 2 H, H-10), 1.70–1.20 (m, 22 H, H-3 to H-9, H-16 to H-19), 0.93–0.83 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3560 (m), 2940 (s), 2860 (s), 1710 (s); MS, $M^{\bullet+}$ 324.

(11*Z*,13*Z*)-15-Hydroxyeicosa-11,13-dienoic Acid (52). Bromine (5.6 mL, 0.11 mol) was added, with stirring and dropwise at 0 °C, to a solution of sodium hydroxide (13.7 g, 0.34 mol) in water (150 mL). A cold solution of sodium hypobromite was added dropwise to 1-octyn-3-ol (12.6 g, 0.10 mol) cooled at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and for an additional 30 min at room temperature. The mixture was extracted twice with ether. The extracts were dried, concentrated in vacuo, and distilled to give 1-bromo-1-octyn-3-ol (11.39 g); bp 85–86 °C (0.6 mmHg).

1-Bromo-1-octyn-3-ol (3.89 g, 19 mmol) was added dropwise to a solution of 11-dodecynoic acid (3.93 g, 20 mmol), copper(I) chloride (0.010 g), hydroxylamine hydrochloride (0.050 g) in methanol (10 mL), and 70% ethylamine (9 mL) cooled at 0 °C. The mixture was stirred for 20 min at 0 °C and then allowed to reach room temperature. Additional small portions of hydroxylamine hydrochloride were added whenever the color of the reaction mixture became green or blue/green. After 40 min at room temperature, the mixture was diluted with water, acidified to pH 2 with 3 N hydrochloric acid, and extracted with ether. The extracts were washed with water, dried, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, hexane–ether–acetic acid (3:1:0.1, v/v/v)) to give eicosa-11,13-diynoic acid (**55**) (2.19 g): ^1H NMR ($CDCl_3$) δ 4.45–4.37 (t, 1 H, H-15), 2.40–2.31 (t, 2 H, H-2), 2.31–2.03 (t, 2 H, H-10), 1.78–1.20 (m, 22 H, H-3 to H-9, H-19), 0.95–0.82 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3600 (m), 3010 (m), 2940 (s), 2860 (s), 1715 (s); MS, $[M - H_2O]^{\bullet+}$ 302.

A solution of eicosa-11,13-diynoic acid (1.28 g, 4.0 mmol) in ether (5 mL) was treated with ethereal diazomethane to give after evaporation of the solvent methyl eicosa-11,13-diynoate (1.28 g). This was dissolved in *n*-propyl alcohol (90 mL), and to the solution were added, with mechanical stirring, water (510 mL) and iron powder (120 g). The mixture was refluxed for 4 h, cooled to room temperature, and filtered. The filter cake was washed several times with ether. The ether washings were washed with dilute HCl and then water, dried, and concentrated in vacuo. The crude mixture was dissolved in isopropyl alcohol (5 mL) and treated with lithium hyroxide monohydrate (0.25 g) in water (6 mL) at room temperature for 2 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in water, and the solution was acidified with dilute HCl to pH 2 and extracted with ether. The extracts were washed with water, dried, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, pentane–acetic acid (10:1, v/v)) to give **52** (0.082 g): ^1H NMR ($CDCl_3$) δ 6.40–6.20 (m, 2 H, H-14 and H-12), 5.60–5.47 (m, 1 H, H-11), 5.47–5.33 (m, 1 H, H-13), 4.66–4.52 (q, 1 H, H-15), 2.40–2.30 (t, 2 H, H-2), 2.25–2.10 (m, 2 H, H-10),

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(29) Corey, E. J.; Hashimoto, S. *Tetrahedron Lett.* **1981**, *22*, 299.

1.75–1.10 (m, H-22, H-3 to H-9, H-16 to H-19), 1.0–0.8 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3600 (m), 2930 (s), 2860 (s), 1710 (s); MS, $M^{\bullet+}$ 324.

(6*E*,8*Z*,11*Z*,13*E*)-5,15(*S*)-Dihydroxyeicosa-6,8,11,13-tetraenoic Acid (58). (6*E*,8*Z*,11*Z*,14*Z*)-5-Hydroxyeicosa-6,8,11,14-tetraenoic acid (0.825 g), prepared according to Corey and Hashimoto,[28] was treated with soybean lipoxygenase, by using similar conditions to those previously described for **1**, to give crude **58**. The crude product was purified by flash column chromatography (silica gel, hexane–ether–acetic acid (1:3:0.01, v/v/v)) to give the desired product as a colorless oil (0.266 g): ^1H NMR ($CDCl_3$) ν_{max} 6.72–6.50 (m, 2 H, H-6 and H-14), 6.10–5.92 (m, 2 H, H-7 and H-13), 5.80–5.65 (m, 2 H, H-8 and H-12), 5.55–5.35 (m, 2 H, H-9 and H-11), 4.30–4.10 (m, 2 H, H-15 and H-5), 3.25–2.95 (m, 2 H, H-10), 2.50–2.30 (t, 2 H, H-2), 2.0–1.20 (m, 12 H, H-3, H-4, H-16 to H-19), 1.00–0.82(t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3600 (w), 3010 (m), 2960 (s), 2940 (s), 2860 (m), 1710 (s); MS, $[M - H_{20}]^{\bullet+}$ 318.

(6*E*,8*Z*,11*Z*,13*E*)-5,15(*S*)-Dihydroxyeicosa-6,8,11,13-tetraene δ-Lactone (59). A solution of compound **58** (0.170 g, 0.5 mmol) in benzene (10 mL) was heated under reflux for 4 h in the presence of one crystal of *p*-toluenesulfonic acid and 3A molecular sieves. The solution was filtered and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, hexane–ether–acetic acid (1:3:0.01, v/v/v)) to give **59** (0.080 g): ^1H NMR ($CDCl_3$) δ 6.70–6.45 (m, 2 H, H-6 and H-14), 6.15–5.90 (m, 2 H, H-7 and H-13), 5.85–5.60 (m, 2 H, H-8 and H-12), 5.60–5.45 (q, 2 H, H-11 or H-9), 5.60–5.28 (q, 2 H, H-9 or H-11), 5.00–4.85 (m, 1 H, H-5), 4.28–4.10 (q, 1 H, H-15), 3.20–2.95 (m, 2 H, H-10), 2.70–2.45 (m, 2 H, H-2), 2.20–1.10 (m, 12 H, H-3 and H-4, H-16 to H-19), 1.00–0.8 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3610 (m), 3040 (m), 2960 (s), 2940 (s), 2860 (m), 1730 (s); MS, $M^{\bullet+}$ 318.

(11*Z*,13*E*)-15-Hydroxyeicosa-11,13-diene Lactone (60). A solution of **16** (0.324 g, 1 mmol), diethyl azodicarboxylate (0.3567 g, 2.05 mmol), and triphenylphosphine (0.524 g, 2 mmol) in dry benzene was stirred under argon at room temperature overnight. The solvent was removed in vacuo and the residue was purified by flash column chromatography (silica gel, hexane–ether (9:1, v/v)) to give lactone **60** (0.053 g): ^1H NMR ($CDCl_3$) δ 6.66–6.52 (q, 1 H, H-14), 6.07–5.90 (m, 1 H, H-12), 5.72–5.60 (m, 1 H, H-13), 5.60–5.32 (m, 2 H, H-11 and H-15), 2.45–2.10 (m, 4 H, H-2 and H-10), 2.10–1.0 (m, 22 H, H-3 to H-9, H-16 to H-19), 1.00–0.80 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3020 (w), 2940 (s), 2860 (s), 1720 (s); MS, $M^{\bullet+}$ 306.

(13*E*)-15-Hydroxyeicosa-13-en-11-yne Lactone (61). By use of the same synthetic procedure described above for **60**, enyne **34** (0.322 g, 1 mmol) was lactonized to give **61** (0.079 g): ^1H NMR ($CDCl_3$) δ 5.80–5.67 (q, 1 H, H-14, $J_{14,13}$ = 15 Hz, $J_{14,15}$ = 7.5 Hz), 5.67–5.57 (td, 1 H, H-13, $J_{13,14}$ = 15 Hz), 5.18–5.05 (q, 1 H, H-15, $J_{15,14}$ = 7.5 Hz), 2.35–2.05 (m, 4 H, H-2 and H-10), 1.70–1.05 (m, 22 H, H-3 to H-9, H-16 to H-19), 0.87–0.90 (t, 3 H, CH_3); IR ($CHCl_3$) ν_{max} (cm^{-1}) 2930 (s), 2860 (s), 1730 (s); MS, $M^{\bullet+}$ 304.

8-[*m*-(1-Hydroxy-1-hexyl)phenoxy]octanoic Acid (62). Iodopentane (86.0 g, 0.44 mol) was added dropwise under argon and with stirring to a mixture of Mg (10.2 g, 0.42 mol) in dry ether (100 mL) containing a few crystals of iodine. The mixture was refluxed for 1 h until all magnesium reacted. 3-Hydroxybenzaldehyde (25.6 g, 0.197 mol) in dry THF (150 mL) was added at room temperature and dropwise to the cooled reaction mixture. Formation of a heavy precipitate and gas evolution were observed. The reaction slurry was stirred overnight and then cooled to 0 °C. A solution of NH_4Cl (38 g) in water (100 mL) was added dropwise and the organic phase was separated. The aqueous phase was extracted twice with ethyl acetate. Both organic phases were combined, dried, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, methylene chloride–ethanol (50:1, v/v)) to give 1-(*m*-hydroxyphenyl)hexanol (5.50 g).

Anhydrous potassium carbonate (3.04 g, 22 mmol) was added to a solution of 1-(*m*-hydroxyphenyl)hexanol (1.94 g, 10 mmol) and 8-bromooctanoic acid (2.45 g, 11 mmol) in dry acetone (75 mL). The mixture was heated under reflux with stirring for 4 days. The solvent was removed in vacuo and the residue was dissolved in water, acidified with dilute HCl to pH 4, and extracted with ether. The extracts were dried and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, methylene chloride–ethanol–acetic acid (20:0:15:0.15, v/v/v)) to give compound **62** (0.7912 g) contaminated with 8-hydroxyoctanoic acid. This impure compound was treated with ethereal diazomethane solution to give impure methyl ester of **62**, which was further purified by flash column chromatography (silica gel, hexane–ether (2:1, v/v)). The pure methyl ester of **62** (0.4735 g) was dissolved in isopropyl alcohol (15 mL) and treated with a solution of lithium hydroxide monohydrate (0.1913 g, 4.56 mmol) in water (7.5 mL) at room temperature with stirring for 2 h. The solvent was removed in vacuo and the residue was dissolved in cold water. The aqueous solution was acidified with 3 N HCl to pH 3 and extracted with ether three times. The extracts were dried and concentrated to give pure compound **62** (0.3790 g): ^1H NMR ($CDCl_3$) δ 7.30–7.20 (m, 1 H, aromatic), 7.00–6.85 (m, 2 H, aromatic), 6.85–6.75 (m, 1 H, aromatic), 4.70–4.60 (t, 1 H, CHOH), 4.05–3.70 (t, 2 H, CH_2O), 2.40–2.30 (t, 2 H, CH_2CO_2H), 1.85–1.15 (m, 18 H, methylenes), 0.95–0.80 (t, 3 H, CH_3); IR ($CHCl_3$) δ (cm^{-1}) 3610 (m), 2940 (s), 2860 (s), 1710 (s); MS, $M^{\bullet+}$ 336.

5-Lipoxygenase Inhibition Assay. Test compounds or vehicle (2% dimethyl sulfoxide) were preincubated for 20 min at 37 °C with the 20000*g* supernatant from sonicated RBL-1 cells (1.5×10^6 cell equivalents in 200 μL) in assay buffer (10 mM BES, 20 mM PIPES, 1 mM EDTA, 0.7 mM $CaCl_2$, 0.1 M NaCl, pH 6.8). The 5-lipoxygenase reaction was initiated by adding 66 μM arachidonic acid containing 0.025 μCi [^{14}C]arachidonic acid; 0.003 μCi [^3H]-5-HETE was added as internal recovery standard. Reactions were incubated for 5 min and terminated by acidification with HCl to pH 3.5. Mass standards (20 μg each of 5-HETE and arachidonic acid) were added to the samples, which were subsequently extracted with 6 mL of ether. The extracts were evaporated under a stream of nitrogen and the residue dissolved in 140 μL chloroform–methanol (1:1). The reconstituted extracts were applied to silica gel impregnated glass fiber thin-layer chromatography (TLC) sheets (Gelman Sciences, Inc., Ann Arbor, MI), which were developed to a height of 15 cm with hexane–ethyl acetate–acetic acid (90:10:0.25, v/v/v). The 5-HETE (R_f 0.4) and arachidonic acid (R_f 0.95) spots were detected by brief exposure to I_2 vapor. After sublimation of I_2 from the plate the 5-HETE spots were cut from the TLC sheets and transferred to scintillation vials. Product was eluted from the TLC medium by agitating with 2.0 mL of methanol–water (90:10, v/v) for 10 min. Radioactivity was measured by liquid scintillation spectroscopy with 10 mL of Insta Gel (Packard, Downers Grove, IL). Product formation in the incubation was measured as ^{14}C (cpm) comigrating with 5-HETE corrected for recovery of [^3H]-5-HETE. Typically uninhibited control incubations convert 15% of added substrate to products.

Compounds were evaluated at concentration up to 300 μM or their solubility limit in the assay buffer. Product formation in the treatment groups (N = 2) were compared to the mean level in the control group (N = 8). An average inhibition of 15% was significantly different from control as evaluated by one-way analysis of variance and Duncan's multiple range test ($p < 0.05$). Lower responses are considered inactive. IC_{50} values were computed as the 50% intercept from linear regression analysis of percent inhibition vs. log concentrations.

Molecular Modeling Methods. To find the low-energy conformation of the lactone ring of **60**, the bonds from C_1 to C_{10} were rotated in 120° increments with CHEMLAB V8.0. This coarse scan is permissible because none of the carbon atoms are branched. The 34 conformations for which the O_{15}–C_1 distance is between 1.0 and 2.0 Å were then energy minimized with MMP2, Revision 5.0. The conformation that differs from each of these by a 120° rotation about C_{10}–C_{11} and C_{14}–C_{15} was also minimized. The conformation shown in Figure 1 is the minimum energy structure.

The conformation of **16** shown was energy minimized from one prepared from **60** by rotations about C_1–C_2 and C_2–C_3 to relieve the close contact of O_1 and O_{15}.

The conformation of **59** was prepared by forming the 6,7 and 8,9 double bonds on the **60** structure and adding the OH_5. The pucker and orientation of the ring was chosen by a systematic scan of the minimized structure about C_1–C_2, C_2–C_3, C_3–C_4, C_4–C_5, and C_5–C_6. The conformation shown was chosen as the low energy-structure for which O_1 is roughly in the same position as that in **60** and **16**.

The conformation of **55** shown was minimized from a starting

structure that resembled **60** as much as possible: because of the length of the diacetylene group, extra volume in the C_8–C_{10} region of the molecule was unavoidable.

The starting structures for the other compounds were prepared by fusing an MMP2 minimized structure of the C_6–C_{16} region of the molecule with the C_1–C_8 fragment of **16** or **55**. The starting structure of each C_9–C_{16} fragment was built from templates of standard geometry. The starting conformations were chosen as follows: (1) for the dienes **50′–52′**, monoene **51′**, and saturated fragment **9′**, H_{12} was set trans to H_{13}; (2) for **9′** and **50′–54′**, H_{10} was set trans to H_{11}, and for **9′**, **34′**, and **50′–53′**, H_{14} was set trans to H_{15}; and (3) similar rotations about the C_9–C_{10} and C_{15}–C_{16} bonds were selected for all fragments modeled. For certain compounds it was necessary to add parameters: the V_2 torsional parameter for the following bond, C(sp^3)–C(sp^2)–C(sp^2)–C(sp) was set equal to 15.0 kcal/mol while V_1 and V_3 were set at 0.0. For C(sp)–C(sp^3)–O(sp^3)–H and C(sp)–C(sp^3)–O(sp^3)–lone pair all three torsional parameters were set at 0.0. The bending constant for the C(sp^2)–C(sp^2)–C(sp) bond was set at 0.40 kcal/mol with a natural bond angle 120°; the values for C(sp)–C(sp^3)–O(sp^3) were 0.20° and 109.5° and those for C(sp)–C(sp)–C(sp) were 0.40° and 180°. The molecular graphics and manipulation program was written in our laboratory.

Supplementary Material Available: Tables listing Cartesian coordinates for compounds **9**, **12**, **16**, **20**, **34**, **50–56**, and **59–62** (16 pages). Ordering information is given on any current masthead page.

1-[[[5-(Substituted phenyl)-2-oxazolyl]methylene]amino]-2,4-imidazolidinediones, a New Class of Skeletal Muscle Relaxants

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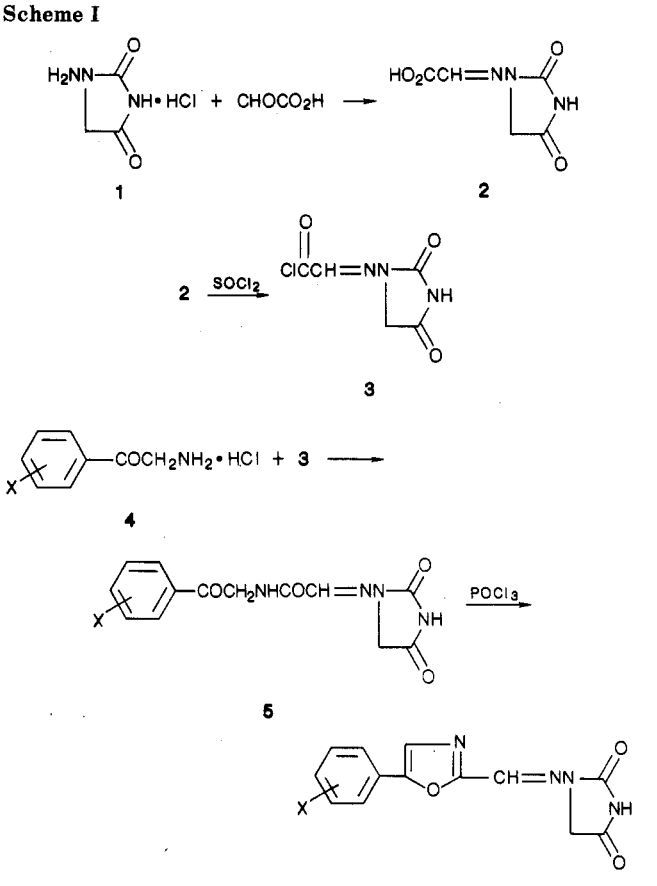
A series of 1-[[[5-(substituted phenyl)-2-oxazolyl]methylene]amino]-2,4-imidazolidinediones (**6a–t**) was synthesized, and the compounds were evaluated for direct skeletal muscle inhibition in the pithed rat gastrocnemius muscle preparation. The correctness of structural assignment of the new series was verified by alternate, unequivocal synthesis of one representative structure (**6f**). The phenyloxazoles **6d**, **6g**, **6j**, **6k**, and **6l** exhibited significant skeletal muscle relaxant activity when administered intravenously and orally. The skeletal muscle relaxant effect of these five compounds is similar to that of other direct-acting skeletal muscle relaxants. The oxazole moiety proved to be an acceptable isosteric replacement for furan, as the biological activity in the oxazole series was retained. The synthesis of this new class of compounds is described, and pharmacologic evaluation data are presented. A discussion of structure–activity relationships is also presented.

In the search for compounds for the treatment of skeletomuscular disorders, a series designed to be direct skeletal muscle relaxants was synthesized and pharmacologically evaluated. Dantrolene sodium[1] and other similar 1-[[[5-(substituted phenyl)-2-furanyl]methylene]amino]-2,4-imidazolidinediones have been found to exhibit direct skeletal muscle relaxant activity.[2,3] It was decided to determine whether structures with an oxazole group would result in compounds that exhibit direct skeletal muscle inhibition. This paper describes the synthesis and pharmacologic evaluation of a series of 1-[[[5-(substituted phenyl)-2-oxazolyl]methylene]amino]-2,4-imidazolidinediones,[4] a new class of skeletal muscle relaxants.

Chemistry

The methodology employed in the past[3] to prepare phenylfuranyliminoimidazolidinediones was not applicable for the proposed corresponding oxazole counterparts. Prior syntheses took advantage of the Meerwein coupling reaction of aryldiazonium salts with 2-furancarboxaldehyde. In this instance, 2-oxazolecarboxaldehyde was not accessible in quantities practical for synthesis, the outcome of a Meerwein reaction was unknown, and 5-phenyl-2-furancarboxaldehydes were nonexistent. Consequently, a new synthetic approach was sought. Two reviews on oxazole chemistry[5,6] yielded useful information on alternative synthetic approaches. The Robinson–Gabriel synthesis[5,6] effects cyclization of α-acylamino carbonyl com-

Scheme I



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