of a normal C–C single bond. Interestingly the four-membered ring is not planar but puckered and results in a skewed conformation of the two $Os(CO)_4$ moieties. The average twist angle about the Os–Os bond is 21°. The observed geometry is presumably due to the flexibility of the four-membered ring, which allows movement of the $Os(CO)_4$ moieties out of the sterically congested eclipsed configuration. The structure closely resembles, including the value of the twist angle, the unsubstituted parent molecule $Os_2(CO)_8(\mu\text{-}\eta^1,\eta^1\text{-}C_2H_4)$, **3c**.[4] Although two other examples of 1,2-dimetallacyclobutanes have been reported,[16] the photostability of **3a** is especially noteworthy and may indicate an extensive series of related 1,2-diosmacycles.[17]

The difference in the long-wavelength photoactivity of **1**-Os and **1**-Ru in the presence of olefins is striking and merits some considerations. Recent UV photoelectron spectral studies and theoretical investigations[18] deduced similar electronic structures for the two $M_3(CO)_{12}$ compounds. Thus the primary photoexcitation probably leads to a common photochemical intermediate (eq 2). Although there is no general agreement concerning the

$$M_3(CO)_{12} \underset{M = Ru,\ Os}{\overset{h\nu,\ \lambda > 370\ nm}{\rightleftharpoons}} M_3(CO)_{12}^* \qquad (2)$$

nature of the intermediate nor about the subsequent steps in the photoreaction,[19] we believe that with the present results, a previously postulated mechanism[2,19a] must receive serious consideration at least for the reaction with olefins (eq 3). The difference

$$M_3(CO)_{12}^* \rightleftharpoons \left\{ \begin{array}{l} M(CO)_4 \xrightarrow{\text{olefin}} M(CO)_4(\eta^2\text{-olefin}) \quad \mathbf{2} \\ \uparrow\downarrow \\ M_2(CO)_8 \xrightarrow{\text{olefin}} M_2(CO)_8(\mu\text{-}\eta^1,\ \eta^1\text{-olefin}) \quad \mathbf{3} \end{array} \right. \qquad (3)$$

in reactivity between **1**-Os and **1**-Ru is best explained by the relative M–M bond strength in these species, Os–Os > Ru–Ru, which may also account for the facile breakdown of $Ru_2(CO)_8$ and formation of **2**-Ru only.

The synthetic implications of the mechanism depicted by eq 2 are being evaluated by exploring the photoreaction of $Os_3(CO)_{12}$ with other unsaturated substrates and the interaction of olefinic ligands with other known metal-metal multiple bonded species.[20] The reactivity of **3a** is also under intense scrutiny.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada, the University of Alberta and the Max-Planck-Institute für Strahlenchemie for financial support, Professor J. R. Norton for a preprint of ref 4, and Professor R. Hoffmann for some relevant comments and preprints.

Registry No. **1**-Os, 15696-40-9; **2a**, 85702-38-1; **2b**, 85719-06-8; **3a**, 85702-39-2; methyl acrylate, 96-33-3; dimethyl fumarate, 624-49-7; osmium, 7440-04-2.

Supplementary Material Available: Detailed results of the X-ray molecular structure of $Os_2(CO)_8(\mu\text{-}\eta^1,\eta^1\text{-}CH_2CHCO_2CH_3)$, tables of experimental details, positional and thermal parameters (U, B), bond distances, and bond angles (5 pages). Ordering information is given on any current masthead page.

(16) (a) Green, M.; Laguna, A.; Spencer, J. L.; Stone, F. G. A. *J. Chem. Soc., Dalton Trans.* **1977**, 1010 ($Pd_2(1,5\text{-}C_8H_{12})(\mu\text{-}\eta^1,\eta^1\text{-}C_2F_4)$). (b) Theopold, K. H.; Bergman, R. G. *Organometallics* **1982**, *1*, 1571 ($Co_2(CO)_2(\eta\text{-}C_5H_5)_2(\mu\text{-}\eta^1,\eta^1\text{-}$benzocyclobutene)).

(17) Preliminary results indicate that photolysis ($\lambda \geq 370$ nm) of $Os_3(CO)_{12}$ with continuous ethylene purge gives conversion to a mixture of **2c** and **3c**.

(18) (a) Green, J. C.; Seddon, E. A.; Mingos, D. M. P. *J. Chem. Soc., Chem. Commun.* **1979**, 94. (b) Ajò, D.; Granozzi, G.; Tondello, E.; Fragalà, I. *Inorg. Chim. Acta* **1979**, *37*, 191. (c) Sherwood, D. E., Jr.; Hall, M. B. *Inorg. Chem.* **1982**, *21*, 3458. (d) Sherwood, D. E., Jr.; Hall, M. B. *Organometallics* **1982**, *1*, 1519. (e) Delley, B.; Manning, M. C.; Ellis, D. E.; Berkowitz, J.; Trogler, W. C. *Inorg. Chem.* **1982**, *21*, 2247.

(19) (a) Austin, R. G.; Paonessa, R. S.; Giordano, P. J.; Wrighton, M. S. *Adv. Chem. Ser.* **1978**, *168*, 189. (b) Malito, J.; Markiewicz, S.; Poë, A. *Inorg. Chem.* **1982**, *21*, 4337. (c) Desrosiers, M. F.; Ford, P. C. *Organometallics* **1982**, *1*, 1715.

(20) Preliminary work indicates that the reaction of ethylene and methyl acrylate with $[(\eta\text{-}C_5Me_5)Rh(CO)]_2$ results in cleavage of the Rh=Rh bond and formation of $(\eta\text{-}C_5Me_5)Rh(CO)$(olefin)); Lin, G.-Y. unpublished observations.

Stereochemical Course of 5-Lipoxygenation of Arachidonate by Rat Basophil Leukemic Cell (RBL-1) and Potato Enzymes

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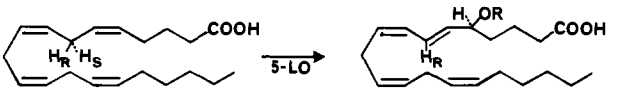
Metabolism of arachidonic acid by the 5-lipoxygenase pathway in response to triggering by IgE-dependent stimuli or by phagocytic leukocytes leads to the production of leukotrienes, a family of potent biological agents that function as regulators of the mammalian cellular microenvironment.[1] We report herein on the absolute stereochemistry of the conversion of arachidonate (**1**)

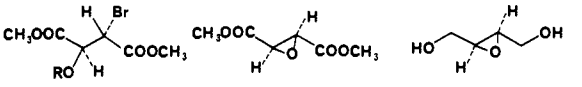


1 **2** R=OH

4 H_R=D **3** R=H

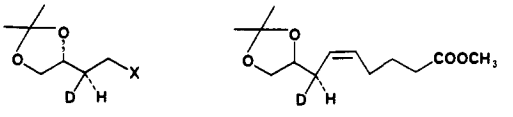
to (5*S*)-hydroperoxy-6*E*-8*Z*,11*Z*,14*Z*-eicosatetraenoic acid (5-HPETE, **2**), the initial step in the leukotriene pathway, in two different enzyme systems—the 5-lipoxygenases (5-LO) derived from rat basophil leukemic (RBL-1) cells and potato tubers.[2] In this study (7*R*)-deuterioarachidonic acid was obtained by total synthesis and converted enzymically to 5-HPETE, which was reduced to the corresponding alcohol (5-HETE, **3**) and analyzed by mass spectrometry as a stable silyl derivative.

Synthesis of (7*R*)-Deuterioarachidonic Acid (4). The readily



5 **6** **7**

available dimethyl (2*R*)-acetoxy-(3*S*)-bromosuccinate (**5**)[4] was deacetylated (0.3 M hydrochloric acid in methanol, 0.1 M **5**, 46 h at 22 °C, 82% yield) and cyclized (6 equiv of potassium carbonate in acetone at 22 °C for 14 h, 69% yield) to the epoxy ester **6** [mp 70–73 °C; $[\alpha]^{25}_D$ −136.9° (*c* 1.07, methanol); enantiomeric purity >97%].[5] Reduction with sodium borohydride (3.7 equiv in ethanol at 0 °C for 4.5 h, 0.2 M **6**) gave 64% of the crystalline, C_2 symmetric diol epoxide **7**.[6] Stereospecific introduction of the deuterium label was achieved by epoxide opening with 95% ^2H lithium aluminum deuteride (2.5 equiv, tetrahydrofuran (THF), [**7**] = 0.06 M, 10 h at reflux), followed by in situ protection of



8 X=OH **10**

9 X=$PPh_3^+Br^-$

(1) For recent reviews see: (a) Bailey, D. M.; Casey, F. B. *Ann. Rep. Med. Chem.* **1982**, *17*, 203. (b) Clark, D. A.; Marfat, A. *Ibid.* **1982**, *17*, 291. (c) Corey, E. J. *Experientia* **1982**, *38*, 1259.

(2) Corey, E. J.; Albright, J. O.; Barton, A. E.; Hashimoto, S. *J. Am. Chem. Soc.* **1980**, *102*, 1435.

(3) Reactions involving air-sensitive reagents or substances were conducted under an Ar atmosphere. Satisfactory spectroscopic data (infrared, proton magnetic resonance, and mass spectral) were obtained for each synthetic intermediate by using chromatographically purified and homogeneous samples.

(4) Corey, E. J.; Marfat, A.; Hoover, D. J. *Tetrahedron Lett.* **1981**, *22*, 1587.

(5) Seebach, D.; Wasmuth, D. *Helv. Chim. Acta* **1980**, *63*, 197.

(6) Found for (2*S*,3*S*)-epoxy-1,4-butanediol (**7**): ^1H NMR (acetone-d_6 with water-d_2) δ 3.69 (dd, J = 12, 3 Hz, 2 H, CHHOH), 3.49 (dd, J = 12, 5 Hz, 2 H, CHHOH), 2.98 (7, 2 H); IR (neat) 3080 (OH), 1040, 875 cm^{-1}; MS, *m/e* 105 (M + 1), 104 (M^+), 73 (M − CH_2OH); $[\alpha]^{25}_D$ −30.57° (*c* 0.168, ethanol); mp 79–82 °C.

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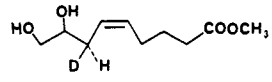
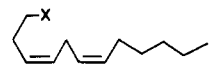
the product triol as the 1,2-acetonide **8** (catalytic *p*-toluenesulfonic acid, acetone, 24 h, 22 °C). Chromatographically purified **8** was converted to phosphonium salt **9** in 65% yield in two steps:[7] (1) reaction with 2 equiv of carbon tetrabromide, 2 equiv of triphenylphosphine (12:1 methylene chloride–pyridine, [**8**] = 0.35 M, 6 h, 22 °C); (2) reaction with 2 equiv of triphenylphosphine (1 M) in acetonitrile containing 0.3 equiv of calcium carbonate for 18 h at reflux. Phosphonium salt **9** was converted to the Wittig ylide (0.95 equiv of sodium hexamethyldisilazide, toluene, [**9**] = 0.1 M, 0 °C)[8] and treated with 1.1 equiv of methyl 4-formylbutyrate[9] at −78 °C (1:1 THF–toluene)[10] to provide acetonide ester **10**, which was deprotected in situ (0.75 M hydrochloric acid



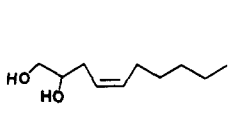
11



12 X=$PPh_3^+Br^-$
15 X=OH

in 15:1 methanol–water for 8 h at 22 °C) to afford pure diol **11** in 55% yield.

Phosphonium salt **12**, corresponding to the C(9)–C(20) segment of **4**, was constructed in the following way: diol **13**[7] was cleaved



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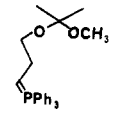

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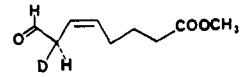


16

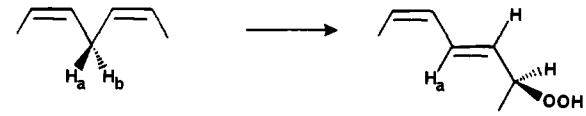
to the corresponding β,γ-unsaturated aldehyde (1.42 equiv of lead tetraacetate, [13] = 0.2 M in methylene chloride for 1.5 h at −65 °C), which was allowed to react with ylide **14**[11] (1:1 THF–toluene at −78 °C) to afford, after deprotection (1.25 mM hydrochloric acid in 3:1 THF–water, 1 h at 22 °C), dienol **15** (30% from **13**). Dienol **15** was converted to phosphonium salt **12** in 82% yield as described above for **9**. The complete carbon skeleton of **4** was assembled by Wittig coupling[10] of aldehyde **16**[7,12] with the ylide derived from deprotonation of **12** (0.87 equiv of sodium hexamethyldisilazide, toluene, 0 °C) to afford after chromatography **4** methyl ester. Deuterium incorporation in **4** methyl ester was determined to be 93.7% from mass spectral analysis.[13] **4** methyl ester was stored in frozen benzene under argon and hydrolyzed (1:1 1 M lithium hydroxide–THF, 20 °C, 12 h, 0.03 M **4**) immediately prior to use.

Enzymatic Conversion of 4 to 5-HETE by 5-LO from RBL-1 Cells. (7*R*)-Deuterioarachidonic acid (**4**) was converted to 5-HETE by the 5-LO in RBL-1 cell supernatant[14] according to the following procedure: an ethanolic solution of **4** (140 μg in 70 μL) was introduced into 1 mL of supernatant, calcium chloride was added to afford a final concentration of 2 μM (calcium is required for 5-LO activity in this RBL-1 supernatant preparation),[15] and the mixture was incubated in air for 2 h at 20 °C. After extractive isolation, the crude product was taken up in THF and esterified with ethereal diazomethane. 5-HETE methyl ester was purified by preparative normal-phase high-pressure liquid chromatography (HPLC)[16] and silylated with bis(trimethylsilyl)trifluoroacetamide (12 h, 4 °C), and its mass spectrum was measured (electron impact ionization, 70 eV, probe temperature = 175 °C, Kratos MS-50 spectrometer).

Enzymatic Conversion of 4 to 5-HPETE by 5-LO from Potato.[2] The 5-LO-catalyzed oxygenation of **4** by potato was effected by using 2 mL of crude potato 5-LO[2] ([protein] = 8 mg/mL) and **4** (2.5 mg) in 400 μL of 6 mM ammonium hydroxide with traces of Triton X-100 and 4-hydroxy-2,2,6,6-tetramethylpiperidinooxy free radical under an oxygen atmosphere for 30 min (20 °C). Methanol was added, and the reaction mixture was brought to pH 9 (1 N sodium hydroxide) at 0 °C. Sodium borohydride (30 mg) was added to reduce 5-HPETE to 5-HETE (30 min, 0 °C). The mixture was brought to pH 2, and insoluble protein was separated by centrifugation (1500*g*, 10 min). The 5-HETE methyl ester was isolated, silylated, and analyzed for deuterium as described for the RBL-1 system.

Deuterium incorporation in the trimethylsilyl ether of 5-HETE methyl ester was determined by using the relative peak heights of the parent ions of the unlabeled (M^+ *m/e* 406) and labeled (M^+ *m/e* 407) compounds.[13] The 5-HETE obtained from **4** in both RBL-1 and potato systems retained 100% (±3%) of the original deuterium label. Thus, in *both* the vegetable and mammalian lipoxygenase reactions the 7-H_s hydrogen is removed in the conversion of arachidonate to (5*S*)-HPETE (**2**).

All known lipoxygenase reactions produce hydroperoxide in which the product has the absolute chirality expressed in subunit formula B by removal of H_b in the starting unsaturated acid of



A **B**

subunit formula A. This absolute stereochemistry is followed not only by the two arachidonate 5-LO systems of the present study but also by the arachidonate 12-LO of platelets,[17] the 5,6-dihydroarachidonate 15-LO soybean,[18] and the linoleate 9- and 11-LO of corn and soybean.[19] As the number of observed LO reactions that proceed by the absolute route A → B increases, it is tempting to speculate that this may be a specificity inherent in lipoxygenase catalysis.

We have recently devised two potent inhibitors of leukotriene biosynthesis that irreversibly deactivate the 5-LO enzyme, 5,6-dehydro-[7] and 4,5-dehydroarachidonate.[14] The available evidence supports the proposition that these inhibitors function by undergoing the 5-LO transformation to give unstable 5,6- or 4,5-dehydro-5-HPETE, which decompose homolytically near the catalytic site to destroy enzyme competence. The results of the present study suggest a crucial test of this mechanism since they predict that (7*R*)-deuterio-4,5- or 5,6-dehydroarachidonic acids should show a diminished rate of enzyme deactivation.[20]

Registry No. 1, 506-32-1; **2**, 71774-08-8; **3**, 70608-72-9; **4**, 85735-74-6; **5**, 78816-00-9; **6**, 85798-25-0; **7**, 85798-26-1; **8**, 85735-75-7; **9**, 85735-76-8; **10**, 85735-77-9; **11**, 85735-78-0; **12**, 83606-41-1; **13**, 85798-27-2; **14**, 79065-14-8; **15**, 29125-78-8; **16**, 85735-79-1; 3-bromopropan-1-ol, 627-18-9; arachidonate 5-lipoxygenase, 80619-02-9.

(7) Corey, E. J.; Munroe, J. E. *J. Am. Chem. Soc.* **1982**, *104*, 1752.
(8) Wannagat, U.; Niederprum, H. *Chem. Ber.* **1961**, *94*, 1540.
(9) Prepared by ozonolysis of 1-methoxycyclopentene.
(10) Bestmann, H.; Stransky, W.; Vostrowsky, O. *Chem. Ber.* **1976**, *109*, 1694.
(11) The phosphonium salt from which **14** was derived was prepared from 3-bromopropan-1-ol in 91% yield by a two-step procedure: (1) reaction with 1.1 equiv of triphenylphosphine in toluene at reflux for 44 h; (2) protection of the alcohol by treatment with 3 equiv of 2-methoxypropene in 3:1 methylene chloride–2,2-dimethoxypropane for 3 h at −20 °C with 0.002 equiv of pyridinium tosylate as catalyst.
(12) Aldehyde **16** was derived from diol **11** by treatment with 1.5 equiv of lead tetraacetate at −78 °C in methylene chloride. The proton magnetic resonance spectrum of **16** showed no contamination with the α,β-unsaturated isomer.
(13) Percent deuterium was calculated from %D = $R - Q/(R - Q + 1)$, where $R = M^+ + 1/M^+$ (experimentally determined) and $Q = M^+ + 1/M^+$ (calculated for unlabeled compound).
(14) RBL-1 cells were broken by using a tissue disrupter, further homogenized in a blender and centrifuged at 10000*g*. See, for example: Corey, E. J.; Kantner, S. S.; Lansbury, Jr., P. T. *Tetrahedron Lett.* **1983**, *24*, 265.
(15) Parker, C. W.; Aykent, S. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1011.
(16) A solution of the esterified crude lipoxygenase products in benzene was injected onto a 25-cm analytical silica gel (du Pont Zorbax) column and eluted HETES were detected by UV absorbance at 235 nm. Two solvent systems were found to be effective in separating the isomeric HETEs: 0.6% propan-2-ol in hexane, retention volume (5-HETE) ≃ 36 mL; 15:1 hexane–THF, retention volume (15-HETE) ≃ 18 mL, retention volume (5-HETE) ≃ 36 mL.
(17) Hamberg, M.; Hamberg, G. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1090.
(18) Samuelsson, B.; Hamberg, M. *J. Biol. Chem.* **1967**, *242*, 5329.
(19) Egmond, M. R.; Vliegenthart, J. F. G.; Boldingh, J. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 1055.
(20) This research was assisted financially by a grant from the National Science Foundation. We are grateful to Dr. Robert A. Lewis for RBL-1 cells.