The ready availability of enantiomerically pure **1** and its analogues should open up the exploration of other cyclopentadienylmetal-catalyzed enantioselective transformations.

Acknowledgment. This work was supported by NIH-GM 22479. R.L.H. was the recipient of a University of California Reagents' Fellowship (1982–1984). M.E.W. was an Exxon Research and Engineering Company and NIH postdoctoral fellow (NRSA GM 11105; 10.1.–12.11.1986). K.P.C.V. was a Miller Research Professor in residence (1985–1986). We thank Dr. A. Mori for carrying out the kinetic experiments.

Supplementary Material Available: A listing of positional and thermal parameters, and tables of bond lengths and angles for (+)-**2**, the dicamphorsulfonate of (1*S*,2*S*,4*S*,5*S*)-2,5-diphenylcyclohexane-1,4-diol, and (±)-Cp*CpTiCl$_2$, including SHELXTL renditions of the structures of the latter two compounds (16 pages); tables of observed and calculated structure factors for the three previously listed compounds (34 pages). Ordering information is given on any current masthead page.

(15) This picture presupposes that the stereochemistry of hydrogenation is set at this stage, an assumption which may not be valid: Halpern, J. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: Orlando, Fl, 1985; Vol. 5, p 41. Indeed, the mechanism of this process has not been established. See, also: Lehmkuhl, H.; Tsien, Y.-L.; Janssen, E.; Mynott, R. *Chem. Ber.* **1983**, *116*, 2426.

(16) An X-ray structural analysis of this compound (in the racemic series) reveals the openness of one of the metal faces to a nonstereodifferentiating substrate approach (see Supplementary Material).

Evidence in Favor of an Organoiron-Mediated Pathway for Lipoxygenation of Fatty Acids by Soybean Lipoxygenase

E. J. Corey* and Ryu Nagata

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138
Received May 26, 1987

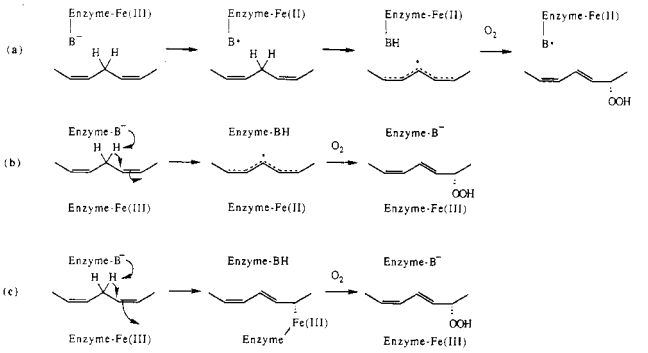
The enzymatic lipoxygenation of polyunsaturated fatty acids is of interest both mechanistically and because of its fundamental role in the biosynthesis of physiologically important compounds such as prostaglandins and leukotrienes.[1] A still unresolved mechanistic question is whether lipoxygenation proceeds via free-radical or organoiron intermediates formed during rate-limiting C–H bond cleavage.[2] Free carbon radicals might reasonably arise by processes (**a**) or (**b**) in Scheme I. In the case of (**a**), the reactive high-spin Fe(III) form of the enzyme activates itself by electron transfer to form an H atom abstracting group, whereas in the case of (**b**) a proton acceptor and the enzyme-bound Fe(III) participate in a concerted proton–electron-transfer reaction with substrate. The alternative organoiron pathway involves concerted deprotonation and electrophilic addition of Fe(III) to carbon giving an organoiron intermediate (coordinated to enzyme) from which product can be formed by σ bond insertion of dioxygen as depicted in (**c**). Described herein are three lines of evidence favoring process (**c**) for soybean lipoxygenase (SBLO).

The first argument is based on the assessment of the self-inactivation of SBLO during fatty acid oxidation as a function of substrate structure and reaction conditions by using the total turnover number (TTN) for lipoxygenation, i.e., the maximum number of molecules of lipoxygenation product produced per molecule of enzyme, as a measure of the frequency of self-inactivation during lipoxygenation. The enzyme utilized in these and all other experiments described herein was Sigma Co. type I SBLO further purified by DEAE-Sephadex column chromatography.[3]

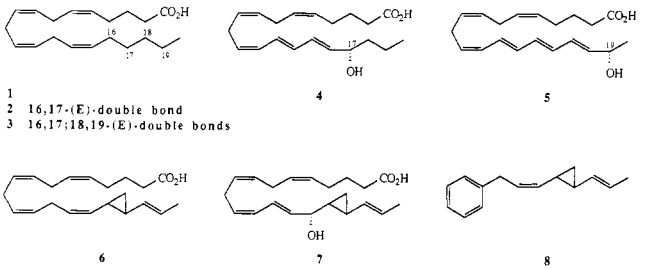
(1) For a recent review, see: Corey, E. J. In *Stereochemistry of Organic and Bioorganic Transformations*; Bartmann, W., Sharpless, K. B., Eds.; VCH Publishers: 1986; pp 1–12.

(2) See, for example; Corey, E. J.; d'Alarcao, M.; Matsuda, S. P. T. *Tetrahedron Lett.* **1986**, *27*, 3585–3588.

Scheme I



Three substrates were selected to probe the issue of radical vs organoiron intermediates, arachidonic acid (**1**), 16,17-(*E*)-



dehydroarachidonic acid (**2**), and 16,17-(*E*);18,19-(*E*)-bis-dehydroarachidonic acid (**3**). Dramatic effects of structure, O_2 pressure, and temperature on TTN values were observed. All enzymatic experiments were conducted in pH 9.2 0.2 M sodium borate buffer by using 0.04–10 nM purified SBLO with substrate at concentrations in the 4–20 μM range.

It was determined (at 23 °C in air) that substrate **2** was bound more tightly but oxidized more slowly (K_m 5.7 μM, V_{max} 2700 min^{-1}) than arachidonate (**1**, K_m 13.3 μM, V_{max} 11 000 min^{-1}).[4] This result runs counter to expectations based on a free-radical intermediate (paths (**a**) or (**b**)) that V_{max} for **2** should exceed that for **1** because of the considerably lower C_{13}–H bond dissociation energy (ca. 10–14 kcal/mol).[4] Values for TTN of **1** and **2**, which were found to be 50 000 and 2200 (air, 23 °C), respectively, also do not accord with the idea of a free-radical intermediate which occasionally causes enzymic inactivation by attack on the enzyme in competition with oxygenation.[5] Clearly the more stabilized radical formed by processes (**a**) or (**b**) operating on **2** as compared with **1** should produce less inactivation of SBLO rather than more. On the other hand, the organoiron intermediate from **2** should homolyze more readily than that from **1** and lead to more frequent deactivation of SBLO. Thus process (**c**) is consistent with these experimental observations. Further, as expected for process (**c**), in which capture of the organoiron intermediate ought to be favored by increasing O_2 pressure, the TTN for arachidonate can be increased to 185 000 at 10 atm of O_2 and further to 340 000 at 50 atm of O_2 (all at 23 °C). Similarly, TTN values for **2** increase from 500 at 0.02 atm of O_2 to 3500 at 1 atm of O_2 and further to 12 800 at 50 atm of O_2 (all at 23 °C).[6] Values of TTN

(3) Axelrod, B.; Cheesbrough, T. M.; Laakso, S. *Methods Enzymol.* **1981**, *71*, 441–451.

(4) Reaction rates were measured by ultraviolet absorption and values of K_m and V_{max} were determined by Lineweaver–Burk analysis.

(5) There is much evidence[1] that free radicals formed from substrate can effectively inactivate SBLO. The values of TTN measured in this work were generally unaffected by carrying out the lipoxygenation in the presence of sodium borohydride which serves to reduce immediately the product hydroperoxide to the corresponding alcohol. This fact argues against enzyme inactivation by alkoxy radicals derived from LO product.

(6) Incubation experiments performed at pressures of O_2 above 1 atm were carried out in a Teflon vessel within a Parr stainless steel autoclave. Reaction was initiated under pressure by magnetically lowering a small vial containing substrate into the oxygenated solution of SBLO. Maximum conversion of substrate to product generally occurred in less than 60 min. Concentrations of SBLO were 0.04–1.0 nM. Control experiments showed no measurable oxidation in the absence of enzyme. At low O_2 pressures (0.02 atm) reaction was initiated by adding a trace amount of 15-HPETE.

0002-7863/87/1509-8107$01.50/0

Table I. Products from the Reactions of 2 with SBLO at 23 °C

pressure O_2, atm	11-LO (%)	15-LO (%)	17-LO (%)	17:15 LO ratio
0.05	2.3	1.3	96.4	74
0.2	2.6	6	92.2	15.4
50	2.3	9	89	9.9

generally *increase* with decreasing temperature. For example, for **2**, TTN values in air changed from 1000 at 33 °C to 2200 at 23 °C to 4300 at 13 °C. The strong variation of TTN with temperature is more easily understood in terms of organoiron rather than free-radical intermediates.

The behavior of substrate **3** falls exactly into line with the results just described for **1** and **2**. In air at 23 °C no conversion of **3** to lipoxygenated product can be observed by ultraviolet absorption measurement. A modest reaction can be initiated by addition of 15-HPETE as activator (TTN ca. 100 in air increasing to 350 in 1 atm of O_2). Although TTN values increase further with pressure, they remain well below those observed for **2** under comparable conditions. Thus **3** was found to be an excellent time dependent inactivator of SBLO as shown by kinetic measurements which reveal K_i 1.0 μM, k_{inact} 0.14 min^{-1} (in air at 23 °C).[7] This result is readily understood in terms of the enhanced susceptibility of the organoiron intermediate from **3** to homolytic decomposition.

The lipoxygenation (LO) products obtained by the action of SBLO on **2** and **3** are of considerable interest from a mechanistic viewpoint,[8] especially because the product distribution was found to depend on O_2 pressure. The distributions of LO products from **2** at various O_2 pressures are summarized in Table I. Although arachidonate (**1**) is converted almost exclusively to the 15-LO product (15-HPETE) by SBLO, **2** affords mainly 17-LO product along with small amounts of 11- and 15-LO products. As O_2 pressure is increased the ratio of 17-LO to 15-LO products decreases, consistent with the trapping of more 15-organoiron intermediate at higher O_2 pressure. The data indicate that the minor 11-LO product probably arises independently of the 15-LO product (e.g., from the 11-organoiron intermediate). In addition it appears that some of the 17-LO product comes from the 15-organoiron intermediate and the remainder from a directly formed (and thermodynamically more stable) 17-organoiron intermediate. The major 17-LO product (after hydroperoxide reduction) has been shown to be the 17-(*S*)-isomer **4**.[8,9] These results are not readily reconciled with the free-radical processes (**a**) and (**b**). The bisdehydroarachidonate analogue **3** is converted by SBLO at 50 atm O_2 and 23 °C mainly into the 19-LO product **5** (94.7%), although smaller amounts of 11-LO (2.9%), 15-LO (0.7%), and 17-LO (1.5%) have been isolated and identified.

Informative results have also been obtained by a study of the "radical clock" substrate analogue **6**. At 23 °C in air **6** behaved not as a substrate but as a time-dependent inactivator of SBLO; K_i 21 μM and k_{inact} 0.28 min^{-1}.[7] At higher O_2 pressures, as with **3**, lipoxygenation could be observed. Thus at 23 °C and 50 atm of O_2 the principal pathway was 15-lipoxygenation *without* cyclopropane cleavage to give (after hydroperoxide reduction) **7** (51%). Analogous products of 11-, 8-, and 5-lipoxygenation *without* cyclopropane cleavage amounted to another 28%. Cyclopropyl cleavage products totaled only 14%. The other reaction product, 14-formyl-5,8,11,13-tetradecatetraenoic acid, was formed in 7% yield. In contrast, peroxide-induced, free-radical chain oxidation of the model olefin **8** in an oxygen atmosphere produced *only* cyclopropyl cleavage products.[10-12]

(7) For method of measurement, see: Corey, E. J.; Lansbury, P. T., Jr.; Cashman, J. R.; Kantner, S. S. *J. Am. Chem. Soc.* **1984**, *106*, 1501-1503.
(8) Syntheses of substrates **2**, **3**, and **6** and the isolation and identification of products of reaction with SBLO are described in a separate paper, see: Corey, E. J.; Nagata, R. *Tetrahedron Lett.*, in press.
(9) Small amounts of the 11,12-(*E*)-isomer of **4** can also be detected.[8]
(10) Griller, D.; Ingold, K. *Acc. Chem. Res.* **1980**, *13*, 317-323.
(11) Conditions: benzene solution, 21 °C, di-*tert*-butyl peroxyoxylate as initiator. HPLC analysis and spectroscopic identification of all products.
(12) Bartlett, P. D.; Benzing, E. P.; Pincock, R. E. *J. Am. Chem. Soc.* **1960**, *82*, 1753-1768.

Finally, an independent study has provided a chemical analogy for the organoiron-mediated process (**c**).[13] Although, further research is required to establish the pathway of the lipoxygenation of fatty acids, the feasibility of mechanism (**c**) is strongly supported by the results outlined above.[14]

(13) Corey, E. J.; Walker, J. C., following publication.
(14) This research was supported in part by grants from the National Institutes of Health and the National Science Foundation.

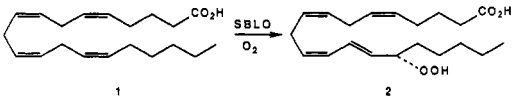
Organoiron-Mediated Oxygenation of Allylic Organotin Compounds. A Possible Chemical Model for Enzymatic Lipoxygenation

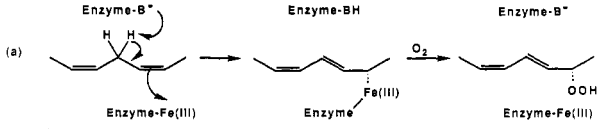
E. J. Corey* and Jonathan C. Walker

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

Received May 26, 1987

Evidence has been presented in the preceding paper[1] that the allylic dioxygenation of fatty acids by soybean lipoxygenase (SBLO), for example, the conversion of arachidonic acid (**1**) to the 15(*S*)-hydroperoxide **2**, follows the pathway summarized in (**a**). The rate-limiting C-H cleavage step in this scheme involves





proton abstraction by a basic group on the enzyme which is facilitated by concurrent electrophilic attack by the Fe(III) unit at the catalytic site. The allylic organoiron intermediate which results can then react with O_2 by σ-bond insertion to form product. A consequence of enzymatic control of the attachment of iron to the substrate would be position and stereospecific delivery of oxygen. Because there are no previously known purely chemical analogues of this lipoxygenation process and, indeed, essentially no knowledge of organoiron-mediated oxygenation reactions of this general sort, the studies described herein were undertaken. The results obtained in this investigation support the feasibility of mechanism (**a**) for enzymatic lipoxygenation and, at the same time, demonstrate a new approach to transition-metal-mediated chemical oxidation.

The realization of a biomimetic chemical allylic oxidation by the mechanism outlined in (**a**) is complicated by the difficulty of arranging for the concerted attack on the substrate of two species which are incompatible in solution, i.e., a potent base (B^-) and a strong Lewis acid (Fe(III) having a vacant coordination site). For this reason a simpler system was chosen for study which consisted of $FeBr_3$ as the Fe(III) electrophile and the allylic tin compounds **3** and **4** as substrates. Because of the excellence of cationic tin as a leaving group, it seemed likely that the required organoiron intermediate could be generated in organic solvents without assistance from a strongly basic reagent. 1-Phenylprop-2-enyltributyltin (**3**) was prepared as previously described.[2]

(1) Corey, E. J.; Nagata, R. *J. Am. Chem. Soc.*, preceding paper in this issue.

0002-7863/87/1509-8108$01.50/0