was added. A yellow-orange precipitate was formed instantly. After stirring for another 15 min, the precipitate was filtered off and washed successively with H_2O (5 × 10 mL), EtOH (3 × 10 mL), ethyl ether (5 × 10 mL), and benzene (2 × 5 mL). It was then suspended in benzene (15 mL) and stored at −20 °C.

Methyl 13,13-Difluoroeicosa-11,14-dien-5,8-diynoate, 29. The dinyecuprate (140 mg) suspension in benzene (7 mL) was centrifuged, the solvent was decanted, and the solid was further washed 4–6 times with ether followed by centrifugation and decanting the ether. It was then dried over N_2, mixed with 26.5 mg (0.54 mmol) of sodium cyanide and dissolved in 1.0 mL of dry DMF. The bromide **27b** (103 mg, 0.36 mmol) in 200 μL of dry DMF was then added, and the mixture was stirred at 25 °C for 2 h. It was worked up by quenching with H_2O and extracting with EtOAc. The ethyl acetate extract was concentrated and passed through a short silica gel column by using CH_2Cl_2 as eluent to remove most of the DMF. The methylene chloride extracts were concentrated and purified by flash chromatography by using CH_2Cl_2/hexane (1:1) as eluent. The crude methyl ester weighed 36 mg (29%): ^{1}H NMR δ 5.5–5.8 (m, 4 H, H-11, H-12, H-14, H-15), 3.68 (s, 3 H, $-OCH_3$), 3.16 (m, 2 H, H-10), 3.11 (m, 2 H, H-7), 2.44 (t, J = 6.8, 2, H, H-2), 2.24 (m, 4 H, H-4), H-16), 1.83 (p, J = 7.0, 2 H, H-3), 1.40 (p, J = 7.2, 2 H, H-17), 1.31 (m, 4 H, H-18, H-19); 0.90 (t, J = 7, 3 H, H-20); ^{19}F NMR ($CDCl_3$, 376.3 MHz) φ 82.62 (t, J = 11.4).

13,13-Difluoroeicosa-11,14-dien-5,8-diynoic Acid, 29a. The methyl ester **29** (36 mg) was stirred vigorously with *Rhizopus arrhizus* lipase (540 μL of suspension in 3.2 M ammonium sulfate, 0.01 M pH 6.0 potassium phosphate, 27 000 units) in potassium phosphate buffer (14.4 mL, 0.1 M pH 7.00) containing 14.4 mg of NaCl and 21.6 mg of gum arabic. After 50 min, over 90% hydrolysis was observed (according to NMR integration). The mixture was acidified with 1.4 mL of 0.1 N HCl to pH 3 and then extracted with ethyl acetate. The ethyl acetate extract was dried over Na_2SO_4, filtered, and concentrated. It was passed through a short silica gel column by using EtOAc/hexane (1:4) as eluent to remove the unreacted methyl ester, followed by EtOAc/hexane (1:1) + 0.5% AcOH as eluent to obtain the acid. The acid was further purified by reversed phase HPLC (65% CH_3CN/H_2O) to give 21.1 mg (59%) of the pure diene diyne acid **29a**: ^{1}H NMR δ 5.55–5.78 (m, 4 H, H-11, H-12, H-14, H-15), 3.17 (m, 2 H, H-10), 3.12 (m, 2 H, H-7), 2.50 (t, J = 7.2, 2 H, H-2), 2.27 (m, 2 H, H-4), 2.23 (m, 2 H, H-16), 1.84 (p, J = 7.1, 2 H, H-3), 1.40 (p, J = 7.1, 2 H, H-17), 1.31 (m, 4 H, H-18, H-19), 0.90 (t, J = 6.7, 3 H, H-20); ^{19}F NMR ($CDCl_3$, 376.3 MHz) φ 82.61 (t, J = 12).

13,13-Difluoroarachidonic Acid 30. The diene diyne acid **29a** (10.4 mg, 0.031 mmol) was hydrogenated over 5% palladium on calcium carbonate poisoned with lead (Lindlar's catalyst, 5.2 mg) in toluene (500 μL) containing 5 μL of 5% synthetic quinoline in toluene. The reaction was terminated after 30 min at 25 °C, during which time 1.6 mL of hydrogen was taken up (theory: 1.5 mL). The reaction mixture was filtered through Celite, and the filtrate was concentrated under a stream of nitrogen to give the crude product. This was purified by reversed phase HPLC (65% CH_3CN/H_2O) to give 6.4 mg (62%) of pure 13,13-difluoroarachidonic acid (**30**): ^{1}H NMR δ 5.6–5.78 (m, 4 H, H-11, H-12, H-14, H-15), 5.34–5.43 (m, 4 H, H-5, H-6, H-8, H-9), 3.03 (m, 2 H, H-10), 2.80 (t, J = 5.8, 2 H, H-7), 2.36 (t, J = 7.2, 2 H, H-2), 2.24 (m, 2 H, H-16), 2.13 (dt, $J_{3,4}$ = 7.2, $J_{4,5}$ = 6.8, 2 H, H-4), 1.72 (p, J = 7.2, 2 H, H-3), 1.40 (p, J = 7.2, 2 H, H-17), 1.31 (m, 4 H, H-18, H-19), 0.90 (t, J = 7.0, 3 H, H-20); ^{19}F NMR ($CDCl_3$, 376.3 MHz) φ 81.80 (t, J = 12.4).

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Enzymatic Conversions of 10,10-Difluoroarachidonic Acid with PGH Synthase and Soybean Lipoxygenase

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Abstract: 10,10-Difluoroarachidonic acid (**1**) was found to be a substrate for PGH synthase and soybean lipoxidase. PGH synthase catalyzed the conversion of this substrate to (11*S*)-10,10-difluoro-11-hydroxyeicosa-5(*Z*),8(*Z*),12(*E*),14(*Z*)-tetraenoic acid (10,10-difluoro-11*S*-HETE, **4**) and (8,15*S*)-10-fluoro-8,15-dihydroxyeicosa-5(*Z*),9(*Z*),11(*Z*),13(*E*)-tetraenoic acid (10-fluoro-8,15-diHETE, **5**), the latter as a mixture of 8-epimers. Cyclization to prostaglandins was not observed. The same epimeric mixture **5** was also obtained on incubation of **1** with soybean lipoxidase, a 15-lipoxygenase, followed by reduction with sodium borohydride. When exposed to aqueous buffer solutions between pH 7 and 9 diallylic difluorides such as **1** or 7,7-difluoroarachidonic acid (**6**) underwent S_N' substitution of fluoride by water with the formation of the fluoroHETES **2**, **3**, **7**, and **9**. In the case of the 7,7-acid, attack by carboxylate anion furnished the 1,5-lactone **8** in addition to **7**. The formation of diHETE **5** is the result of both enzymatic oxygenation and S_N' substitution.

In the accompanying paper we have described a general synthesis of polyunsaturated fatty acids, in which a methylene group residing between two double bonds is replaced by a CF_2 group.[1] Polyunsaturated acids are substrates for a variety of oxygenase enzymes of both plant and animal origin. Our interest in these fluorinated acids was to examine their ability to serve as substrates of such enzymes, most notably of PGH synthase,[2] the enzyme responsible for the biosynthesis of PGH_2, which in turn is the precursor for all the prostaglandins. If successful, fluorinated prostaglandins could be prepared rapidly for biological studies by such a procedure.

We describe here our results of incubations of 10,10-difluoroarachidonic acid (10,10-DFAA) with PGH synthase derived from ram seminal vesicle microsomes (RSVM) and with soybean lipoxygenase. Prior to this work several investigators have examined the substrate specificity of PGH synthease as measured by prostaglandin formation by varying the chain length and number or position of double bonds[3-7] of the fatty acid and by

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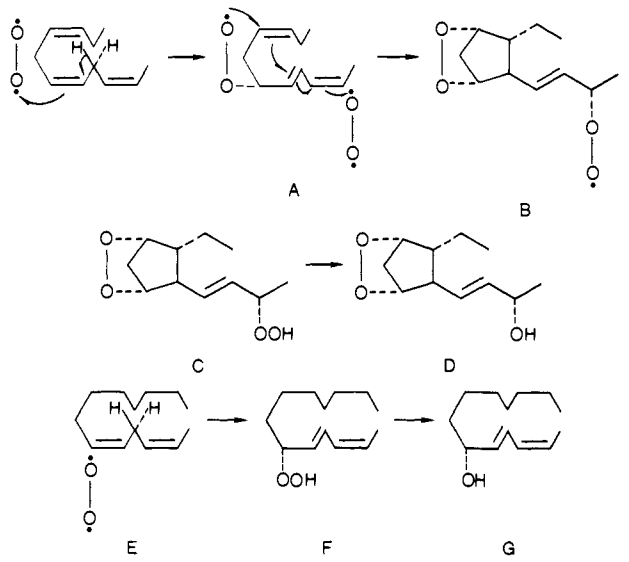
Scheme I

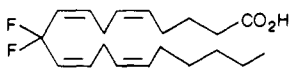


introducing a variety of substituents.[8,9] It was concluded from these studies that polyunsaturated acids ranging in chain length from 18–22 carbon atoms and possessing from 2–5 cis double bonds were substrates for PGH synthease.[10] If the triene system shown in Scheme I is present, the acid reacts with two molecules of oxygen to form the peroxy radical B,[11] which after abstraction of a hydrogen atom forms a hydroperoxy-PGG (C). Substrates lacking the third double bond (E) react with one molecule of oxygen and form the hydroperoxy acid F.[12] The peroxy acids C and F are subject to further attack by PGH synthase which also acts as a peroxidase to form the hydroxy acids D and G, respectively. Even in the presence of the third double bond the sequence E to G is observed as a minor reaction.[12]

As to effects of substitution of the fatty acid, extensive work by the Unilever group[8] showed that the PGH synthase tolerates a large variety of substituents in the 2-position, including fluorine. Closer approach to the portion of the molecule where chemical change takes place during the enzyme reaction[9] proved detrimental. 10,10-Dimethylarachidonic acid[13] and its 10,10-ethano analogue[14] have been prepared, but no data are available as to their ability to serve as substrates for PGH synthase. Not unexpected was the finding that 13,13-dimethylarachidonic acid proved to be a competitive inhibitor of the enzyme.[15]

Substituents which could exert their influence mainly by electronic interactions as is the case with fluorine have never been tested. The case of 10,10-DFAA is particularly striking since the position of substitution adjoins two of the sites specific for enzymatic oxygenation, C-9 and C-11.

To determine whether 10,10-difluoroarachidonic acid **1** was a substrate in the prostaglandin pathway, it was treated with PGH synthase, both as a crude preparation for ram seminal vesicular



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microsomes (RSVM) and a highly purified PGH synthase.[16,17]

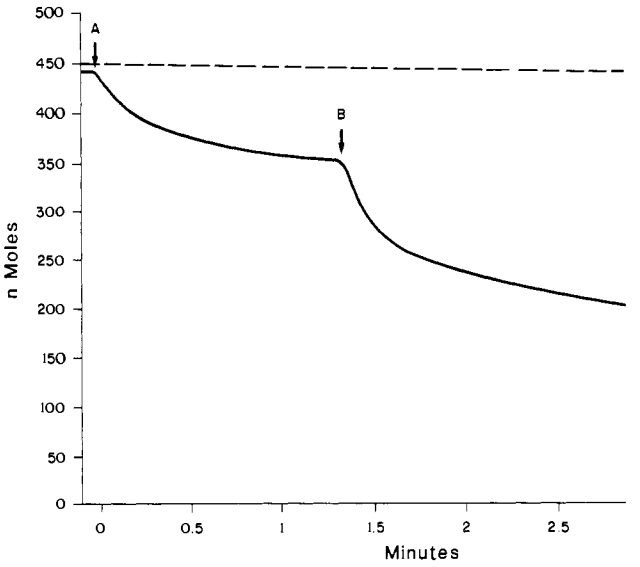

Figure 1. Oxygen uptake monitored by an oxygen electrode. (—) A. The substrate (175 μg of 10,10-DFAA in 6 μL of ethanol) was added to 2.5 mL of 0.1 M potassium phosphate buffer (pH 7.8, kept at 37 °C) containing 0.5 mM phenol, 1 μM hematin, and 122 μg of PGH synthase. Initial rate: 2275 nmol O_2/min/mg enzyme. B. At 1.3 min, 68.9 μg of PGH synthase was added to the reaction mixture. Initial rate: 7140 nmol O_2/min/mg enzyme. The increased initial rate at B is ascribed to the presence of peroxides formed during the first 1.3 min of the reaction. (---) Same as A + 1 μM indomethacin.

Table I. Oxygen Uptake Assay for RSVM & PGH Synthase Using Arachidonic Acid (AA) and 10,10-Difuloroarachidonic Acid (10,10-DFAA)

substrate (μM)		enzyme	oxygen uptake[a]
AA	(130)	RSVM	1080
AA	(160)	RSVM + 1 μM indomethacin	50
10,10-DFAA	(150)	RSVM	142
10,10-DFAA	(150)[b]	RSVM	381
10,10-DFAA	(150)	RSVM + 1 μM indomethacin	9
AA	(130)[b]	PGH synthase	105200
AA	(160)	PGH synthase + 1 μM indomethacin	2270
10,10-DFAA	(210)	PGH synthase	2275[c]
10,10-DFAA	(210)	PGH synthase	7140[c]
10,10-DFAA	(150)	PGH synthase + 1 μM indomethacin	100

[a] Initial rate of oxygen uptake in nmol oxygen/min/mg protein, assuming the concentration of oxygen in a saturated aqueous buffer at 37 °C to be 0.185 nM. [b] Incubation done at pH 7.02. [c] Rate data obtained from the experiment shown in Figure 1 at A and B, respectively.

The reactions were monitored by an oxygen electrode (Figure 1), and the products were analyzed by UV, HPLC, proton and fluorine NMR. The procedure was typical for the preparation of PGG_2 and PGH_2.[18] The enzyme was incubated for 60 s at 37 °C in a 100 mM potassium phosphate buffer (pH 7.8) containing 0.5 mM phenol. Hematin was added to make a 1.0 μmolar solution, and the enzyme was incubated for an additional 60–120 s before the substrate was added in a small amount of ethanol (see Experimental Section). The results of oxygen uptake experiments are shown in Table I. Rapid oxygen consumption was observed in all cases although the initial rate of oxygen uptake for 10,10-DFAA was slower than that of natural arachidonic acid (AA). On average, AA consumed oxygen 5 times faster than 10,10-DFAA when incubated with RSVM and more than 20 times faster when incubated with PGH synthase. Caution is indicated,

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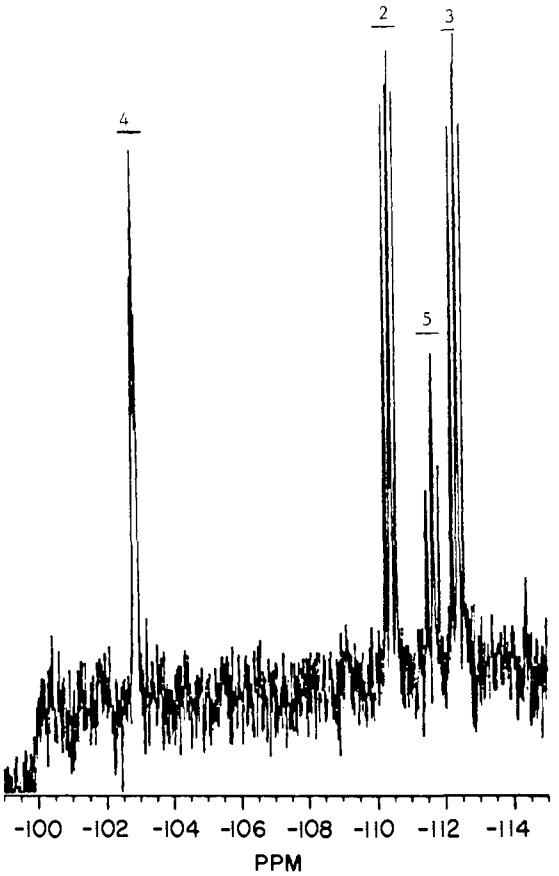

Figure 2. ^{19}F NMR spectrum of crude products obtained from incubation of 10,10-DFAA (700 μg in 25 μL ethanol) with PGH synthase (765 μg) at 37 °C for 15 min. Incubation performed in 0.1 M potassium phosphate buffer (pH 7.8, 10 mL) containing 0.5 mM phenol and 1 μM hematin.

however, when comparing the rates of reaction for the two substrates. Since peroxides are necessary to initiate the reaction,[19] and since arachidonic acid is more readily contaminated by peroxides due to autoxidation than 10,10-DFAA,[1] the rates shown in Table I are probably biased in favor of the natural substrate. The rapid oxygen uptake was inhibited when AA or 10,10-DFAA was incubated with either of the two enzyme preparations in the presence of a 1 μM concentration of the PGH synthase inhibitor indomethacin[20] (Figure 1).

A recurring feature observed in these incubations was the characteristic slowing and finally termination of the reaction due to destruction of the enzyme. The reaction resumed immediately upon the addition of fresh enzyme (Figure 1).

Encouraged by the fact that oxygen consumption was initiated on addition of PGH synthase, several preparative-scale reactions (ca. 1 mg each) were performed in order to obtain sufficient material for structural identification of the products. Fluorine NMR spectra of the crude mixture of products from these reactions showed signals due to four fluorine-containing products (**2**, **3**, **4**, and **5**), in addition to varying amounts of unreacted starting material. A representative spectrum is shown in Figure 2. The starting material was completely consumed in this particular experiment.

In order to determine whether these four products were indeed the result of enzymatic action or of nonenzymatic processes, blank reactions were run. In buffers of various pH values (6.8–9.0) and temperatures (22 or 37 °C), with or without phenol and/or hematin, two of the products, **2** and **3**, were always formed from 10,10-DFAA within 1 min, and the conversion was almost complete within 5 min at 37 °C and within 2 h at room temperature,

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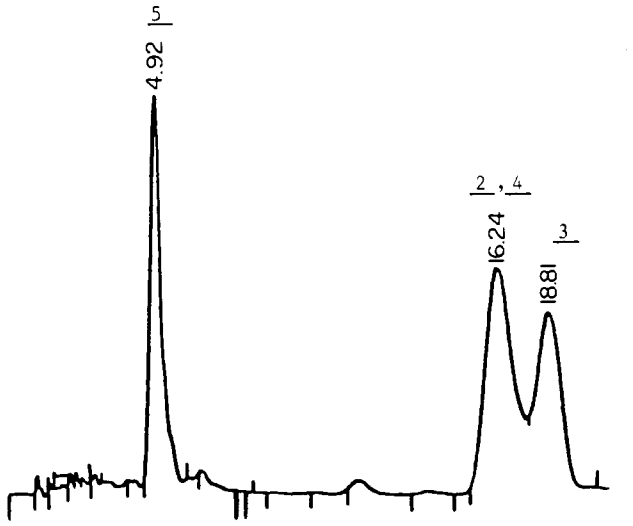


Figure 3. RP-HPLC profile of products from reaction of RSVM and 10,10-DFAA. Incubation conditions are as in Figure 2. Eluted with 1:1 acetonitrile/water (pH 3.5) at 2 mL/min.

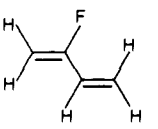
as shown by RP-HPLC. The ^{19}F NMR spectrum of the products isolated from such mixtures lacking the enzyme showed the presence of two signals identical with those of **2** and **3** observed in the reaction of 10,10-DFAA with PGH synthase. They were also found in the ^{19}F NMR spectrum of the crude product isolated from the enzymatic hydrolysis of methyl 10,10-DFAA with the fungal lipase from *Rhizopus arrhizus*. The formation of these products could be suppressed by quenching the PGH synthase reaction within 1.5 min after the addition of the substrate to the buffer. Compounds **4** and **5** were never observed when 10,10-DFAA was incubated with the buffers alone or with buffers containing phenol and/or hematin. They must therefore be products derived from enzymatic catalysis.

When the crude reaction mixture from incubating 10,10-DFAA with RSVM was analyzed by RP-HPLC, the results are shown in Figure 3. Monitored at 192 nm, the order of elution was **5** (t_R = 4.92 min), **2** and **4** (t_R = 16.24 min), and **3** (t_R = 18.81 min). This strongly suggested that **2**, **3**, and **4** were monohydroxy acids while **5** was a dihydroxy acid. Only **5** was detected when the UV monitor was set at 270 nm. All four products were detected at 234 nm.

The dihydroxy acid **5** and the monohydroxy acid **3** were isolated in pure form by semipreparative RP-HPLC. The monohydroxy acids **2** and **4** were collected from the column as a mixture. They were then converted to the corresponding methyl esters **2a** and **4a** (CH_2N_2, ether, 0–23 °C, 1 h) which were easily separated by preparative thin-layer chromatography. Larger quantities of the monohydroxy acids **2** and **4** could be isolated in pure form from the extended lipase hydrolysis of 10,10-DFAA. The structures assigned to the four products are based on a detailed analysis of their UV and proton and fluorine NMR spectra.

The UV spectra of monohydroxy acids **2**, **3**, and **4** exhibited maxima at 235 nm in ethanol, while that of dihydroxy acid **5** showed a maximum at 269.8 nm with shoulders at 260.0 and 280.0 nm, indicating conjugated diene chromophores for monohydroxy acids **2**, **3**, and **4** and a conjugated triene chromophore for dihydroxy acid **5**.

The fluorine NMR spectra of monohydroxy acids **2** and **3** showed doublet of doublets (J_{HF} = 28, 35) while that of dihydroxy acid **5** exhibited a triplet (J_{HF} = 35), all with chemical shifts between ϕ 110 and 112. These values were similar to those reported for fluoroprene **6**,[21] which exhibits a fluorine signal at



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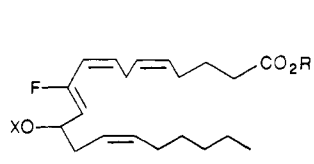
ϕ 114 with coupling constants of 16 Hz (cis H—F), 48 Hz (trans H—F), and 25 Hz (CH_2=C*F*—C*H*=CH_2). Compounds **2**, **3**, and **5** are therefore monofluorides containing vinylic fluorine in a conjugated olefinic system.

The ^{19}F NMR spectrum of the monohydroxy acid **4** showed a multiplet at ϕ 102.94, indicating that **4** was a difluoro compound with equivalent fluorines (i.e., noncyclic) and with only one double bond adjacent to the difluoromethylene moiety (the chemical shift of 10,10-DFAA was ϕ 81.95).

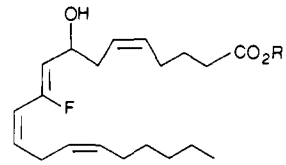
The proton NMR experiments confirmed the conclusions based on the fluorine data and showed further that compounds **2**, **3**, and **5** contained a (—CH=CHCF=CHCHOHCH$_2$CH=CH—) moiety. The evidence for this structural assignment was based on the three signals in the vicinity of δ 4.9, 4.6, and 2.4. The relevant signals in the ^{1}H NMR spectrum of monohydroxy acid **3** were found at δ 4.89 (dd, 1 H, J = 35.7, 8.5), δ 4.69 (dt, 1 H, J = 8.5, 5.6), and δ 2.36 (ABdt, 2 H, J = 15.8, 5.6, 6.6). The signal at δ 4.89 was consistent with that of a vinyl proton trans coupled to fluorine on the same double bond (J_{HF} = 35.7) and coupled to one vicinal proton on the other side. Decoupling experiments showed that the signal at δ 4.89 was coupled to the signal at δ 4.69, which belonged to the methine proton on a carbon bearing an allylic hydroxyl group. Its downfield shift relative to that of a typical methine proton (ca. δ 4.3) attached to a carbon bearing a secondary allylic hydroxyl group may be ascribed to the inductive effect of fluorine. The signal at 4.69 could be decoupled from the signal at δ 2.36, which had an integral for two protons. This signal represented a complicated AB multiplet, indicating that it was due to a methylene group adjacent to a chiral center. This methylene signal at δ 2.36 could be linked by decoupling to protons in the vinyl region of the NMR spectrum.

The ^{1}H NMR spectra of monohydroxy acid **2** and dihydroxy acid **5** contained signals with similar chemical shifts and coupling patterns, indicating that all three compounds contained the (—CH=CHCF=CHCHOHCH$_2$CH=CH—) moiety. The spectrum of monohydroxy acid **4** confirmed the existence of a conjugated diene moiety (signals at δ 6.69, 5.99, 5.65, and 5.51).

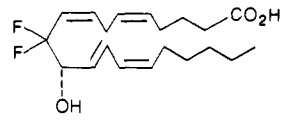
Finally, systematic proton–proton decoupling experiments were performed on compounds **2**–**5** to establish the precise locations of these functional groups. Proceeding sequentially from both ends of the molecule the complete structures could be deduced. Monohydroxy acids **3** and **4** were dealt with as the free acids. The monohydroxy acid **2** was converted to its methyl ester acetate **2b** which facilitated the process by spreading out the vinyl signals. These experiments led to the structures shown below.



2, R=H; X=H
2a, R=CH_3; X=H
2b, R=CH_3; X=CH_3CO



3, R=H
3a, R=CH_3



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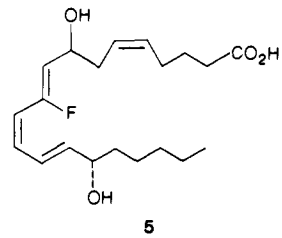

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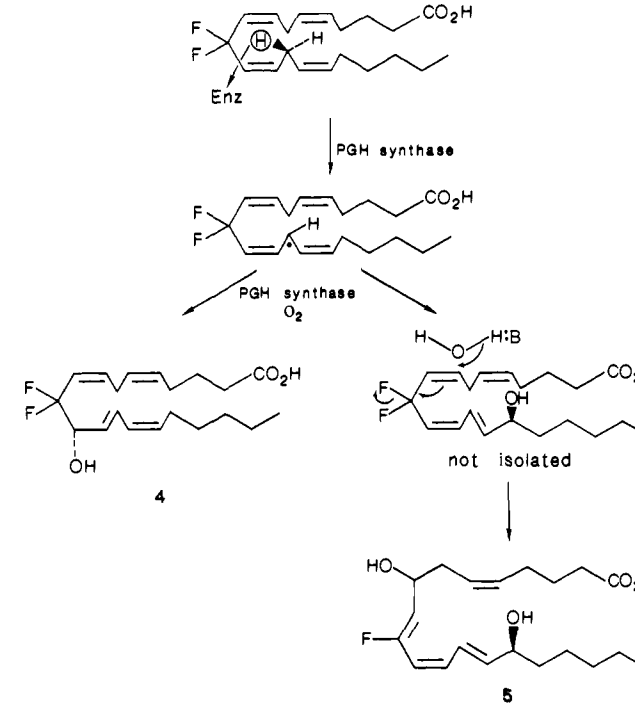
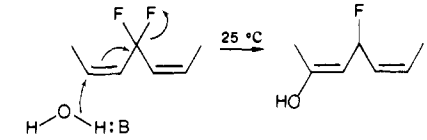
These structures were fully supported by the high resolution mass spectra of free acids **3**, **4**, and **5** and the methyl ester acetate **2b**. The assignment of olefinic geometry was based on the H–H and H–F coupling constants. The stereochemistry of the hydroxyl groups in 10-fluoro-12-HETE (**2**) and 10-fluoro-8-HETE (**3**) must be (*RS*) since they arise by nonenzymatic reactions. On the other hand, the hydroxy groups at C-11 in 10,10-difluoro-11-HETE (**4**) and at C-15 in 10-fluoro-8,15-diHETE (**5**) are assigned the *S* configuration characteristic of the reactions catalyzed by the enzyme PGH synthase.

Scheme II

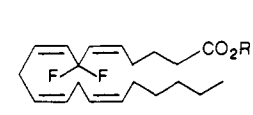


4 **5**

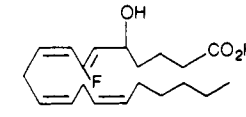
The mechanism for the formation of HETE's **2** and **3** is postulated to be a general base-catalyzed S_N' reaction, the driving force being the formation of the stable conjugated fluorodiene system. This surprisingly facile reaction is characteristic for the



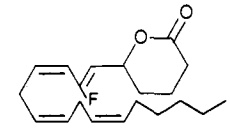
diallylic difluoride system shown. Its generality was demonstrated by the formation of the 7-fluoro HETE's **7**, **8**, and **9** during the enzymatic hydrolysis of the 7,7-DFAA methyl ester **6a** at pH 7,



6, R=H
6a, R=CH_3



7



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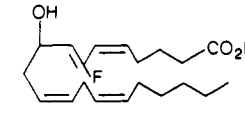


9

described in the accompanying paper.[1] These compounds became the exclusive products when the reaction time was extended to 24 h at 25 °C. As in the case of 10,10-DFAA these structures were secured by UV, ^{1}H, and ^{19}F NMR spectroscopy.

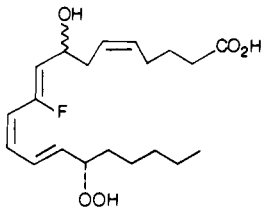
Plausible mechanisms leading to the enzymatic products are shown in Scheme II. The isolation of 10,10-difluoro-11-HETE (**4**) implies that the PGH synthase reaction is "frozen" after the first step, abstraction of the *pro-S*-hydrogen at C-13 and addition of molecular oxygen at C-11 with shift of the double bond from C-11 to C-12. The presence of the fluorines at C-10 sufficiently retards the formation of the 9,10-endoperoxide, permitting release of the intermediate peroxy radical from the enzyme and conversion

(21) Bothner-By, A. A.; Harris, A. K. *J. Am. Chem. Soc.* **1965**, *87*, 3445.

to the alcohol. This retardation may be the result of repulsion between the peroxidic oxygen(s) and the α-fluorosubstituent in the transition state of the cyclization reaction. It should be noted that once 11-oxygenation has taken place, the 3,3-difluoro-1,4-diene system is destroyed. S_N' attack at C-8 can no longer lead to a conjugated system, and no 8,11-diHETE is formed in the enzymatic reaction.

The formation of 10-fluoro-8,15-diHETE (**5**) is rationalized to proceed by initial enzymatic oxygenation at C-15, followed by nonenzymatic S_N' attack at C-8. This mechanism requires that the 11,12-double bond remain in its original cis geometry, which agrees with experimental fact. It must be pointed out that 15-oxygenation is a minor reaction when arachidonic acid and its analogues are incubated with RSVM.[12] Thus, when 8,11,14-eicosatrienoic acid was incubated with RSVM, 15-oxygenation accounted for less than 3% of the products while 11-oxygenation accounted for 37%.[12] In the case of 10,10-DFAA, the amounts of diHETE **5** and 11-HETE **4** were of the same order. Apparently, the presence of fluorine at C-10 retards the rate of oxygenation at C-11 to such an extent that oxygenation at C-15 becomes a more significant competitive reaction. This is not unreasonable since the first step of the PGH synthase reaction is the abstraction of hydrogen at C-13 and the resulting radical can delocalize toward either C-11 or C-15.

To test the hypothesis that diHETE **5** could be formed by enzymatic 15-oxygenation followed by nonenzymatic S_N' attack at C-8, 10,10-DFAA was incubated with soybean lipoxidase, a 15-lipoxygenase which catalyzes the introduction of a hydroperoxy group into arachidonic acid in the 15*S* configuration.[22] Incubation at 0 °C for 30 min in 0.1 M sodium borate buffer (pH 9.12) saturated with oxygen led to rapid oxygen uptake. The crude reaction mixture gave a positive ferrous thiocyanate test,[23] indicating the presence of peroxidic oxygen. Two compounds **10** and **11**, were found to absorb at 270 nm. When 10-fluoro-



10, t_R = 17.6 min
11, t_R = 14.5 min

8,15-diHETE (**5**) was cochromatographed with the reaction mixture, three closely associated peaks were observed. The mixture derived from the 15-lipoxygenase reaction was then reduced with sodium borohydride, and when the crude product was once again cochromatographed with **5**, only a single peak was observed.

In a preparative experiment the peroxidase **10** and **11** were isolated in essentially pure form by HPLC, and their NMR spectra were shown to be virtually identical. These spectra were similar to that of diHETE **5**, except that the signal for the proton at C-15 appeared at δ 4.35, essentially as reported for H-15 in 15-HPETE (δ 4.16).[24] The ^{1}H NMR spectrum of the borohydride reduction product of **11** proved identical with that of diHETE **5**. These results indicated that **10** and **11** were the 8*R* and 8*S* diastereomers of 10-fluoro-15*S*-hydroperoxy-8-hydroxyeicosa-5(*Z*),9(*Z*),11-(*Z*),13(*E*)-tetraenoic acid. At the same time, these findings provide evidence that enzymatic oxygenation at C-15 followed by S_N' attack of water on the primary 15-lipoxygenase product represents the most likely route for the formation of the two diasteromeric 10-fluoro-8,15-diHETE's (**5**).

In conclusion, 10,10-DFAA was shown to be a substrate for PGH synthase and soybean lipoxygenase. The resulting products are fluorinated, conjugated hydroxy acids typical of arachidonic acid metabolism. Cyclization to prostaglandinlike products has not been observed. The fact that enzymatic oxygenation occurs in α position to a CF_2 group is remarkable. Our findings support the generally accepted mechanistic concept, in which the process is initiated by abstraction of a hydrogen atom at C-13 rather than of a proton or hydride. Proton abstraction would have resulted in a C-13 carbanion, which in view of the facile S_N' displacement of fluoride observed at pH 7 should have led to elimination of fluoride and yielded a conjugated tetraene, which was searched for but not found. Initial abstraction of hydride would have led to a carbonium ion allylic to two fluorines, a process energetically too unfavorable to occur.

Experimental Section

Methods and Instrumentation. Reagent or HPLC grade solvents were used as is upon initial opening. ^{1}H NMR spectra were obtained in $CDCl_3$ on a University of Chicago DS-1000 spectrometer at 500 MHz and were processed by Fourier transformation by using a Nicolet Instrument Corporation (now G.E. Magnetics) 1280 Data Acquisition system. The ^{19}F NMR spectra were obtained on a Nicolet Instrument Corporation NTC-200 at 188.2 MHz (in $CDCl_3$) by using a 1280 Data Acquisition system. The chemical shifts of the ^{1}H NMR signals were reported in δ parts per million (ppm) with chloroform as internal standard (δ 7.25). The chemical shifts of the ^{19}F NMR signals were reported in ϕ ppm upfield from the internal standard $CFCl_3$. Coupling constants, *J*, are reported in Hz. The abbreviations s, d, t, q, p, m, and br signify singlet, doublet, triplet, quartet, quintet, multiplet, and broad, respectively. High resolution mass spectra were determined at 70 eV by using a VG 70-250HF mass spectrometer equipped with GLC, gas, and solid probe inlets. Purification and separation of the 10,10-DFAA metabolites were performed on a Waters Associates RCM-100 radical compression module fitted with an analytical 5 mm × 10 cm Radial Pak-A (C-18, 5 μm) cartride or on a Rainin Instruments semipreparative 10 mm × 25 cm Dynamax Macro-HPLC (C-18, 8 μm) column by using a Waters Associates Model 590 Programmable Solvent Delivery Module, a U6K injector, and a 0.2 μm prefilter. Elution rates were 2 or 3 mL/min with varying ratios of water (double deionized, adjusted to pH 3.5 with acetic acid) and HPLC grade acetonitrile. Elution was followed by the measurement of the absorbance with a Lambda-Max Model 481 LD spectrophotometer and a Hewlett Packard HP-3390A integrator. The pH value of aqueous buffers was determined by the use of a Fisher Accumet Model 815MP pH meter. Oxygen uptake was measured by a Yellow Springs Instrument Model 53 Biological Oxygen Monitor equipped with a YSI 5331 oxygen electrode. UV spectra were obtained on a Perkin-Elmer Lambda 5 UV–vis spectrophotometer. The lipase from *Rhizopus arrhizus* was purchased from Boehringer Mannheim Biochemicals as a suspension in ammonium sulfate solution, 3.2 M, potassium phosphate 0.01 M, pH approximately 6. Two preparations of PGH synthase were used, namely, highly purified PGH synthase (referred to as PGH synthase in the text) and crude ram seminal vesicular microsomes (RSVM). PGH synthase was kept frozen at −78 °C in 0.1% Tween-20, 300 μM diethyl dithiocarbamate, 40 mM Tris buffer (pH 8.0). RSVM was also kept frozen at −78 °C in 10 mM sodium phosphate buffer (pH 7.8) containing 250 mM mannitol. The lipoxidase (Type IV) from soybean was purchased from Sigma Chemical Co. as a 2× crystallized suspension in 2.3 M ammonium sulfate solution, pH approximately 6.

Preparation of Buffers and Solutions. a. Potassium phosphate buffer (0.1 M) was prepared by dissolving 1.36 g of KH_2PO_4 in 100 mL of double deionized water and adjusting the pH to the desired value by addition of concentrated aqueous potassium hydroxide. b. Sodium borate buffer (0.1 M, pH 9.12) was prepared by dissolving 3.81 g of sodium borate in 100 mL of double deionized water. c. A stock solution of phenol (10 mM) was prepared by dissolving 18.8 mg of solid phenol in 20 mL of double deionized water. A final concentration of 0.5 mM in phenol was achieved by adding 50 μL of the stock solution to each mL of the incubation mixture. d. A stock solution of hematin (0.5 mM) was prepared by dissolving 6.4 mg of hematin (purchased from Sigma) in 20 mL of 1 mM NaOH. It was stirred (capped) until the solution was homogeneous (about 1 h). The stock solution was stable for about a month when kept at 4 °C. A working solution was prepared fresh each day by adding 100 μL of the stock solution to 400 μL of double deionized water. Addition of 10 μL per mL of incubation mixture would result in a final hematin concentration of 1.0 μM, which would saturate the apoenzyme.

General Procedure for the Incubation of 10,10-DFAA with RSVM and PGH Synthase. The enzyme preparation was added to potassium phosphate buffer (0.1 M, pH 7.8) containing 0.5 M phenol. After preincubation for 1 min at 37 °C, hematin was added to a final con-

(22) Hemberg, M.; Samuelsson, B. *J. Biol. Chem.* **1967**, *242*, 5329.
(23) Gorman, R. R.; Sun, F. F.; Miller, O. V.; Johnson, R. A. *Prostaglandins* **1977**, *13*, 1043.
(24) Van Os, C. P. A.; Rijke-Schilder, C. P. M.; Van Halbeck, H.; Verhagen, H.; Vliegenthart, F. G. *Biochim. Biophys. Acta.* **1981**, *663*, 177.

Table II

expt	10,10-difluoro-11-HETE (4), %	10-fluoro-8,15-diHETE (5), %	10-fluoro-12-HETE (2), %	10-fluoro-8-HETE (3), %	10,10-DFAA, %
a	25.6	6.8	6.1	6.1	55.3
b	23.5	23.5	10.0	10.0	33.0
c	33.9	31.9	4.6	4.6	25.0
d	16.8	18.8	32.2	32.2	0.0

centration of 1 μM, and the mixture was incubated for an additional 60–120 s. The reaction was initiated by addition of the 10,10-DFAA in absolute ethanol (15–50 μL). When inhibitors of the enzyme were added, they were introduced immediately following the addition of hematin and at least 30 s prior to the addition of 10,10-DFAA.

Oxygen Uptake Assay for RSVM and PGH Synthase Using 10,10-DFAA as Substrate. To the sample chamber of an oxygen electrode kept at 37 °C containing 2 mL of phosphate buffer and phenol was added the enzyme preparation (1.1 mg of RSVM in 50 μL buffer or 15.3 μg of PGH synthase in 10 μL of buffer). The mixture was incubated for 1 min with vigorous stirring. Hematin (20 μL of 0.1 mM solution) was introduced, and the mixture was incubated for an additional 60–120 s while the oxygen electrode was lowered into the chamber, carefully expelling all air bubbles from the chamber. The substrate, 10,10-DFAA, was added by a syringe into the chamber, and the uptake of oxygen was monitored. In all cases, rapid oxygen uptake was observed initially but slowed to a halt within 1 min. The reaction was allowed to proceed for a total of 5 min and was then quenched by the addition of 1 M HCl, adjusting the pH to about 3. The mixture was then extracted with dichloromethane (3 × 2 mL). The organic phase was dried and concentrated to give a crude product, which was analyzed by RP-HPLC. RP-HPLC showed the formation of enzymatic products 10,10-difluoro-11-HETE (**4**) and 10-fluoro-8,15-diHETE (**5**) in comparable amounts with varying amounts of nonenzymatic products 10-fluoro-12-HETE (**2**) and 10-fluoro-8-HETE (**3**).

Preparation of 10,10-Difluoro-11-HETE and 10-Fluoro-8,15-diHETE. The incubations were carried out as above by using 10 mL of phosphate buffer containing phenol and hematin at 37 °C. The following reactions were performed: a. 1000 μg of 10,10-DFAA, 11 mg of RSVM, 1 min; b. 900 μg of 10,10-DFAA, 11 mg of RSVM, 5 min; c. 1000 μg of 10,10-DFAA, 1.56 mg of PGH synthase, 5 min; and d. 700 μg of 10,10-DFAA, 765 μg of PGH synthase, 15 min.

The reaction was quenched and worked up as above, and the crude product mixture was analyzed by RP-HPLC and ^{19}F NMR. The results are shown in Table II.

Purification of Products from Enzymatic Reactions. Products from the incubation of 10,10-DFAA with PGH synthase or RSVM were isolated by RP-HPLC on a semipreparative Dynamax column eluted at 3 mL/min, and the absorbance was monitored at 192 nm. The mobile phase consisted of first 50% CH_3CN/50% H_2O (30 min) and then 65% CHCN/35% H_2O (30 min) and followed by 80% CH_3CN/20% H_2O (30 min). The more polar enzymatic product, 10-fluoro-8,15-diHETE (**5**), was eluted first (t_R = 15.5 min). The HETE's were eluted after the second solvent was switched on. The enzymatic product 10,10-difluoro-11-HETE (**4**) was eluted together with the nonenzymatic product, 10-fluoro-12-HETE (**2**) (t_R = 48.6 min, 18.6 min after solvent change) followed by the other nonenzymatic product, 10-fluoro-8-HETE (**3**) (t_R = 51.2 min, 21.2 min after solvent change). The unchanged 10,10-DFAA (**1**) was eluted after the third solvent was switched on (t_R = 75.77 min, 16.8 min after second solvent change). In most cases, the amount of 10-fluoro-12-HETE formed was insignificant, and 10,10-difluoro-11-HETE was used in structural studies without further purification. The fractions containing these products were collected, and the acetonitrile was removed under a stream of nitrogen. The aqueous layer was then extracted 3 times with dichloromethane, and the organic layer was dried and concentrated to give the desired products.

In cases where 10,10-difluoro-11-HETE (**4**) and 10-fluoro-12-HETE (**2**) were in comparable amounts, they were converted to the corresponding methyl esters (CH_2N_2 in Et_2O containing 10% MeOH, 0–23 °C, 1 h; followed by evaporation of solvent under a stream of N_2) and separated by preparative TLC (R_f = 0.37, 0.28, respectively, in 25% EtOAc/hexane).

(*R*,*S*)-Methyl 12-Acetoxy-10-fluoroeicosa-5(*Z*),8(*Z*),10(*Z*)-tetraenoate, 2b: ^{1}H NMR H_2 δ 2.32, $J_{2,3}$ = 7.5, H_3 1.72, $J_{3,4}$ = 7.2, H_4 2.12, $J_{4,5}$ = 6.1, H_5, H_6 5.41, m, H_7 3.10, $J_{7,8}$ = 7.4, H_8 5.51, $J_{8,9}$ = 11.8, H_9 5.64, $J_{9,10}$ = 29.0, H_{11} 4.83, $J_{10,11}$ = 33.2, $J_{11,12}$ = 8.5, H_{12} 5.71, $J_{12,13a}$ = $J_{12,13b}$ = 6.7, H_{13a} 2.47, $J_{13a,13b}$ = 15.2, $J_{13a,14}$ = 6.3, H_{13b} 2.37, $J_{13b,14}$ = 6.3, H_{14} 5.32, $J_{14,15}$ = 11.1, H_{15} 5.52, $J_{15,16}$ = 7.0, H_{16} 2.04, $J_{16,17}$ = 7.5, H_{17} 1.37, $J_{17,18}$ = 7.4, $H_{18,19}$ 1.31, m, H_{20} 0.90, $J_{19,20}$ = 6.8, $-CO_2CH_3$, 3.69, s, -OAc, 2.04, s; ^{19}F NMR ϕ 109.86 (dd, J = 28.2, 34.5); UV (ethanol) 234.9 nm; high resolution MS (70 eV, m/z), calcd for $C_{23}H_{36}O_4F$ 394.2520, found 394.2521 (0.08%); calcd for $C_{21}H_{34}O_3F$ (M – CH_3CO_2H) 334.2308, found 334.2315 (18%); calcd for $C_{15}H_{21}O_4F$ (M – $C_5H_{11}CH$=$CHCH_2$) 283.1346, found 283.1355 (19%).

(*R*,*S*)-10-Fluoro-8-hydroxyeicosa-5(*Z*),9(*Z*),11(*Z*),14(*Z*)-tetraenoic Acid, 3: ^{1}H NMR H_2 δ 2.38, $J_{2,3}$ = 6.7, H_3 1.74, $J_{3,4}$ = 7.1, H_4 2.16, $J_{4,5}$ = 6.7, H_5 5.51, $J_{5,6}$ = 10.4, H_6 5.47, $J_{6,7}$ = 6.6, H_7 2.36, $J_{7a,7b}$ = 15.8, $J_{7,8}$ = 5.6, H_8 4.69, $J_{8,9}$ = 8.5; H_9 4.89, $J_{9,10}$ = 35.7, H_{11} 5.65, $J_{10,11}$ = 29.3, $J_{11,12}$ = 11.8, H_{12} 5.51, $J_{12,13}$ = 6.8, H_{13} 3.12, $J_{13,14}$ = 6.4, H_{14} 5.36, $J_{14,15}$ = 10.7, H_{15} 5.44, $J_{15,16}$ = 6.9, H_{16} 2.06, $J_{16,17}$ = 7.2, H_{17} 1.37, $J_{17,18}$ = 7.1, H_{18}, H_{19} 1.31, m, H_{20} 0.90, $J_{19,20}$ = 6.8; ^{19}F NMR ϕ 112.35 (dd, J = 28.5, 34.9); UV (ethanol) 235.0 nm; high resolution MS (70 eV, m/z), calcd for $C_{20}H_{31}O_3F$ (M – H_2O) 320.2152, found 320.2183 (57%); calcd for $C_{12}H_{15}O_3F$ (M – $C_5H_{11}CH$=$CHCH_2$) 227.1084, found 227.1101 (10%); calcd for $C_{13}H_{20}OF$ (M – CH_2CH=$CHC_3H_6CO_2H$) 211.1498, found 211.1496 (58%); calcd for $C_{10}H_{17}$ ($C_5H_{11}CH$=$CHCH_2CH$=CH) 137.1330, found 137.1333 (9%).

(*S*)-10,10-Difluoro-11-hydroxyeicosa-5(*Z*),8(*Z*),12(*E*),14(*Z*)-tetraenoic Acid 4: ^{1}H NMR H_2 δ 2.36, $J_{2,3}$ = 7.3, H_3 1.72, $J_{3,4}$ = 7.3, H_4 2.14, $J_{4,5}$ = 6.9, H_5, H_6 5.41, m, H_7 3.05, $J_{7,8}$ = 7.0, H_8 5.81, $J_{8,9}$ = 11.6, H_9 5.46, $J_{9,10}$ = 15.2, H_{11} 4.39, $J_{10,11}$ = 9.4, $J_{11,12}$ = 6.4, H_{12} 5.65, $J_{12,13}$ = 15.3, H_{13} 6.69, $J_{13,14}$ = 11.2, H_{14} 5.99, $J_{14,15}$ = 11.2, H_{15} 5.51, $J_{15,16}$ = 7.4, H_{16} 2.20, $J_{16,17}$ = 7.0, H_{17} 1.40, $J_{17,18}$ = 7.4, H_{18}, H_{19} 1.32, m, H_{20} 0.90, $J_{19,20}$ = 6.8; ^{19}F NMR ϕ 102.9 (m); UV (ethanol) 235.5 nm; high resolution MS (70 eV, m/z), calcd for $C_{20}H_{30}O_3F_2$ (M^+) 356.2163, found 356.2150; calcd for $C_{20}H_{28}O_2F_2$ (M – H_2O) 338.0257, found 338.2103 (0.38%); calcd for $C_{20}H_{29}O_3F$ (M – HF) 336.2100, found 336.2112 (0.90%).

(15*S*)-10-Fluoro-8,15-dihydroxyeicosa-5(*Z*),9(*Z*),11(*Z*),13(*E*)-tetraenoic Acid and 8-Epimer 5: ^{1}H NMR (CD_2Cl_2, –20 °C) H_2 δ 2.30, $J_{2,3}$ = 7.2, H_3 1.63, $J_{3,4}$ = 6.8, H_4 2.06, $J_{4,5}$ = 7.0, H_5, H_6 5.3–5.4, m, H_7 2.30, $J_{7,8}$ = 6.6, H_8 4.59, $J_{8,9}$ = 6.7, H_9 4.95, $J_{9,10}$ = 37.0, H_{11} 5.56, $J_{10,11}$ = 32.5, $J_{11,12}$ = 11.9, H_{12} 6.02, $J_{12,13}$ = 11.9, H_{13} 6.83, $J_{13,14}$ = 14.3, H_{14} 5.79, $J_{14,15}$ = 6.2, H_{15} 4.16; $J_{15,16}$ = 7.4, H_{16} 1.47, m, H_{17}, H_{18}, H_{19} 1.23, m, H_{20} 0.84, $J_{19,20}$ = 6.6; ^{19}F NMR (CD_2Cl_2, –20 °C, 188.4 MHz) ϕ 112.07 (t, J = 35); high resolution MS (70 eV, m/z) calcd for $C_{20}H_{29}O_3F$ (M – H_2O) 336.2101, found 336.2108 (7%); calcd for $C_{20}H_{27}O_2F$ (M – $2H_2O$) 318.1995, found 318.1951 (11%); calcd for $C_{13}H_{23}O_2F$ (M–CH_2CH=$CHC_0H_6CO_2H$) 227.1447, found 227.1467 (33%).

Incubation of 10,10-DFAA with PGH Synthase or RSVM in the Presence of 1 μM Indomethacin. The reaction was performed as in the oxygen uptake assay above with indomethacin (20 μL of 0.1 mM solution) introduced immediately following the addition of hematin. The substrate (100 μg) was added in 12.5 μL of ethanol. A steady but slow oxygen uptake was observed in all cases, in contrast to the rapid oxygen uptake observed when 10,10-DFAA was incubated with PGH synthase in the absence of indomethacin.

The reaction was quenched and worked up as above, and the RP-HPLC of the crude products showed the formation of 10-fluoro-12-HETE (**2**) and 10-fluoro-8-HETE (**3**) (major products >50%) and very small amounts of 10,10-difluoro-11-HETE (**4**) and 10-fluoro-8,15-diHETE (**5**).

Incubation of 10,10-DFAA with Soybean Lipoxidase. To soybean lipoxidase (Sigma, 300 000 units) in sodium borate buffer (0.1 M, pH 9.12, 10 mL) saturated with oxygen at 0 °C was added 10,10-DFAA (1 mg). The reaction mixture was stirred for 30 min at 0 °C. The reaction was quenched by acidifying the mixture to ca. pH 3 with 1 M HCl. Workup with dichloromethane gave a crude product which yielded a positive thiocyanate test.[23] Two epimeric products were isolated by RP-HPLC (t_R = 17.6 min for **10**, 19.5 min for **11**) by using a Dynamax macro HPLC column (C-18, 10 mm × 25 cm, 8 μm) eluted with 50% CH_3CN/50% H_2O (pH 3.5) at 3 mL/min. These products were closely related to, but distinct from, the PGH synthase product, 10-fluoro-8,15-diHETE (**5**), as shown by RP-HPLC profile and proton NMR spectra.

^{1}H NMR spectra of the two epimers **10** and **11** were essentially identical.

10: ^{1}H NMR δ 6.92 (t, 1 H, J = 15, H-13), 6.04 (t, 1 H, J = 12, H-12), 5.68 (m, 1 H, H-14), 5.63 (dd, 1 H, J = 12, 30, H-11), 4.98 (dd, 1 H, J = 12, 35, H-9), 4.63 (m, 1 H, H-8), 4.35 (m, 1 H, H-15), 2.32 (t, 2 H, J = 7, H-2), 2.30 (ABm, 2 H, H-7), 2.07 (m, 2 H, H-4), 1.39 (m, 2 H, H-16), 1.22 (m, 6 H, H-17, H-18, H-19), 0.80 (t, 3 H, J = 7, H-20).

Reduction of the Peroxyacids 10 and 11. To the purified peroxyacids **10** and **11** in MeOH (1 mL) at 0 °C was added $NaBH_4$ (10 mg). The mixtures were stirred at 0 °C for 20 min and then at 23 °C for 30 min. The reaction mixtures were then acidified to pH 3 with 1 M HCl. The methanol was removed under a stream of nitrogen, and the aqueous layers were extracted with dichloromethane (3 × 2 mL). The organic

layers were dried and concentrated under a stream of nitrogen. The residues from the two samples were taken up in dichloromethane-*d* for ^{1}H NMR spectroscopy. The sample derived from **11** yielded sufficient material to produce an NMR spectrum identical with that of fluoro-diHETE **5**.

Preparation of 7-Fluoro-5-hydroxyeicosa-6,8,11,14-tetraenoic Acid, 7, Its Lactone 8, and 7-Fluoro-9-hydroxyeicosa-5,7,11,14-tetraenoic Acid, 9, from Methyl 7,7-Difluoroarachidonate, 6a. Methyl 7,7-difluoroarachidonate (**6a**, 10 mg) was hydrolyzed with *Rhizopus arrhizus* lipase (800 μL, 40 000 units) in potassium phosphate buffer (9.9 mL, 0.1 M, 7.02) containing 17.3 mg of NaCl and 107.7 mg of gum arabic (1% solution). The mixture was stirred at 25 °C for 18 h. The reaction was terminated by acidification with 1 N HCl to pH 3.0 and extraction of the product with CH_2Cl_2. Separation of products was achieved by RP-HPLC on a Rainin Instruments Dynamax macro-HPLC column (C-18, 10 mm × 25 cm, 8 μm). Elution occurred with 65% CH_3CN/35% H_2O (pH 3.5) at 3 mL/min, and absorbance was monitored at 205 nm. Three fractions were collected and worked up as follows: The acetonitrile was removed under a stream of nitrogen. The residual aqueous solutions were then extracted 5 times with equal volumes of dichloromethane, and the extracts were dried by passing through a small column of anhydrous sodium sulfate in a disposable pasteur pipette and concentrated under a stream of nitrogen. The products are listed in the order of their elution from the column.

7-Fluoro-9-hydroxyeicosa-5(*Z*),7(*Z*),11(*Z*),14(*Z*)-tetraenoic Acid, 9 (24.0 min): ^{1}H NMR δ 5.68 (dd, 1 H, *J* = 30.4, *J* = 11.4, H-6), 5.55 (m, 1 H, H-5), 5.41 (m, 2 H, vinyl), 5.32 (m, 2 H, vinyl), 4.89 (dd, 1 H, *J* = 35.9, *J* = 8.1, H-8), 4.67 (m, 1 H, H-9), 2.81 (t, 2 H, *J* = 7.1, H-13), 2.46-2.31 (m, 6 H, H-2, H-4, H-10), 2.06 (q, 2 H, *J* = 6.9, H-16), 1.77 (m, 2 H, H-3), 1.39-1.27 (m, 6 H, H-17, H-18, H-19), 0.90 (t, 3 H, *J* = 6.9, H-20); ^{19}F NMR φ 112.6 (dd, *J* = 29.9, *J* = 33.7); UV (ethanol) 231.2 nm; high resolution MS (70 eV, *m/z*), calcd for $C_{20}H_{29}O_2F$ (M - H_2O) 320.2152, found 320.2157 (6.0%); FAB$^-$ 337 (M - 1).

7-Fluoro-5-hydroxyeicosa-6(*Z*),8(*Z*),11(*Z*),14(*Z*)-tetraenoic Acid, 7 (26.8 min): ^{1}H NMR δ 5.65 (dd, 1 H, *J* = 29.4, *J* = 11.2, H-8), 5.53 (m, 1 H, H-9), 5.40 (m, 3 H, vinyl), 5.36 (m, 1 H, vinyl), 4.86 (dd, 1 H, *J* = 35.6, *J* = 9.6, H-6), 4.66 (m, 1 H, H-5), 3.14 (t, 2 H, *J* = 6.0, H-10), 2.81 (t, 2 H, *J* = 6.2, H-13), 2.43 (t, 2 H, *J* = 7.5, H-2), 2.05 (q, 2 H, *J* = 7.2, H-16), 1.70 (m, 4 H, H-3, H-4), 1.40-1.27 (m, 6 H, H-17, H-18, H-19), 0.90 (t, 3 H, *J* = 6.6, H-20); ^{19}F NMR φ 112.8 (dd, *J* = 29.9, *J* = 33.8); UV (ethanol) 231.3 nm; high resolution MS (70 eV, *m/z*), calcd for $C_{20}H_{29}O_2F$ (M - H_2O) 320.2152, found 320.2161 (2.2%); FAB$^-$ 337 (M - 1).

7-Fluoro-5-hydroxyeicosa-6(*Z*),8(*Z*),11(*Z*),14(*Z*)-tetraenoic Acid 1,5-Lactone, 8 (104 min): ^{1}H NMR δ 5.67 (dd, 1 H, *J* = 28.7, *J* = 12.2, H-8), 5.63 (m, 1 H, H-9), 5.46-5.28 (m, 5 H, H-5, H-11, H-12, H-14, H-15), 4.94 (dd, 1 H, *J* = 34.5, *J* = 8.2, H-6), 3.15 (t, 2 H, *J* = 6.1, H-10), 2.81 (t, 2 H, *J* = 6.7, H-13), 2.63 (dt, 1 H, *J* = 17.7, *J* = 7.3, H-2), 2.49 (dt, 1 H, *J* = 17.7, *J* = 7.9, H-2), 2.06 (q, 2 H, *J* = 7.1, H-16), 1.69 (m, 4 H, H-3, H-4), 1.41-1.26 (m, 6 H, H-17, H-18, H-19), 0.91 (t, 3 H, *J* = 7.3, H-20); ^{19}F NMR φ 110.34 (dd, *J* = 28.1, *J* = 34.7); UV (ethanol) 232.8; high resolution MS (70 eV, *m/z*) calcd for $C_{20}H_{29}O_2F$ (M^+) 320.2152, found 320.2151 (4.5%).

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On the Origin of Proximity Effects on Reactivity: A Modified MM2 Model for the Rates of Acid-Catalyzed Lactonizations of Hydroxy Acids

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Abstract: In order to determine the role of functional group proximity and orientation upon reactivity, we have carried out a computational study of the acid-catalyzed lactonizations of hydroxy acids. Ab initio calculations on the attack of water on protonated formic acid gave information about the preferred geometry of nucleophilic attack. On the basis of the results of these calculations, new parameters have been added to Allinger's MM2 force field, so that the steric energies of the transition states of lactonizations of hydroxy acids can be calculated. Relative activation energies found experimentally are reproduced by this force field. Some of the various models which have been proposed to explain the very high rates of certain intramolecular and enzymatic reactions are evaluated. In particular, our calculations show that there is no relationship between (i) the angle of nucleophilic attack and the reactivity of a hydroxy acid or (ii) the distance between reacting atoms in the starting material and the rate of reaction. Both (i) and (ii) are true even for rigid systems where the spatial relationships between reacting atoms are fixed. Most importantly, our results show that very diverse reactivities (with rate constants varying over ten orders of magnitude) can be accounted for quantitatively by our force field. This constitutes a dramatic improvement over previous qualitative models. Our force field also allows us to make quantitative reactivity predictions for conformationally rigid hydroxy acids.

During the last 20 years, the rates of acid-catalyzed lactonizations of hydroxy acids have been used to assess the importance of proximity and mutual orientation of functional groups upon rates of intramolecular reactions and as models to evaluate the importance of these effects upon the catalytic power of enzymes. We have devised a force field for the transition states of these reactions and describe here the computational model and the general implications of this study for the understanding of proximity and orientation effects on reaction rates.

In a series of recent reports[1-4] it has been suggested that the rates of intramolecular organic reactions are dependent primarily upon the distance between the reacting atoms in the starting

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