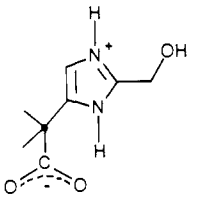

16

The study also shows that the carboxylate in **2d**, although not optimally aligned with the imidazole in the sense described by Gandour,[26] enhances the imidazole basicity by 1.2 pK_a units relative to comparison ester **2e**. For imidazolium ionizations wherein an intramolecular carboxylate is *syn* oriented, reference has been made to large ΔpK_a values in comparison with a corresponding imidazolium ester[40a] or other comparison materials[40b] containing an *anti* disposed carboxylate- - -imidazolium couple. However, the data herein indicate that a *syn* orientation is not required for a large ΔpK_a. Moreover, we note that large ΔpK_a values are conditional upon the nature of the amine (e.g., pK_a^{NH} serine–ethyl serinate = 2.06, Table I) and solvation (e.g., pK_a^{Im}-H^+ **2d–2e** is 7.45 - 6.02 = 1.43 in 40% EtOH/H_2O and 7.33 - 5.74 = 1.59 in 80% EtOH/H_2O). A *syn* orientation of the carboxylate may be of evolutionary advantage to the enzymes that employ it not only for the basicity arguments[26,41] but also because that arrangement places the center of (-)-charge in the carboxylate closer to the imidazole H–N thereby optimizing the electrostatic interaction.

To term this system a model for the acylation of the SPases invites comparison with the enzyme which may not be justified given the unorthodox geometry of the acylating agent and perhaps non-optimal alignment of the functional groups in serine or **2d**. Furthermore, even with the enzymes the mechanism of acylation shows subtle substrate dependent diversities so that in some cases the Asp CO_2^- may act electrostatically and in others it may act as a bonafide general base.[3] What is certain from the above study is a demonstration of a role for carboxylate that enhances the amine basicity and allows the latter to more effectively influence direct CH_2OH acylation. This may be viewed as a small molecule precedent for a similar role suggested to occur in the SPases.[4]

Acknowledgment. The authors acknowledge the financial support of the University of Alberta and Natural Sciences and Engineering Research Council of Canada.

Supplementary Material Available: Tables of k_2^{obsd} and k_2^{max} values for the serine derivatives and **2a–f** reacting with **1**, k_2^{max} data used in the calculation of Brønsted lines in Figure 3, and product IR, NMR, and MS data (14 pages). Ordering information is given on any current masthead page.

(40) (a) Zimmerman, S. C.; Cramer, K. D. *J. Am. Chem. Soc.* **1988**, *110*, 5906. (b) Huff, J. B.; Askew, B.; Duff, R. J.; Rebek, J., Jr. *Ibid.* **1988**, *110*, 5908.

(41) The basicity argument requires that a proton be transfered from the proximal imidazole N–H to carboxylate concurrent with the distal N: acting as a general base on SerOH. This is thermodynamically favorable only if the normal pK_a values of imidazolium and the carboxylic acid be reversed in the enzyme active site as the transition state for the acylation is approached.[4]

Stereochemistry and Mechanism of the Biosynthesis of Leukotriene A_4 from 5(*S*)-Hydroperoxy-6(*E*),8,11,14(*Z*)-eicosatetraenoic Acid. Evidence for an Organoiron Intermediate

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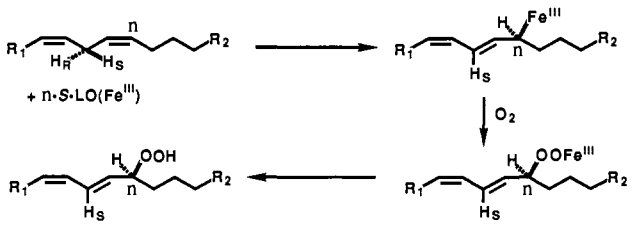
Contribution from the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138. *Received July 5, 1988*

Abstract: The pathway of biosynthesis of leukotriene A_4 (LTA_4, **2**) from 5(*S*)-hydroperoxy-6(*E*),8,11,14(*Z*)-eicosatetraenoic acid (5-*S*-HPETE, **1**) has been explored by the comparative study of (*S*)- and (*R*)-lipoxygenase (LO) enzymes as catalysts. The purified LO from potato, an *S*-lipoxygenase, converts (anaerobically) **1** to **2** (determined as the characteristic hydrolysis mixture of two epimeric 5,6-diols and two epimeric 5,12-diols), as previously reported by Samuelsson et al. However, the 8-*R*-LO from the coral *Plexaura homomalla* transforms **1** (anaerobically) into 6-*epi*-LTA_4 (**6**). The observed divergence of stereopathways agrees with predictions based on the intermediacy of organoiron intermediates in enzymic lipoxygenation (Scheme I) and detailed in Schemes II and III. Further evidence for the intervention of such intermediates has been obtained by trapping experiments under pure O_2 at pressures of 1–60 atm. Under O_2 pressure **1** is converted by the potato LO to a new product, the bis(hydroperoxide) **7**, whereas the coral LO converts **1** to the diastereomeric bis(hydroperoxide) **9**.

The biosynthesis of the physiologically and medically important leukotrienes[1] from arachidonate is initiated by two processes which are also of great mechanistic interest: (1) the conversion of arachidonate by a (5*S*)-lipoxygenase (5-LO) to 5(*S*)-hydroperoxy-6(*E*),8,11,14(*Z*)-eicosatetraenoic acid (5-*S*-HPETE, **1**), and (2) the transformation of 5-*S*-HPETE to leukotriene A_4 (LTA_4, **2**).[1] Evidence has been obtained that the 5-LO, either from murine mast cells[2] or from potato,[3,4] catalyzes the conversion of arachidonate to 5-*S*-HPETE *and also of 5-S-HPETE to* LTA_4. Further, it has been reported that the enyzmic transformation of

Scheme I



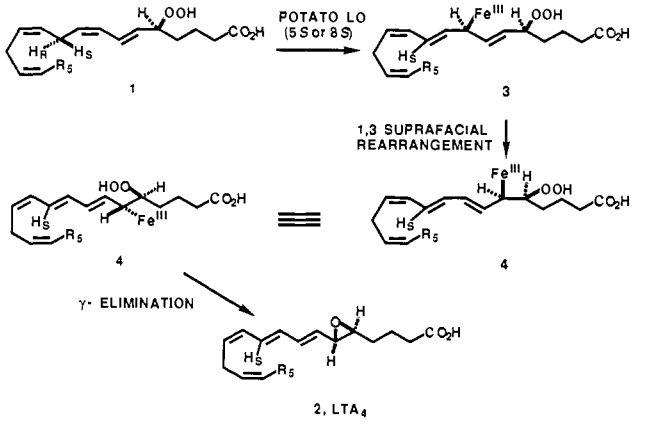
(1) See: *The Leukotrienes, Chemistry and Biology*, Chakrin, L. W., Bailey, D. M., Eds.; Academic Press: New York, 1984.

(2) Shimizu, T.; Izumi, T.; Seyama, Y.; Tadokoro, K.; Rådmark, O.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4175–4179. Because LTA_4 undergoes rapid hydrolysis in neutral aqueous media, it was not isolated from incubation experiments but was analyzed as the mixture of 5,6- and 5,12-diols which results from hydrolysis.

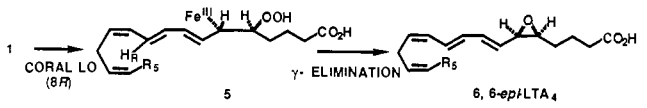
(3) Shimizu, T.; Rådmark, O.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 689–693.

0002-7863/89/1511-1452$01.50/0

Scheme II



Scheme III

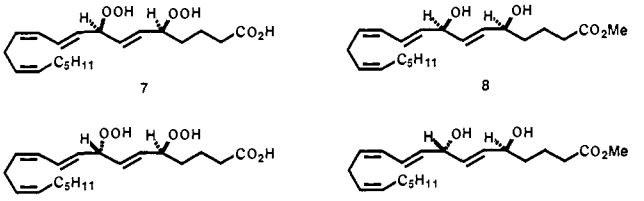


5-*S*-HPETE to LTA_4 by human leukocytes involves stereoselective removal of the *pro-R* hydrogen at C(10) with a kinetic isotope effect, $k_H/k_T > 10$.[5] Both lines of evidence point to the possibility that the reaction which results in the formation of LTA_4 from 5-*S*-HPETE may be related mechanistically to and have features in common with the enyzmic lipoxygenation step. Recent studies in this laboratory have provided evidence for an enzyme-linked organoiron (Fe^{III}) intermediate in the LO reaction which gives rise to product hydroperoxide by insertion of O_2 with retention of configuration followed by the equivalent of hydrolytic Fe^{III}–O bond cleavage,[6-8] as illustrated in Scheme I. On the basis of these considerations a possible mechanism could be derived for the biosynthesis of LTA_4 from 5-*S*-HPETE, which is outlined in Scheme II.

The first step in Scheme II involves the potato LO, which is a 5-*S*-LO with respect to arachidonate, reacting with **1** as an 8-*S*-LO to form the organoiron intermediate **3**, which then rearranges to the more stable conjugated triene system **4**[9] by suprafacial 1,3-shift via a π-allyl structure. Epoxide-forming γ-elimination from the conformer of **4** with Fe and OOH groups antiperiplanar would then produce LTA_4 (**2**). If this mechanism were correct, it would be expected that an (8*R*)-lipoxygenase would react with 5-*S*-HPETE by the pathway shown in Scheme III to form 6-*epi*-LTA_4 (**5**). The recent discovery of a marine route to prostanoids from arachidonate in which the first step is formation of the 8-*R*-lipoxygenation product, 8-*R*-HPETE, and the availability of the 8-*R*-LO from coral[10] allowed the testing of this hypothesis.

First of all, it was confirmed that the *aerobic or anaerobic* incubation of 5-*S*-HPETE (**1**) at pH 8 with the purified lipoxygenase from potato[3,4] cleanly converted (**1**) to LTA_4, as evidenced by the isolation of *exactly* the same mixture of diols (both diastereomeric 5,6- and 5,12-diols) as obtained from authentic LTA_4 under the same conditions (HPLC analysis as the methyl esters).[11] Incubation of **1** with the 8-*R*-LO from the coral *Plexaura homomalla*[10] under the same conditions afforded a *different* mixture of diol hydrolysis products which was *identical* with the mixture produced from an authentic sample of 6-*epi*-LTA_4[12] (**6**) in control experiments.[13] The same results were obtained in repeated experiments: the potato LO converted **1** to the characteristic mixture of LTA_4 hydrolysis products, whereas the coral LO converted **1** to the characteristic mixture of 6-*epi*-LTA_4 hydrolysis products.

Encouraged by the concordance of these results with the predictions outlined in Schemes II and III, we sought and gained additional evidence for the intermediacy of the diastereomeric organoiron species **4** and **5** in the reactions of 5-*S*-HPETE with potato and coral enzymes, respectively. When the incubation of **1** with potato 5-*S*-LO was carried out under 60 atm of O_2, a new product, the dihydroperoxide **7**, was obtained in ca. 1% yield.



Identification of **7** was made by esterification with ethereal diazomethane and sodium borohydride reduction to **8** (UV max 235 nm) and further reduction (H_2, Pt) to octahydro **8** and mass spectral analysis. Both **8** and octahydro **8** gave structurally diagnostic mass spectra.

The coral LO transformed **1** to the bis(hydroperoxide) **9** in the presence of O_2; even at 1 atm of O_2 a 5% yield of **9** was obtained. For characterization, **9** was converted into dihydroxy ester **10** and its octahydro derivative (H_2, Pt), and both were analyzed mass spectrally. The bis(hydroperoxides) **7** and **9** and the diols **8** and **10** were all distinguishable chromatographically. The potato LO reaction with **1** gave **7** but not **9**, and conversely the coral LO converted to **1** to **9** but not **7**.

The evidence presented herein is fully consistent with the mechanistic hypothesis for LTA_4 biosynthesis which is outlined in Scheme II. Our data are not easily explained by the assumption of free-radical intermediates in the transformation of 5-*S*-HPETE to LTA_4 or by a direct pathway involving *concerted* removal of hydrogen at C(10), peroxide bond cleavage, and oxirane formation.[15]

Experimental Section

Arachidonic acid was purchased from Nu-Check Prep. and was purified by column chromatography on silica gel prior to use. Hexane, ether, acetone, and benzene were distilled immediately prior to use. Buffers were prepared in glass-distilled water. All chemicals used were reagent grade and were used as received. Potatoes were purchased at local markets. Coral (*P. homomalla*) was collected by hand at ca. 3 m depth off of Freeport, Grand Bahama. High-performance liquid chromatography was carried out with a Waters Associates 6000A pump, using a DuPont Zorbax silica column (4.6 mm × 25 cm) and a Perkin-

(4) Corey, E. J.; Albright, J. O.; Barton, A. E.; Hashimoto, S. *J. Am. Chem. Soc.* **1980**, *102*, 1435–1436.
(5) Maas, R. L.; Ingram, C. D.; Taber, D. F.; Oates, J. A.; Brash, A. R. *J. Biol. Chem.* **1982**, *257*, 13515–13519.
(6) (a) Corey, E. J. *Pure Appl. Chem.* **1987**, *59*, 269–278. (b) Corey, E. J. In *Stereochemistry of Organic and Bioorganic Transformations*; Bartmann, W., Sharpless, K. B., Eds.; VCH Publishers: Deerfield Beach, FL, 1986; pp 1–12.
(7) (a) Corey, E. J.; Nagata, R. *J. Am. Chem. Soc.* **1987**, *109*, 8107–8108. (b) Corey, E. J.; Nagata, R. *Tetrahedron Lett.* **1987**, *28*, 5391–5394.
(8) Corey, E. J.; Walker, J. C. *J. Am. Chem. Soc.* **1987**, *109*, 8108–8109.
(9) Evidence for such rearrangement has been presented earlier.[7] It is also possible that 4 is formed directly from **1**.
(10) (a) Corey, E. J.; d'Alarcao, M.; Matsuda, S. P. T.; Lansbury, P. T., Jr.; Yamada, Y. *J. Am. Chem. Soc.* **1987**, *109*, 289–290. (b) Corey, E. J.; Matsuda, S. P. T. *Tetrahedron Lett.* **1987**, *28*, 4247–4250. (c) Corey, E. J.; Matsuda, S. P. T.; Nagata, R.; Cleaver, M. B. *Tetrahedron Lett.* **1988**, *29*, 2555–2558.
(11) These diols were isolated by extraction, esterified with ethereal diazomethane, and analyzed by HPLC on a DuPont Zorbax silica column with 96:4 hexane–isopropyl alcohol for elution, detecting at 280 nm. HPLC analysis of the product from the anaerobic or aerobic incubation of **1** with the potato LO showed the ratio of 5*S*,6*S*, 5*S*,6*R*, 5*S*,12*S*, and 5*S*,12*R* diols was equal to 1:10:55:55, exactly as obtained from the hydrolysis of a synthetic sample of LTA_4 under the same conditions.
(12) We thank Dr. Bernd Spur for a generous gift of synthetic 6-*epi*-LTA_4 methyl ester.
(13) The mixture obtained both from the incubation of the *P. homomalla* LO with **1** and the hydrolysis of 6 at pH 8 consisted of 5*S*,6*S*, 5*S*,6*R*, 5*S*,12*S*, and 5*S*,12*R* diols in a ratio of 3:1:18:18.
(14) By the technique described in ref 7.
(15) This research was assisted financially by the National Institutes of Health.

Elmer LC-235 array detector. Mass spectral data were recorded on a Kratos Erba MS-50 instrument. NMR spectra were obtained on a JEOL FX-270 spectrometer. Infrared and ultraviolet spectra were recorded on Perkin-Elmer 683 and 559A spectrometers, respectively.

Preparation of Potato Lipoxygenase. Approximately 500 g of potatoes were scrubbed to remove the dirt. They were not peeled. The potatoes were removed to a cold room and all further work was carried out at 4 °C. Each tuber was sliced into pieces, about 1.5 cm thick, and placed in a blender with 300 mL of pH 4.5 0.1 M acetate buffer (containing 2 mM sodium ascorbate, 2 mM sodium thiosulfate, and 1 mM disodium EDTA). The cover was replaced and the blender was purged with nitrogen for 5 min. The nitrogen flow was continued while the blender was operated for 2 min. The contents of the blender were then poured into two layers of clean cheesecloth in a large glass funnel. The mash was allowed to drain as much as possible. The filtrate was centrifuged at 15000*g* for 20 min. The supernatant was placed in a 1-L Erlenmeyer flask and was stirred gently under nitrogen while being brought to 25% saturation by the addition of solid ammonium sulfate added in 4 portions. The mixture was centrifuged at 15000*g* for 20 min and the supernatant was quickly filtered with suction through three pieces of Whatman No. 1 filter paper. The clear filtrate was brought to 50% saturation with ammonium sulfate under nitrogen as before. The cloudy solution was centrifuged 15000*g* for 20 min and the protein pellet was dissolved in 10 mL of 0.05 M pH 6.8 phosphate buffer (containing 1 mM disodium EDTA). The solution was dialyzed for 5 h against 1 L of the same buffer that had been deoxygenated with nitrogen. A slow stream of nitrogen was bubbled through the beaker during the dialysis. The contents of the dialysis bag were then centrifuged again, and the clear, brown solution was applied to a column of hydroxylapatite. The column was then eluted with 0.05 M pH 6.8 phosphate buffer at a flow rate of ca. 3 mL min^{-1}. Fractions were collected in disposable 13 × 100 mm culture tubes. The lipoxygenase activity was found in the nonadsorbed fractions.

Preparation of Coral Lipoxygenase. Frozen stems of *P. homomalla* (ca. 50 g) were broken up with a pliers and the hard exterior fragments were placed in 150 mL of acetone at −20 °C. The rubbery inner matter was discarded. The acetone suspension was homogenized for 3 min in a blender with a glass cover. The suspension was allowed to settle for 30 s and then was filtered through a Buchner funnel. The dry powder was stored at −40 °C and was used as needed. Partially purified coral lipoxygenase was prepared by triturating 25 mg of the acetone powder with 3 mL of reagent-grade acetone in a test tube with a glass rod for 5 min followed by centrifugation and removal of the acetone with a Pasteur pipet. This was repeated six times. The coral was reduced to the finest powder possible. A final trituration with ether followed by drying in vacuo completed the extraction process. The powder was dissolved in 5 mL of 0 °C pH 8.0 50 mM phosphate buffer, centrifuged, and applied to a 1 cm × 3 cm column of hydroxylapatite, which was eluted with the same buffer. The clear amber solution was used as coral lipoxygenase.

Preparation of 5-*S*-HPETE (1). Arachidonic acid (41 mg, 135 μmol) was weighed into a small glass cup fastened with fine Nichrome wire to a Teflon-covered stir bar. The acid was dissolved in 0.1 mL of methanol, followed by 0.1 mL of 1 M potassium carbonate. The mixture was stirred with a stainless steel needle until a clear solution was formed. The Parr reactor was fitted with a polyethylene liner (cut from a 250-mL Nalgene bottle) and the reactor was charged with 30 mL of potato lipoxygenase solution. The glass cup and magnet were suspended within the bomb by means of a horseshoe magnet taped to the bomb exterior. The reactor cover was bolted on, taking care not to shake the vessel. The bomb was slowly pressurized to 60 atm with oxygen. After 0.5 min, the magnet was removed from the side of the reactor and the magnetic stirrer was started. The mixture was stirred vigorously for 4 min, and then the bomb was vented and the cover was removed. The contents were acidified with 4 mL of cold 2 M citric acid and divided between two cold 40-mL glass centrifuge tubes. The bomb liner was rinsed with 4 mL of cold ethanol and the washings were added to the tubes. The tubes were removed to the 4 °C cold room and all further work was carried out there. To each tube was added sodium sulfate decahydrate, and the tubes were shaken to saturate the solution with the salt. Each tube was then extracted twice with 15 mL of freshly distilled ether. The tubes were centrfigued to separate the layers, and the ether layers were withdrawn by pipet. The combined ether solutions were washed with water (2 × 25 mL) and brine (1 × 25 mL), dried, and evaporated at 0 °C to give crude hydroperoxide. This was flash chromatographed at 0 °C on silica (3:1 hexane–ether) to give 18.1 mg (40%) of 5-*S*-HPETE (**1**): IR (NaCl) 3500–3200 (OH), 3020 (=CH), 1710 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.58 (d of d, *J* = 15, 11 Hz, 1 H, 7-CH), 6.00 (t, *J* = 11 Hz, 1 H, 8-CH), 5.63 (d of d, *J* = 15, 9 Hz, 1 H, 6-CH), 5.38 (m, 5 H, olefinic H), 4.38 (br q, 1 H, 5-CH), 2.82 (m, 4 H, 10- and 13-CH_2), 2.30 (t, 2 H, 2-CH_2), 1.79 (m, 2 H, 16-CH_2), 1.26 (m, 10 H, 3-, 4-, 17-, 18-, and 19-CH_2), 0.89 (t, 3 H, 20-CH_3); UV (MeOH) λ_{max} 230 nm. A sample of the product was esterified with ethereal diazomethane at 0 °C and then reduced to the corresponding alcohol with sodium borohydride in ethanol at 0 °C for mass spectral and HPLC analysis. MS (70 eV, CI), *m/e* 335 ($M + H^+$), 317 ($-H_2O$); HPLC (16:1 hexane–*tert*-butyl methyl ether, 2 mL min^{-1}) t_R = 16.5 min, identical with a synthetic sample of racemic 5-HETE methyl ester.

Study of LTA_4 Biosynthesis. Controls. These incubations were carried out under nitrogen at 25 °C. The hydrolysis of LTA_4 methyl ester was carried out by adding 10 μg of the ester in benzene to 400 μL of 1:1 THF–water and adding 20 μL of 1 M lithium hydroxide to the mixture. After 0.5 h at 20 °C, the saponification was complete as judged by TLC. The THF was removed in vacuo, and 3 mL of pH 8.0, 50 mM phosphate buffer was added. After 45 min at 25 °C, the mixture was brought to pH 4.0 with 2 M citric acid, saturated with sodium sulfate decahydrate, and extracted with ether (3 × 2.5 mL). The extracts were washed with water (1 × 1 mL) and brine (1 × 1 mL), dried, and filtered. The ether solution was cooled to 0 °C and diazomethane in nitrogen was bubbled through the solution for 10 min. The solution was concentrated and the residue was kept frozen in benzene. The products were analyzed by HPLC (96:4 hexane–isopropyl alcohol, 2 mL min^{-1}, 280 nm). Retention times (min, average of five runs, maximum deviation from the average was ±0.5 min) were as follows: 5*S*,6*S* and 5*R*,6*R*, 12.6; 5*S*,6*R* and 5*R*,6*S*, 14.2; 5,12 and 5,12, 18.9 and 21.1. Hydrolysis of synthetic LTA_4 gave products in the ratio of 1:10:55:55. Hydrolysis of 6-*epi*-LTA_4 gave products in the ratio 3:1:18:18.

Enzyme Transformations. Incubations were carried out under nitrogen by adding 250 μg of 5-*S*-HPETE in ethanol to 5 mL of enzyme and 15 mL of pH 8.0 buffer. The mixture was incubated for 45 min at 25 °C, and then the mixture was brought to pH 4.0 with 2 M citric acid, saturated with sodium sulfate decahydrate, and extracted with ether (3 × 2.5 mL). The extracts were washed with water (1 × 1 mL) and brine (1 × 1 mL), dried, and filtered. The ether solution was cooled to 0 °C and diazomethane in nitrogen was bubbled through the solution for 10 min. The solution was concentrated and the residue was kept frozen in benzene. The products were analyzed by HPLC as above. Incubation of **1** with potato LO gave products in the ratio 1:10:55:55. Incubation of **1** with coral LO gave products in the ratio 3:1:18:18.

Preparation and Identification of 7 and 8. Potato LO (5 mL) was placed in the Parr pressure apparatus in a plastic liner cut from a 30 mL polyethylene bottle and 5-*S*-HPETE (5 mg) was added as a solution in 150 μL of ethanol. The mixture was incubated with magnetic stirring at 25 °C for 45 min under 900 psig of oxygen. The reactor was vented, and the incubation mixture was then saturated with sodium sulfate decahydrate, brought to pH 4.0 with 2 M citric acid, and extracted with ether (3 × 8 mL). The ether extracts were washed with water (1 × 5 mL) and brine (1 × 5 mL), dried, and filtered. The ethereal solution was cooled in ice, and diazomethane in nitrogen was bubbled through the solution for 10 min. The product mixture was analyzed by HPLC (96:4 hexane–isopropyl alcohol, 2 mL min^{-1}), **7**: t_R = 7.3 min; λ_{max} 235 nm. Under these conditions 5-*S*-HPETE methyl ester had t_R = 3.2 min, 5-*S*-HETE methyl ester had t_R = 4.3 min.

The sample of **7** was dissolved in 2 mL of methanol at 0 °C and was treated with 50 mg of sodium borohydride added in 5 portions. After 15 min, the mixture was quenched with 1 mL of acetone and was concentrated to 0.5 mL. Water (1 mL) was added followed by ether (2 mL) and 2 M citric acid to bring the mixture to pH 4.0. The ether solution was washed with water (1 × 0.5 mL) and brine (1 × 0.5 mL), dried, and evaporated. The sample of **8** was stored in benzene. HPLC of **8**: t_R = 20.7 min; λ_{max} 235 nm.

The sample of **8** was hydrogenated over 20 mg of Adam's catalyst in methanol for 1 h to give octahydro **8** after filtration and evaporation: MS (70 eV, DCI, TMS derivative), *m/e* 503 (M^+, 3), 471 (−MeOH, 4), 413 (−TMSOH, 26), 381 (fragmentation at C-5, 5), 323 (−2TMSOH, 37), 291 (−2TMSOH, −MeOH, 12), 271 (fragmentation at C-8, 10), 243 (fragmentation at C-8, 14), 203 (fragmentation at C-5, 7).

Preparation and Identification of 9 and 10. Coral LO (5 mL) was added to 15 mL of pH 8.0 buffer and 5-*S*-HPETE (250 μg) was added as a 5 mM ethanol solution. The incubation mixture was stirred under 1 atm of oxygen for 45 min at 25 °C. The mixture was then saturated with sodium sulfate decahydrate, brought to pH 4.0 with 2 M citric acid, and extracted with ether (3 × 8 mL). The ether extracts were washed with water (1 × 5 mL) and brine (1 × 5 mL), dried, and filtered. The ethereal solution was cooled in ice, and diazomethane in nitrogen was bubbled through the solution for 10 min. The product mixture was analyzed by HPLC (96:4 hexane–isopropyl alcohol, 2 mL min^{-1}), **9**: t_R = 9.4 min; λ_{max} 235 nm.

The sample of **9** was dissolved in 2 mL of methanol at 0 °C and was treated with 50 mg of sodium borohydride added in 5 portions. After 15 min, the mixture was quenched with 1 mL of acetone and was con-

centrated to 0.5 mL. Water (1 mL) was added followed by ether (2 mL) and 2 M citric acid to bring the mixture to pH 4.0. The ether solution was washed with water (1 × 0.5 mL) and brine (1 × 0.5 mL), dried, and evaporated. The sample of **10** was stored in benzene. HPLC of **10**: t_R = 20.5 min; λ_{max} 235 nm. MS (70 eV, DCI), *m/e* 350 (M^+, 20), 333 (-OH, 65), 315 ($-H_2O$, 100).

The sample of **10** was hydrogenated over 20 mg of Adam's catalyst in methanol for 1 h to give octahydro **10** after filtration and evaporation: MS (70 eV, DCI, TMS derivative), *m/e* 503 (M^+, 8), 471 (-MeOH, 12), 413 (-TMSOH, 77), 381 (fragmentation at C-5, 8), 323 (-2TMSOH, 100), 291 (-2TMSOH, -MeOH, 13), 271 (fragmentation at C-8, 6), 243 (fragmentation at C-8, 29), 203 (fragmentation at C-5, 14).

The Extraordinarily Long Lifetimes and Other Properties of Highly Destabilized Ring-Substituted 1-Phenyl-2,2,2-trifluoroethyl Carbocations[1]

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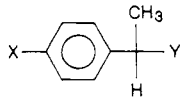
Abstract: A large bromide common ion rate depression is observed for the reactions of 1-(4-methoxyphenyl)- and 1-(4-(methylthio)phenyl)-2,2,2-trifluoroethyl bromides in largely aqueous solutions when the ionic strength is maintained with weakly nucleophilic $NaClO_4$ but not when it is maintained with NaN_3. The rates of the reactions of ring-substituted ($\sigma^+ \leq -0.32$) 1-phenyl-2,2,2-trifluoroethyl tosylates, mesylates, and bromides in 50:50 (v/v) water/trifluoroethanol, at increasing $[I^-]$ or $[N_3^-]$, are independent of nucleophile concentration, but at 0.5 M [Nu] the reactions give yields of the nucleophile adduct which range from 30% to quantitative (≫99%), depending on the ring substituent. These results show that the highly unstable carbocations $XArCH(CF_3)^+$ are readily generated as intermediates of the solvolysis reactions of $XArCH(CF_3)Y$. The rate constant ratios for partitioning of $XArCH(CF_3)^+$ between capture by azide and a solvent of 50:50 (v/v) water/trifluoroethanol (k_{az}/k_s), calculated from product ratios, decrease from 6×10^6 M^{-1} for capture of 4-$N(CH_3)_2ArCH(CF_3)^+$ to 0.8 M^{-1} for capture of 4-$CH_3ArCH(CF_3)^+$ and give k_s values ranging from 800 s^{-1} to 6×10^9 s^{-1}, assuming a diffusion-controlled rate constant of 5×10^9 M^{-1} s^{-1} for k_{az}. There is a modest correlation of log k_s values to σ^+ with a slope $\rho^+_s = 4.8$. The k_s values for the reactions of $XArCH(CF_3)^+$ are nearly identical with k_s values for comparably ring-substituted $XArCH(CH_3)^+$ despite the fact that the former carbocations are at least 8 kcal/mol more unstable relative to a neutral reactant. It is proposed that $XArCH(CF_3)^+$ possess an unusual kinetic stability, which is due to unusually large resonance, electrostatic, or homoconjugative barriers to $XArCH(CF_3)^+$ capture by solvent.

Recently considerable interest has been directed toward the determination and rationalization of the effects of strongly electron-withdrawing α-substituents on the mechanism for solvolysis at saturated carbon and on the stability of the carbocations which may be intermediates in these reactions.[2-5] The studies show that the substitution of an electron-withdrawing group (e.g. cyano,[2a] carbonyl,[2b] or trifluoromethyl[3-5]) for an α-methyl at $XArCH(CH_3)Y$ causes a large decrease in the rate constant for

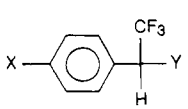


$XArCH(CH_3)Y$

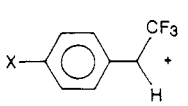
the solvolysis reaction but little change in the structure of the reaction transition state as measured by the structure-reactivity terms ρ^+ and m. This suggests, but does not prove, that the solvolysis reactions proceed by the same S_N1 mechanism observed for the parent compounds $XArCH(CH_3)Y$.[6,7]

In contrast, aliphatic substitution by highly reactive nucleophiles at substrates with strongly electron-withdrawing α-substituents has been little studied in recent years. A thorough examination of these reactions will complement and greatly extend the results from work on the solvent addition reactions to give a fuller description of the effect of electron-withdrawing groups on the substitution reaction mechanism and on the stability of the putative carbocation reaction intermediates.

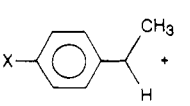
First, a determination of the dependence of the rate of substrate reaction on nucleophile concentration could provide kinetic evidence for the formation of a carbocation reaction intermediate; e.g., the observation of nucleophile adduct formation by a reaction zero order in nucleophile concentration or of a common-ion depression of the reaction rate. It is not generally known whether α-CF_3-substituted carbocations form as intermediates of solvolysis reactions. The recent structure-reactivity studies on the solvolysis of $XArCH(CF_3)Y$ provide important information about transi-



$XArCH(CF_3)Y$



$XArCH(CF_3)^+$



$XArCH(CH_3)^+$

Y = Br, I; Mes = CH_3SO_3;
Tos = 4-CH_3ArSO_3;
4-NB = 4-NO_2ArCO_2;
3,5-NB = 3,5-di-NO_2ArCO_2

(1) Dr. Richard was supported by a Summer Research Fellowship (1985) from the University of Kentucky.

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