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(16) This and other new substances described herein were fully characterized by IR, $^1H$ NMR, and mass spectroscopy.
(17) With the development of this synthesis selective conversions of arachidonic acid into the 5,6-, 11,12-, and 14,15-oxides are now available as described in this communication and ref 9.
(18) Separated by high pressure liquid chromatography [Waters Associates $\mu$-Porasil column using heptane–isopropyl alcohol (200:1)]. The trans configuration of the 10,11 double bond in the 12-HETE methyl ester synthesized by the present method is clear from (1) its identity ($^1H$ NMR, IR, mass spectrum, chromatographic mobility) with methyl ester of 12-HETE (**9**) synthesized by the previously described[19] unambiguous route and (2) the $^1H$ NMR spectrum (in $CDCl_3$) which shows peaks at $\delta$ 6.55 (dd, $J_{10,11}$ = 15, $J_{9,10}$ = 10.5 Hz, 1 H) for H(10), 5.95 (dd, $J_{8,9} = J_{9,10}$ = 10.5 Hz, 1 H) for H(9), and 5.70 (dd, $J_{10,11}$ = 15, $J_{11,12}$ = 6 Hz, 1 H) for H(11). See Gardner, H. W.; Weisleder, D. *Lipids* **1970**, *5*, 678; **1972**, *7*, 191. The trans configuration of the 12,13 double bond in synthetic 11-HETE (**8**) and its methyl ester is similarly indicated by $^1H$ NMR data. **8** methyl ester (in $CDCl_3$): $\delta$ 5.64 (dd $J_{12,13}$ = 15.4, $J_{11,12}$ = 6.5 Hz, 1 H) for H(12) and 6.54 (dd, $J_{12,13}$ = 15.4, $J_{13,14}$ = 10.6 Hz, 1 H) for H(13).
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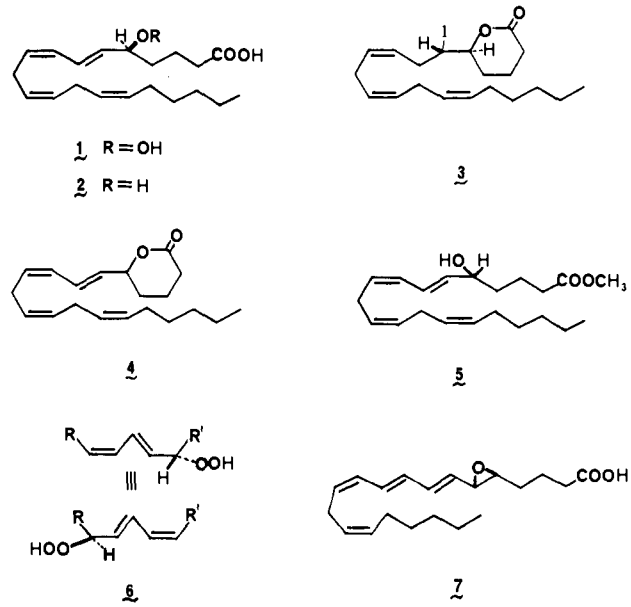
## Chemical and Enzymic Syntheses of 5-HPETE, a Key Biological Precursor of Slow-Reacting Substance of Anaphylaxis (SRS), and 5-HETE

*Sir:*

The hydroperoxide (*S*)-5-HPETE (**1**) is the first intermediate[1,2] in a recently recognized series of biosynthetic processes which lead from arachidonic acid to a number of biologically active compounds, including **7** (leukotriene A) and the slow-reacting substance of anaphylaxis (SRS).[1c,3-7] In this communication we describe a simple chemical synthesis of **1** and the corresponding alcohol (**2**) in racemic form and also a straightforward enzymic preparation of the 5-(*S*)-chiral forms of **1** and **2**.

As previously described, the iodo lactone **3** is easily available from arachidonic acid in 86% yield (8 equiv of KI, 15 equiv of $I_2$, 5 equiv of $KHCO_3$ in 1:2 aqueous tetrahydrofuran (THF) at 0 °C for 18 h). Reaction of **3** with 2.5 equiv of 1,5-diazabicyclo[5.4.0]undec-5-ene in benzene at 23 °C for 7 h produced the unsaturated lactone **4**[8] (72–85% yield), $\lambda_{max}$ in ether 235 nm ($\epsilon$ 28 000), which was transformed into the methyl ester of (±)-5-HETE (**5**), $\lambda_{max}$ in $CH_3OH$ 235 nm ($\epsilon$ 28 600), by treatment with 6 equiv of triethylamine in methanol at 23 °C for 30 min (82% overall yield from iodo lactone **3**). The mass spectrum of the trimethylsilyl ether of **5** was identical with that previously reported for natural material obtained from rabbit leukocytes.[9] Saponification of **5** proceded quantitatively using lithium hydroxide in dimethoxyethane–water at 23 °C to afford (±)-5-HETE (**2**).




Reaction of the methyl ester of (±)-5-HETE in methylene chloride at −65 °C with 1.5 equiv of methanesulfonyl chloride and 1.8 equiv of triethylamine for 30 min produced a solution of the 5-mesylate which was cooled to −110 °C and treated with 50 equiv of dry hydrogen peroxide in ether (3 M) for a reaction time of 15 min. After quenching, extractive isolation, and thin layer chromatography on silica gel, the methyl ester of (±)-**1** was obtained in ~50% yield.[10] Saponification of the methyl ester was effected using a large excess of lithium hydroxide and hydrogen peroxide in dimethoxyethane–water (1:1) at 23 °C for 1.5 h to give after TLC purification on silica gel (with 95:5 $CH_2Cl_2$–$CH_3OH$ for elution) pure (±)-5-HPETE (**1**). Treatment of (±)-**1** with diazomethane in ether afforded cleanly the corresponding methyl ester. Reduction of (±)-**1** with sodium borohydride in water at pH 9 produced (±)-5-HETE (**2**).

With synthetic 5-HETE and 5-HPETE in hand as chromatographic references, the possibility that various plant-derived lipoxygenases might be capable of converting arachidonic acid into 5-HPETE could readily be tested. The lipoxygenase of potato tubers was especially interesting for study since it has been reported to convert linoleic acid almost exclusively (95%) into 9-(*S*)-hydroperoxyoctadeca-*trans*-10,*cis*-12-dienoic acid.[11,12] The strong tendency to attach oxygen at the point nearest to the carboxylic function contrasts, for example, with the much studied soybean lipoxygenase which converts linoleic acid mainly into 13-(*S*)-hydroperoxyoctadeca-*cis*-9,*trans*-11-dienoic acid and arachidonic acid into 15-(*S*)-HPETE.[13] A highly significant (but previously unnoted) stereochemical pattern also emerges from previous studies on the stereochemistry of the hydroperoxides produced from plant lipoxygenases. Thus, soybean-, corn-, and potato-derived lipoxygenases generate the dissymmetric 1,5-disubstituted penta-*trans*-2,*cis*-4-dien-1-ol unit with the absolute configuration depicted in **6**, i.e., with *S* chirality at the oxygenated carbon atom. Obviously, it is this absolute stereochemical specificity which is required for the formation of (*S*)-5-HPETE from arachidonic acid.

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The lipoxygenase derived from potato tubers *in fact* was found to convert arachidonic acid into a mixture of products which included 5-HPETE (15% yield after correction for recovered arachidonic acid). The potato enzyme used was obtained simply by brief homogenization of potato tubers in pH 4.5 acetate buffer, filtration through gauze and precipitation with ammonium sulfate (50% saturation after a prior precipitation at 25% saturation), dissolution in pH 6.8 phosphate buffer, and dialysis as previously described.[12] The solution of lipoxygenase so obtained could be stored at 0 °C for up to 1 week with only minor loss of activity.

Preparative experiments with the enzyme were performed starting with an aqueous solution of ammonium arachidonate at pH 9 adding Triton X-100 (dispersant), Antifoam B and 4-hydroxy-2,2,6,6-tetramethylpiperidinooxy free radical (as radical trap), adjusting the pH to 6.4, adding enzyme solution, and stirring with oxygen for 12 min. Acidification to pH 4.0, extractive isolation with ether, and purification by thin layer chromatography on silica gel (5% $CH_3OH$, 25% hexane, 70% ether at 0 °C) afforded pure (*S*)-5-HPETE (**1**), identical chromatographically and by $^1H$ NMR with (±)-5-HPETE synthesized chemically as described above. Reduction of (*S*)-5-HPETE by cold aqueous sodium borohydride at pH 9 afforded (*S*)-5-HETE (chromatographically and spectroscopically identical with (±)-5-HETE synthesized chemically as described above). The absolute configuration of the enzymatically produced 5-HPETE and 5-HETE was shown to be *S* (as in **1** and **2**) by two different methods. The methyl ester of 5-HETE of enzymatic origin (made with ethereal diazomethane) was converted into the menthyloxycarbonyl derivative using the chloroformate of *l*-menthol[14] and pyridine for 3 h at 23 °C and then subjected to the sequence[15] (1) ozonolysis in methylene chloride at −20 °C; (2) oxidation with peracetic acid in ethyl acetate at 23 °C for 12 h; and (3) esterification with diazomethane in ether. The resulting menthyloxycarbonyl derivative of dimethyl 2-hydroxyadipate was characterized by gas chromatography (2% QF-1 fluorosilicone column at 185 °C) as the diastereomer of shorter retention time,[9a,16] thereby proving the *S* configuration at C-5 in **1** and **2**. The optical rotation measured for the methyl ester of **2** (*c* 0.99 in ethanol) was positive and increased with decreasing wavelength, e.g., $[\alpha]^{23}_{436}$ +12.42°, $[\alpha]^{23}_{D}$ +4.73°. The same dextrorotation with increasing value for decreasing wavelength has been observed previously for several alcohols having the dissymmetric unit corresponding to **6**.[17] The criterion of increasing dextrorotation as a function of decreasing wavelength in the range 589–436 nm is thus a simple and convenient indicator of chirality corresponding to **6** in an HETE methyl ester.

A lipoxygenase which converts arachidonic acid into 5-HPETE has also been found in tomato; however, the preparation using the potato[18] enzyme has been found to be cleaner and more convenient. Research is in progress on the action of a variety of other plant lipoxygenases on arachidonic acid.

By the use of radiolabeled arachidonic acid and the lipoxygenase of potato radiolabeled (*S*)-5-HPETE was readily prepared. This is now under study in the laboratory of Professor B. Samuelsson to check incorporation into SRS and other eicosanoids[2] in this series. The chemical conversion of (*S*)-5-HPETE to 5(*S*),6-oxido-7,9-*trans*-11,14-*cis*-eicosapentaenoic acid (**7**), the direct precursor of SRS, is described in a separate note.[19,20]

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(8) Satisfactory infrared and proton magnetic resonance ($^1H$ NMR) spectra were obtained for a chromatographically homogeneous sample of each substance described herein. In addition mass spectral data were obtained for each substance except for the unstable hydroperoxides. The trans stereochemistry of the newly introduced double bond in **4** was clear from $^1H$ NMR data ($CDCl_3$): protons at C-7 and C-8 were farthest downfield; $\delta$ 6.62 (dd, $J$ = 10.5, 15 Hz, 1 H) for H(7) and 5.99 (dd, $J$ = 10.5, 10.5 Hz, 1 H) for H(8). See Gardner, H. W.; Weisleder, D. *Lipids* **1970,** *5,* 678; **1972,** *7,* 191. In addition the expected trans HC=CH bond was present in the IR spectrum at 985 $cm^{-1}$. Products **1, 2,** and **5** showed the same characteristic $^1H$ NMR peaks for the *cis,trans*-HC=CH—CH=CH– unit; for example, **5** had $\delta$ 6.54 (dd $J$ = 10.5, 15 Hz, 1 H) for H(7) and 5.98 (dd, $J$ = 10.5, 10.5 Hz, 1 H) for H(8).

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(18) Fresh Florida "red-skin" or Maine potatoes have been employed in our work.



(19) This research was assisted by a grant from the National Science Foundation. We are indebted to Mr. P. Malan of this department for helpful advice on the enzymic experiments.


(20) The results outlined in this paper were previously described in a lecture at the Karolinska Institutet, Stockholm, Sept 14, 1979.


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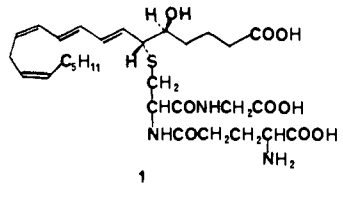
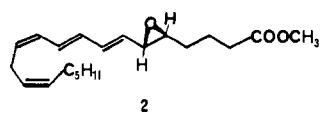
## Stereospecific Total Synthesis of a "Slow Reacting Substance" of Anaphylaxis, Leukotriene C-1

*Sir:*

"Slow reacting substance" (SRS), though known since 1938,[1] has only recently been characterized in terms of molecular structure.[2-5] We record here the first total synthesis of an SRS, leukotriene C-1 (**1**), isolated from mouse mast cell tumor,[6] along with variable amounts of a second less active SRS, leukotriene C-2. Other sources have also been used to




**1**




**2**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