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Reviews

Chemical studies on slow reacting substances/leukotrienes*

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Summary. The family of eicasanoids, biologically active metabolites of polyunsaturated C_{20} fatty acids such as arachidonic acid, has recently been enlarged by the recognition of a new biosynthetic pathway leading to the leukotrienes, including the compounds described two decades ago as 'slow reacting substances'. These biologically potent substances are involved in regulation of the immune response and also as mediators in various disease states. This account presents a brief history of this field, an overview of the biological relevance of leukotrienes, and a discussion of the investigations which led to the clarification of the molecular structures, pathway of biosynthesis and total chemical synthesis of the leukotrienes, including leukotrienes A, B, C, D and E (LTA-LTE). As a result of the synthetic work these rare substances are available for the first time in pure form and in quantities sufficient for biological and medical studies. Also reviewed are recent discoveries with regard to the development of inhibitors of leukotriene biosynthesis and anti-leukotrienes.

Introduction

This article summarizes research at Harvard over the past 5 years on a new class of biologically active mammalian natural products. The 'slow reacting substances', which are also known by the corresponding acronym 'SRS', are a family of chemical substances produced in the body as part of the immunochemical response, that is, the sequence of events by which viruses, bacteria, and also certain indigenous cells such as tumor cells or damaged tissue are recognized and dealt with. The name derives from a characteristic ability to cause slow and prolonged contraction of a test smooth muscle strip maintained under tension in a suitable physiological bath. This response contrasts with the rapid and short lasting contraction produced by substances such as histamine. The SRSs are currently also referred to as 'leukotrienes', a term recently introduced by Prof. Bengt Samuelsson of Karolinska Institutet, Stockholm. Although the knowledge of the roles of the leukotrienes in normal or abnormal physiological states is now only at a rudimentary level, it is already clear that these substances are significantly involved in immunoregulation and in a variety of diseases including asthma and various allergic conditions.

The first evidence for the existence of the SRSs came from immunological experiments with various antigens. It has been known for several decades that whereas the injection of a foreign nontoxic protein such as serum albumin into experimental animals usually produces no ill effects, a 2nd injection of the same substance days or even weeks later can cause a very acute illness and in the extreme, death within minutes. The initial injection in these cases induces an allergic state in which the experimental animal has become hypersensitive to the initially injected 'antigen' or 'allergen'. The same sequence of operations is involved in 'immunization', however, there is a very different response: the production of antibodies which are specific for the antigen and which serve to neutralize additional antigen without harmful affect. By contrast, in hypersensitivity the combination of antigen with antibody triggers a poorly controlled response and an excessive release of various mediators which serve as the immediate cause of pathological change. Among these mediators are the slow reacting substances. The extreme form of hypersensitivity is called anaphylaxis.

The slow reacting substances were first recognized as biological factors in 1938 by Feldberg and Kellaway in England[2] who found that the perfusion of guinea-pig lung with various antigenic materials resulted in the generation of agents which caused contraction of guinea-pig ileum. They introduced the acronym SRS to designate the factor(s) responsible for the observed physiological response of this test muscle. Later it was shown by the same group that the synthesis of these compounds can be induced by challenge with conven-

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tional allergens[3]. Some time later Brocklehurst at Eli Lilly Laboratories in England[4] demonstrated that during an anaphylactic event materials are generated which show biological activity similar to the Feldberg and Kellaway SRS. Because he could not ascertain whether the anaphylactic material and the previously described SRS were the same or different substances, he modified the acronym to SRSA to indicate the former. It is now known that these substances are indeed the same and a distinction between SRS and SRSA is unnecessary.

Work carried out by a number of investigators in the 1960s, most noteworthy Austen's group[5] at Harvard Medical School, demonstrated that the slow reacting substances behave as potent mediators of airway constriction and bronchial spasm in human asthma and of allergic hypersensitivity. The action of this SRS is not blocked by antihistamines, a fact consistent with the ineffectiveness of antihistamines in the treatment of asthma and other forms of hypersensitivity. It is of special interest that the symptoms of asthma have been produced by an aerosol administration of SRSs into the lungs of experimental animals and that SRSs have been detected in fluid from the lungs of asthmatic humans[5c].

For several years it had been known that these slow reacting substances are produced from arachidonic acid as a biochemical predecessor. They are therefore members of the same large biochemical family as the prostaglandins and thromboxanes. I have introduced the term 'eicosanoid' to denote this general class of compounds which is derived from the 20-carbon precursor arachidonic acid.

Evidence has accumulated over the years that various cells of the body, which are either components of circulating blood or initially circulating cells that have become localized in tissue, are involved in the immune response and hypersensitivity. These cells are listed along with certain salient characteristics in table 1.

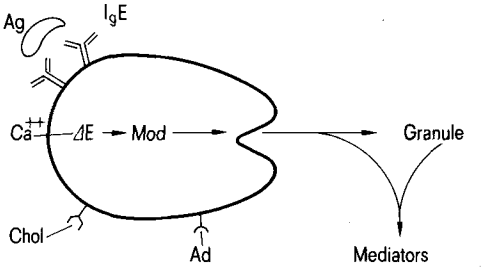
The mast cell is widely distributed and occurs in all kinds of connective tissue being especially abundant in the respiratory system, gastrointestinal tissue, skin and generally those parts of the body that are exposed to external agents. It has been described as a kind of immunological watchdog or sentinel cell because of the ability to detect foreign material. A mechanism for this detection has emerged from extensive biologi-

Table 1. Cells involved in hypersensitivity

1. Mast cell:	widely dispersed in connective tissue; immunological 'lookout' or 'sentinel' function at sites of potential invasion by harmful agents. Receptors at cell surface bind ca. 5×10^5 IgE molecules. Activation causes release of granules/discharge of various mediators and factors. Immediate hypersensititivty (IH*).
2. Polymorphonuclear leukocytes (PMNL's) (white blood cells):	~ 95% neutrophils, ~ 1% basophils, ~ 1% eosinophils. IH*
3. Lymphocytes:	Bursa-produced (B cells) and Thymus-produced (T cells); circulating; Ag specific; delayed hypersensititivty; attract or inhibit migration of macrophages.
4. Macrophages:	develop in marrow, transported to tissues via blood; non-specific destroyer cells; pro- and anti-inflammatory effects.

Table 2. Mast cell activation and mediator release

Activation:

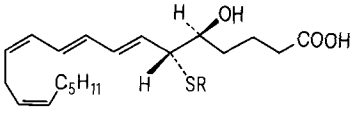


Mediators:	histamine, SRS, platelet activating factor, chemotactic factors (peptide, lipid), heparin, enzymes. SRS can be isolated from cultured mastocytoma cells.
Cell interactions:	attract macrophages, neutrophils, eosinophils.
Modulation:	release inhibited by increase in c-AMP; α-adrenergic, cholinergic activity decrease c-AMP, β-agonists increase c-AMP. (Theophylline blocks PDE, increases c-AMP).

Table 3. Macrophages

Macrophages (MP's): possess IgE receptors; ingest antigens (Ag's) and process them to increase immunogenicity; attach to the surface of mast cells effecting granule transfer.
MP's produce SRS in vitro from arachidonic acid under stimulation by zymosan; also produce PGE_2, PGI_2, TXA_2, PAF.
MP's secrete superoxide, H_2O_2, collagenase, elastase (and other proteases), lipases, phosphatases, complement components, endogenous pyrogens.
MP's inhibit replication of microbes, tumor cells and viruses (interferon).

Table 4. Contractile activities of leukotriene analogs with various saturation of ethylenic bonds (Drazen et al.[38])

	Pulmonary parenchymal strip	Ileum
		
Parent compound		
LTC	1	1
LTD	1	1
Analog		
14,15-dihydro-LTC	0.19	1.0
14,15-dihydro-LTD	0.66	0.66
7-*trans*-hexahydro-LTC	0.19	0.35
7-*trans*-hexahydro-LTD	0.019	0.005
7-*cis*-hexahydro-LTC	0.06	0.35
7-*cis*-hexahydro-LTD	0.03	0.007

Activities of the analogs are expressed relative to those of the parent compounds on guinea-pig tissues.

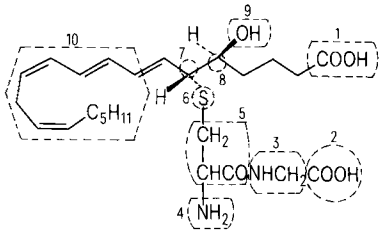
cal and biochemical studies (see table 2). Each mast cell has at its surface about a half a million receptors which are specific for one kind of immunoglobin, the immunoglobin E variety (IgE). These receptors bind IgE and detect foreign substances (allergens) having an affinity for that IgE. Such allergens activate mast cells causing degranulation and secretion of a number of biological agents. Mast cell degranulation has been implicated in immediate hypersensitivity[5c].

A great deal is known about the morphology and function of the mast cell, though much remains to be discovered. Each mast cell possesses roughly half a million surface receptors that are specific for immunoglobin E which binds to these receptors through its 'fixed region'. The 'variable region' of the IgE structure projects outward from the surface of the cell and is available for specific binding to a suitable antigen. Whenever 2 IgE molecules attached to adjacent receptors are bridged by a single molecule of antigen, a signal is sent to the cell wich changes the character of the cell membrane in such a way as to allow influx of calcium ions into the mast cell. This influx of calcium triggers a series of biochemical processes the end result of which is degranulation. The processes of mast cell activation are also regulated within the cell in a rather complicated way. The modulation depends, for example, on the level of cyclic AMP within the cell. This is in turn dependent upon the presence of other modulators such as prostaglandins and on adrenergic and cholinergic agents acting on receptors at the cell surface. In general the higher the concentration of cyclic AMP, the less the tendency for the cell to be activated for the granule release action.

The biological mediators released in degranulation include histamine, slow reacting substances, the so-called platelet activating factor (a phospholipid), heparin, various chemotactic factors which cause the attraction to the mast cell of other types of cells such as the macrophages, and various enzymes, especially protolytic enzymes which have a capacity to break down tissue. SRS compounds can actually be isolated from cultured mast tumor cells (mastocytoma cells). This provides part of the evidence that SRS is produced by the mast cell. Electron microscopy has demonstrated that the release of chemotactic factors can signal macrophages to approach the mast cell surface and accept granules (and mediators) in a direct transfer.

In current therapy theophylline is a widely used drug for the treatment of asthma. It evidently works by blocking the e zyme phosphodiesterase thereby preserving cyclic AMP. That increase tends to diminish

Table 5. Contractile activities of leukotriene analogs; activity ratios to LTD on guinea-pig tissues (Lewis et al.[45])

Entry	Compound	Structural variation	Pulmonary parenchymal strip, EC_{50} ratio	Ileum dose ratio
				
1	LTD	None	1	1
2	LTD monoamide (C-1)	1	1	0.25
3	LTD bisamide (C-1, Gly)	1,2	< 0.001	< 0.002
4	Deamino LTD bisamide (C-1, Gly)	1,2,4	< 0.001	< 0.002
5	LTD monoamide (Gly)	2	0.10	ND
6	LTD monodimethylamide (Gly)	2	0.006	< 0.002
7	D-Ala-LTD	3	0.11	0.33
8	L-Ala-LTD	3	0.09	0.05
9	Pro-LTD	3	0.03	0.03
10	Glu-LTD	3	0.02	0.02
11	Val-LTD	3	0.008	0.01
12	Homocys-LTD	5	0.26	0.20
13	D-Pen-LTD	5,6	< 0.001	< 0.002
14	D-Cys-LTD	6	0.11	0.02
15	D-Pen-LTE	5,6	< 0.001	0.005
16	Deamino-LTD	4	0.05	0.20
19	LTD-sulfoxide, isomer 1	6	0.10	0.10
20	LTD-sulfoxide, isomer 2	6	< 0.001	< 0.002
21	6-Epi-LTD	7	0.004	0.005
22	6-Epi-LTC	7	0.005	0.002
23	5-Epi-LTD	8	0.004	0.005
24	5-Dehydroxy-9-12,14,15-hexahydro-LTD	9,10	< 0.001	< 0.002

the release reaction and the hypersensitivity response in asthmatic individuals. Steroids such as cortisone are also used in medicine. They block arachidonic acid release and SRS synthesis. Thus mast cells occupy a position on the perimeter of the body's defense system and they trigger important cellular events of the immune response. Macrophages (table 3) are produced in the marrow, transported to tissues by way of the blood, and subsequently lodge and reside in tissue for several weeks. They take up their stations and they serve as nonspecific destroyer cells once a signal from the other parts of the immune system, the trigger mechanisms, is received. Because they can produce massive nonselective digestion of the material around them, macrophages can be a major source of inflammation. Macrophages also possess IgE receptors. Further, they are capable of ingesting and modifying antigens (by an obscure process) so as to increase their immunogenicity, thereby amplifying the immune response. Macrophages secrete SRS and also powerfully destructive agents, for example, superoxide ion (O_2^-), hydrogen peroxide, collage ase, galactase, and other chemicals which are capable of breaking down biological matter. Thus they are protectors against viruses, tumor cells, and other biological threats.

The neutrophils which also function as 'killer cells' in many ways are similar to the macrophages. They produce a comparable assortment of powerful cell- and tissue-disintegrating substances: proteases, hydrogen peroxide, hypochlorous acid, and the like. They, too, are attracted by chemotactic factors secreted by the trigger cells and by each other.

Slow reacting substances are now known to be produced in a variety of tissues and cells. The include mast cells and mast tumor cells (mastocytoma cells), rat basophilic leukemia cells, macrophages, human and guinea-pig lung tissue (after sensitization), and cells in the inflamed rat peritoneal cavity. Four different SRSs have now been obtained from these sources.

Structure and synthesis of SRSs

The chemical nature of the SRSs was completely unknown for many years. However, as mentioned above, it has become clear that these materials are formed biosynthetically from arachidonic acid, the same C_{20}-tetraunsaturated fatty acid which gives rise to prostaglandins (PGs) such as $PGF_{2\alpha}$ and PGE_2[6]. SRS biosynthesis can be stimulated by the calcium SRS biosynthesis can be stimulated by the calcium ionophore A23187 (which transports Ca^{++} across membranes[7] and by addition of various thiols (such as cysteine)[8] to certain SRS synthesizing cells. Of considerable importance to the clarification of the structure of SRSs were the findings that these substances could be refined by reversed phase high performance liquid chromatography (RP-HPLC)[9], that they exhibited strong ultraviolet absorption (centered at approximately 280 nm[9], and that they are deactivated by soybean lipoxygenase, an enzyme specific for fatty acids containing the structural subunit: *(cis, cis)*-$CH{=}CH{-}CH_2{-}CH{=}CH{-}$[10].

Although our group at Harvard had long been involved in chemical and synthetic studies on the prostaglandins and had followed developments in the research on SRSs, chance was to play a key role in our activities in this area. Professor Bengt Samuelsson, a former collaborator and also a former postdoctoral associate of mine (1961–1962), happened to be visiting in the Boston area in March of 1977. Over lunch in a restaurant in Harvard Square he described some interesting studies which were in progress in his laboratory involving the metabolism of arachidonic acid in polymorphonuclear leukocytes (PMNs), principally the neutrophil type. In work done collaboratively with a postdoctoral fellow, Pierre Borgeat, he found that arachidonic acid is converted by PMNs in a very low yield to a new substance which could be isolated in μg amounts. It was demonstrated that this metabolite contained 2 hydroxyl groups in addition to the oxygens originally present in arachidonic acid and that these hydroxyl groups were at carbons 5 and 12. Further, it was shown that the absolute chirality of the stereocenters was 5-*(S)*, 12-*(R)*. On the basis of these data and also UV absorption, he proposed the structure in figure 1 (bottom, center), with the geometry of the 3 conjugated bonds being only tentative since the data did not allow a final decision on this point.

This metabolite was found not to have contractile activity in measurements involving a conventional smooth muscle test preparation, guinea-pig ileum. It was therefore conjectured that this substance might be a transformation product of a much less stable, but biologically active and more important molecule. This reasonable hypothesis finds a parallel in Samuelsson's work on metabolites of arachidonic acids in platelets, the thromboxanes A_2 and B_2. Arachidonic acid is converted in platelets into an extremely unstable substance, thromboxane A_2, which has a half life of only approximately 33 sec, but which is a powerful inducer of platelet aggregation. The major metabolite of thromboxane A_2, thromboxane B_2, is a product of hydroly sis and is essentially inactive. Thromboxane A_2 can be trapped by addition of methanol and converted to thromboxane B_2 methyl ether.

A parallel methanol trapping experiment in the neutrophil system led to isolation of a new product, which was formed at the expense of the C_{20}-5,12-dihydroxy acid that would normally be obtained. It was found to be a monomethyl ether having 1-free OH and 1 COOH group. By microchemical degradation and mass spectroscopy, it was shown that the free hydrox-

yl function was attached at C-5 and the methoxy group at C-12. The TLC analysis indicated that the methanol trapping product was homogeneous and it seemed from degradation studies to have the R-configuration at C-12. On the basis of all these data, Samuelsson deduced the formulas shown in figure 1 for the monomethyl ether and the unstable intermediate. It was considered that the reactive oxa-cyclononatriene intermediate combines with methanol by a non-enzymic S_N2 process in the trapping experiment. For various reasons this interpretation seemed to us unlikely. Why should the oxa-cyclononatriene structure react with methanol only at C-12 and not at C-5? Why should it not react by an S_N1 process to form mixtures if isomeric methoxy compounds? Is not the oxa-cyclononatriene structure so strained as to be

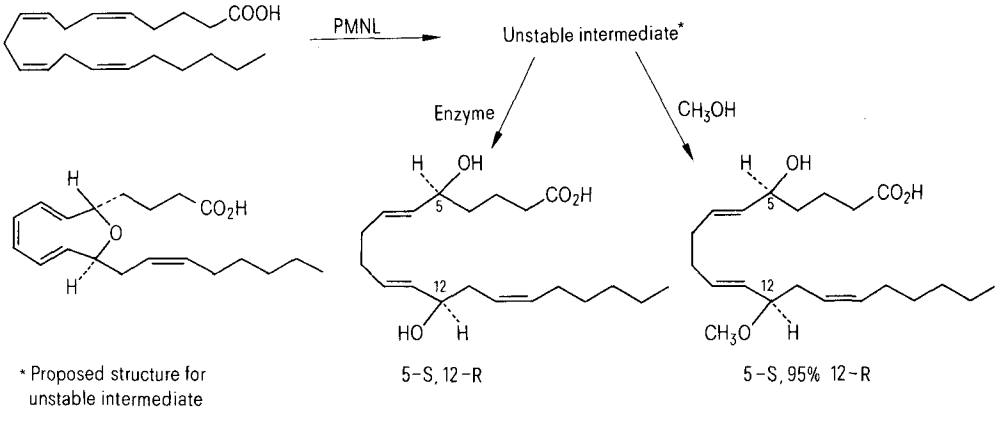


Figure 1. Eicosanoid production in polymorphonuclear leukocytes (PMNL). I. Neutrophils (B. Samuelsson, 1977).

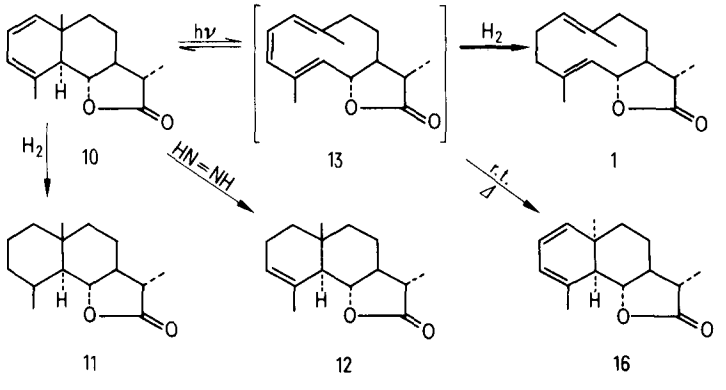


Figure 2. Generation of an unstable cyclodecatriene.

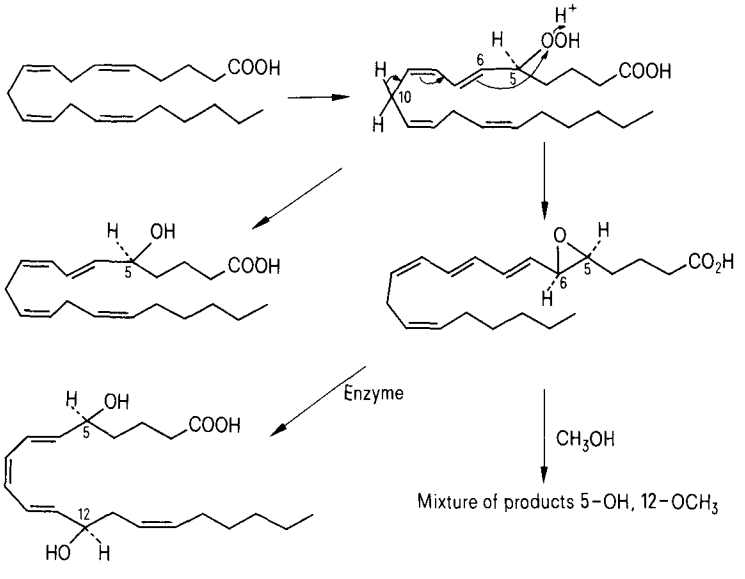
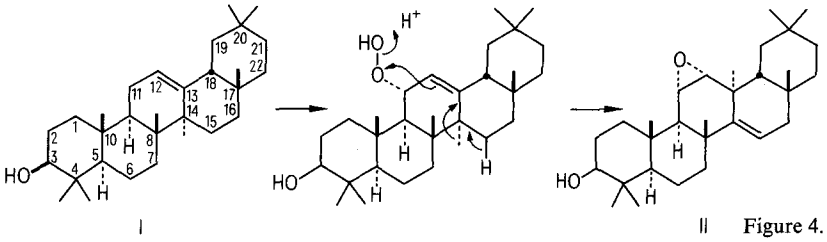


Figure 3. Alternate proposal for PMNL intermediate (March 1977). (Borgeat and Samuelsson[14]).

highly suspect? We had encountered similar structures having one more ring member, cyclodeca-1,3,5-trienes, years before in our synthetic studies on costunolides[11] (fig. 2). These were very unstable even in aprotic solution at 20 °C and it was clear that the oxacyclononatriene structure would be vastly more strained and less stable. Stimulated by these doubts and considering the usual lipoxygenase mode of oxygenation of arachidonic acid, I proposed the alternative structure for the PMN-derived predecessor of the 5,-diol and the pathway for biosynthesis which are shown in figure 3. This scheme could explain enzymic 5, 12-diol formation, but it required that the methanol quenching reaction produce at least a mixture of 2 epimeric C-12 methoxy compounds (an S_N1 mechanism would clearly be expected) and probably other products as well (e.g., 2 C-6 methoxy products). These considerations suggested the desirability of a reexamination of the methanolysis product to ascertain whether more than one isomer could be detected. The proposal outlined in figure 3 is mechanistically reasonable. In fact, some years ago we encountered the allylic hydroperoxide to epoxide conversion with studies in the triterpene series (fig. 4)[12]. The triene-conjugated oxirane structure for the metastable intermediate which is shown in figure 3 was expected to be quite readily hydrolyzed in water at pH 7 (even faster at lower pH) but much more stable at higher pH and therefore an experimental pH-lifetime profile of the intermediate from the neutrophil system was indicated. The olefinic stereochemistry shown in figure 3 for the oxirane intermediate seemed the most likely on mechanistic basis. The oxirane unit in principle could be a either *trans* substituted or *cis* substituted with the



I II Figure 4. Allylic hydroperoxide to epoxide transformation.

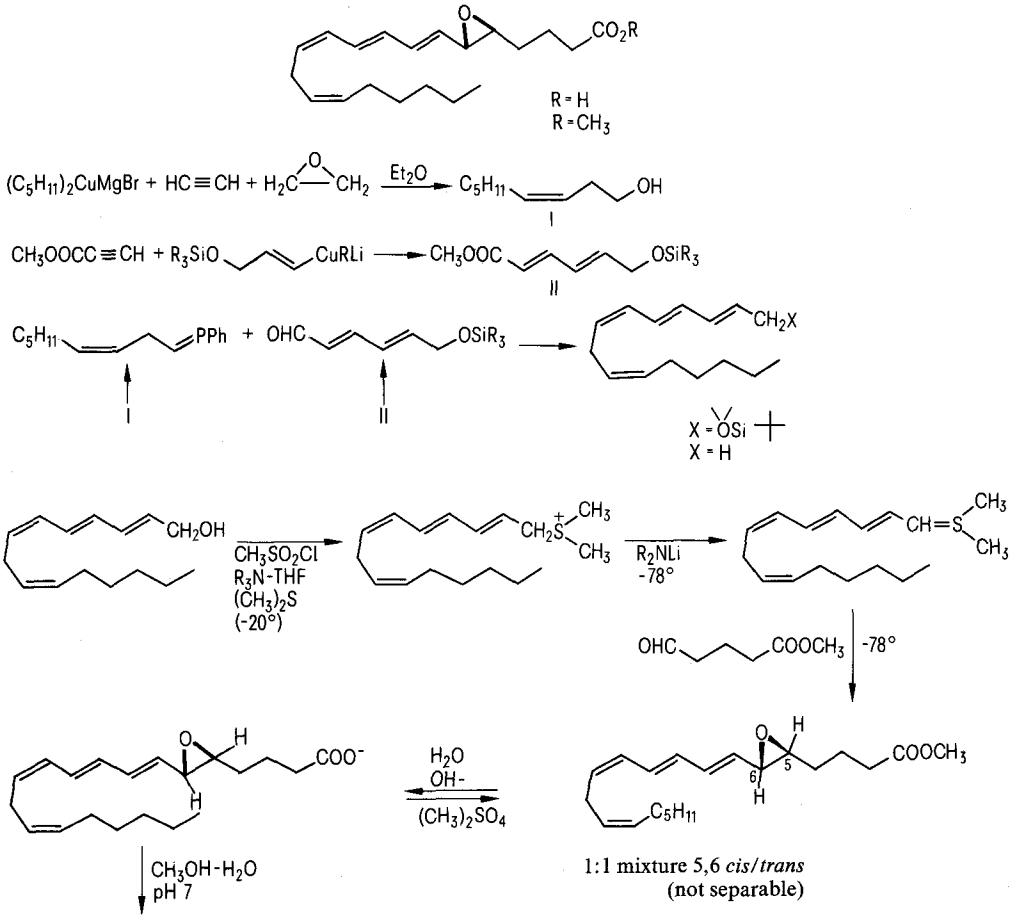


Figure 5. Synthesis of (±)-5,6-oxido-7,9-trans-11,14-cis-eicosapentaenoic acid (Corey et al.[13]).

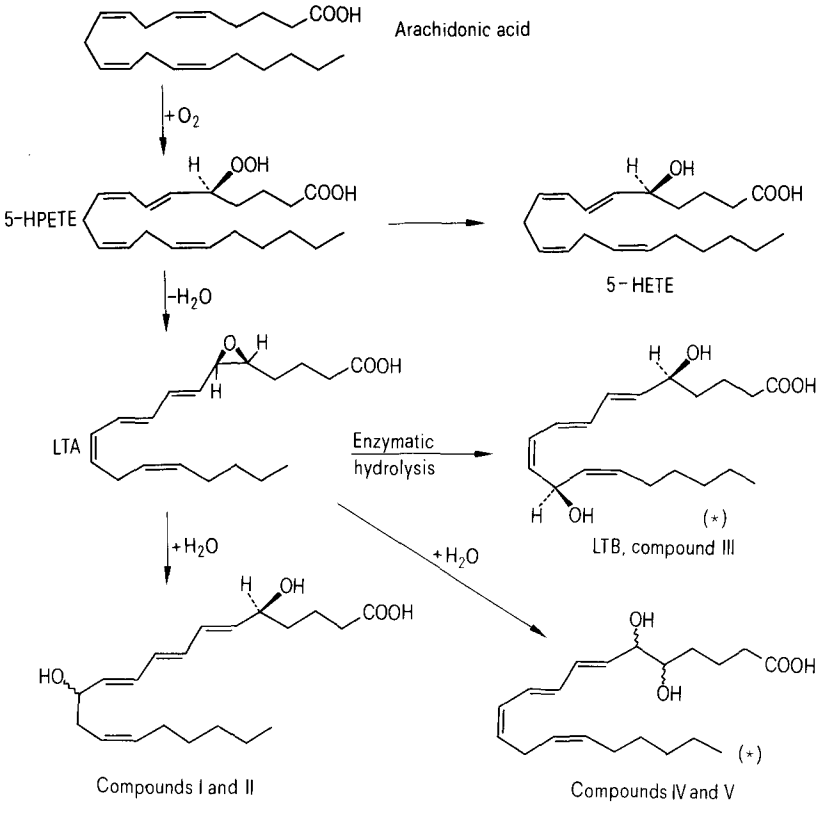


Figure 6. PMNL-neutrophil pathway.

former being more likely on purely energetic grounds. It seemed appropriate to test the oxirane proposal by synthesis and this was subsequently undertaken at Harvard despite the discrepancy between Samuelsson's experimental observation of a single methanolysis product from interception of the unstable neutrophil intermediate and the more complex results expected on the basis of the oxirane structure.

Our first synthesis of the oxirane structure postulated for the metastable neutrophil product is outlined in figure 5. It was designed to produce a (racemic) mixture of *cis* and *trans* substituted oxiranes, but to be unambiguous with regard to the geometry of the 4 double bonds. It was our intention to separate the *cis* and *trans* oxiranes resulting from the synthesis, to assign structures from pmr data and to compare each pure isomer with the Samuelsson intermediate. The synthesis proceeded satisfactorily, as has already been described both in published form[13] and in a lecture presented at the 4th International Meeting on Prostaglandins in Washington D.C. on May 28, 1979. The *cis*- and *trans*-5,6-oxides of methyl eicosa-7,9-*trans*, 11,14-*cis*-pentaenoate were isolated and it was demonstrated that these could be saponified in aqueous base to the corresponding carboxylate ions (from which the starting methyl ester could be obtained with methyl sulfate). Unfortunately, the mixture of *cis* and *trans* epoxy esters could not be preparatively separated by TLC. A mixture of the two was

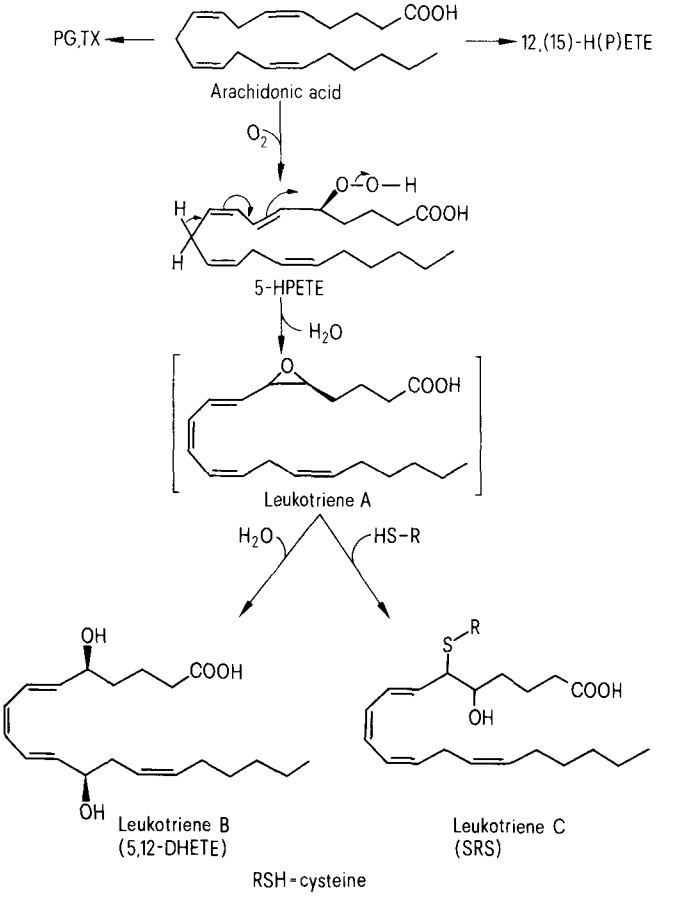


Figure 7. Structure of SRS - Samuelsson proposal (Murphy et al.[15]).

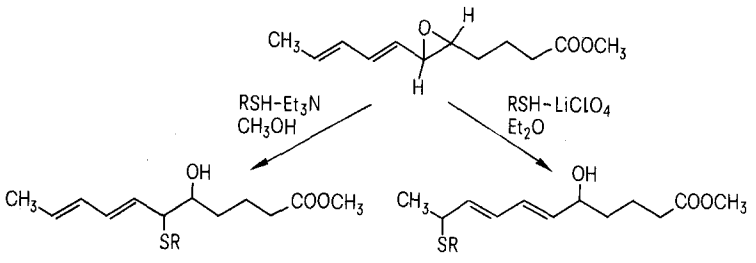


Figure 8. Position-selectivity in displacement of RSH.
R = $CH_2CH_2COOCH_3$
R = $CH_2CHCOOCH_3$ (N-TFA-cys Me)
$NHCOCF_3$
R = N-TFA-glutathione Me_2

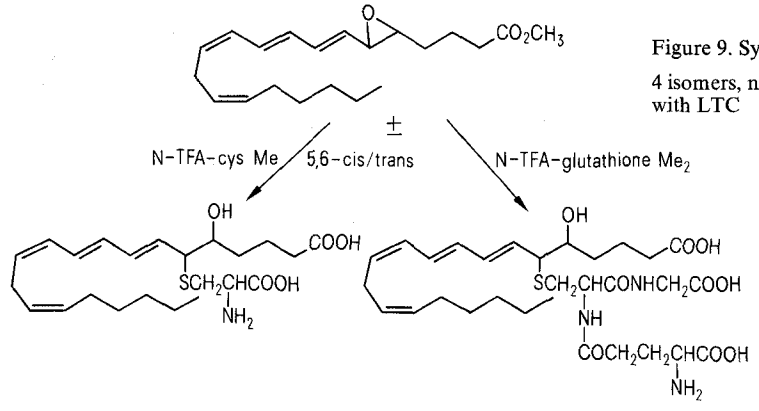


Figure 9. Synthesis of (±)-C_{20}-6-cys and (±)-C_{20}-6-glutathione.
4 isomers, none identical with LTC
4 isomers, 1 active and identical with LTC-1

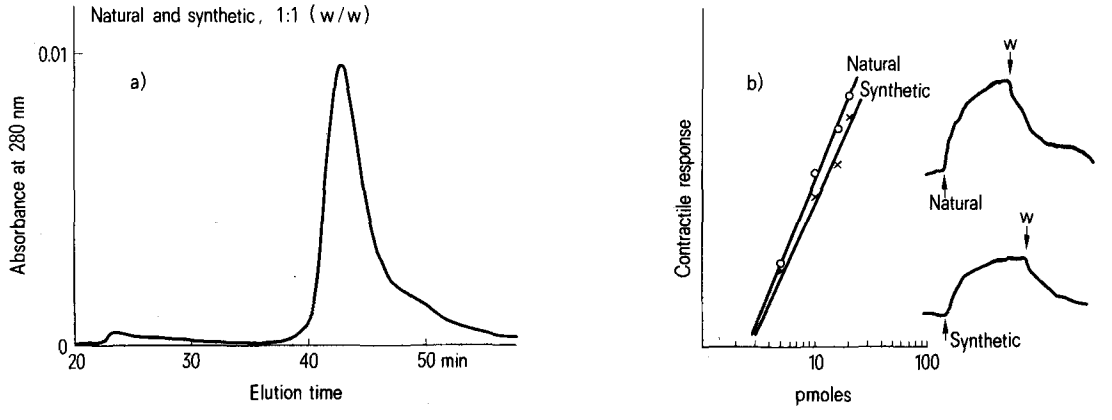


Figure 10. Bioassay on guinea-pig ileum of synthetic and natural leukotriene C-1. *a* and *b* Cochromatography of synthetic and natural leukotriene C-1 on reverse phase high pressure liquid chromatography (Nucleosil C_{18}, 5 µl; methanol/water 65:35 (v/v) + 0.01% acetic acid, 1 ml/min).
c UV spectra of synthetic and natural leukotriene C-1 before (t = 0 min) and after treatment with soybean lipoxygenase (t = 30 min). Spectra were recorded in Tyrode's buffer. (Hammarström et al.[16,17]).

therefore sent to Professor Samuelsson to enable comparison with his metastable intermediate.

In the meantime Borgeat and Samuelsson had completed the reinvestigation of the metastable intermediate from neutrophils using HPLC for analysis and had found that mixtures of several methoxy or dihydroxy compounds are indeed formed in quenching experiments using, respectively, methanol or aqueous acid quencher. In view of these results and others, the 5,6-oxirane formulation was accepted for the intermediate, and they reported the interpretation outlined in figure 6[14]. Comparative quenching experiments with the biosynthesized intermediate and our synthetic oxide showed essentially indistinguishable behavior; both were quite stable in alkaline solution (pH > 10) at 24 °C as expected.

Then a really dramatic development occurred in Stockholm. Professor Samuelsson and his colleagues had the inspired eea that perhaps this oxirane struc-

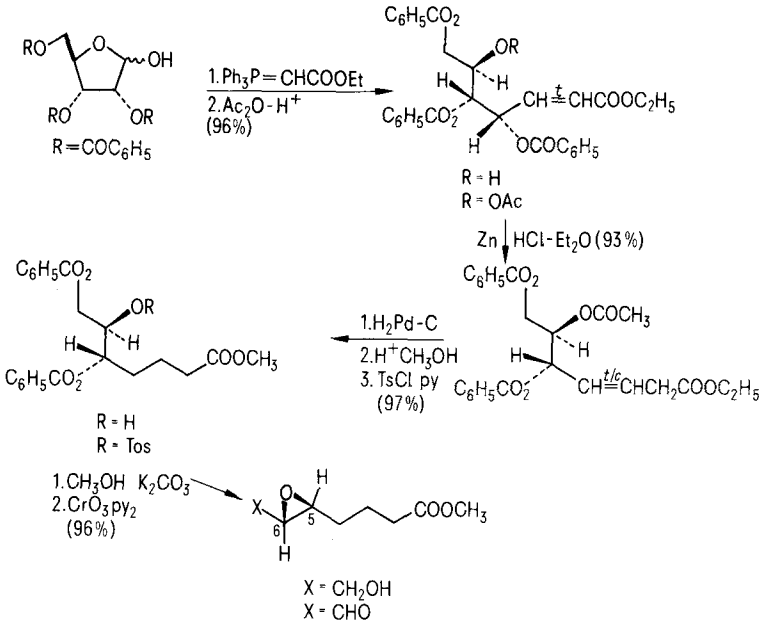


Figure 11. Stereospecific synthesis of leukotriene A and C-1 (Corey et al.[18]).

ture was an intermediate in the formation of the slow reacting substances. They speculated that one could understand the role of the sulfhydryl compounds such as cysteine to increase production of the slow reacting substances as a reaction of the oxirane intermediate, leukotriene A, as it came to be called, with a thiol group in a nucleophilic ring opening to give a sulfur-carbon bond, the resulting product being a conjugate of an amino acid with a fatty acid moiety. Based on this consideration they carried out an incubation of tritiated arachidonic acid with mastocytoma cells in the presence of ^{14}C-labeled cysteine and the ionophore A23187. They were able to separate from the mixture by HPLC a very low yield of material which contained both ^{14}C and tritium, which had the same UV absorption and reactivity toward soybean lipoxygenase as SRSs. Samuelsson and coworkers therefore proposed the scheme shown in figure 7 for the formation of the SRS from mastocytoma cells and this material as leukotriene C[15]. Because of the incorporation of radiocarbon label from cysteine, they suggested that a cysteinyl substituent was present in leukotriene C; this was placed at C-6 in view of the UV data (UV_{max} 280 nm) and the reactivity toward soybean lipoxygenase to form a conjugated tetraene[10,15].

Professor Samuelsson had informed us of his interesting experiment in April of 1979 and since we had synthetic (±)-leukotriene A in hand we embarked on the synthesis of the cysteine and other thiol conjugates so that synthetic and native compounds could be compared. In this way we hoped to establish the SRS structure in all detail (including stereochemistry) and to make adequate amounts of these hitherto rare and elusive agents available for study.

By the use of the readily available and simple model compound shown in figure 8 we were able to establish suitable reaction conditions for effecting the desired S_N2 displacement at the oxirane unit. In addition, other conditions were found for directing oxirane ring opening via an S_N1 pathway, which was found to afford a different structural isomer, as indicated in figure 8. The structures of the S_N2 and S_N1 products were rigorously established by physical measurements, especially PMR spectroscopy.

Using the S_N2 reaction conditions which were developed with the model compound (fig. 8), the synthetic racemic mixture of 5,6-*cis* and 5,6-*trans*-epoxides of methyl eicosa-7,9-*trans*-11,14-*cis*-pentaenoate was transformed into a mixture of 4 diastereomeric 6-*L*-cysteinyl thiol conjugates as shown in figure 9. The displacement was conducted using N-trifluoroacetyl-*L*-cysteine methyl ester in methanol containing excess triethylamine at 23 °C and the resulting material was deprotected by aqueous base (all under an argon atmosphere, since intermediates and products of these

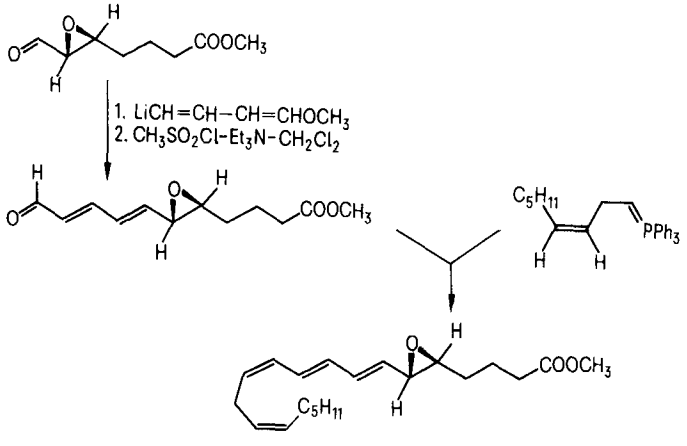


Figure 12. Stereospecific synthesis of leukotriene A and C-1 (Corey et al.[8]). $[\alpha]_D^{25}$ -21.9° UV max (MeOH) 269, 278, 289 nm (ε_{278} 40,000).

reactions were found to be extremely sensitive to air and free radical initiators). Reversed phase HPLC analysis revealed equal amounts of 4 diastereomeric compounds as expected on the basis of the starting material being a mixture of *(+)-cis* and *(+)-trans* epoxides. When these synthetic compounds were compared with the 2 SRSs isolated from mastocytoma cells in Samuelsson's laboratory, it was clear that they were different. In fact, the differences between the synthetic compounds and the native leukotrienes (C-1 and C-2) were so large by RP-HPLC that it seemed likely that a different (and more polar) cysteine-containing substituent was attached to the C_{20} chain in leukotriene C-1 and C-2. It transpired from comparisons performed in Stockholm that one of the 4 isomeric glutathione conjugates was identical with naturally derived leukotriene C-1 (LTC-1) as shown by UV absorption, RP-HPLC behavior, bioassay using guinea-pig ileum and reaction with lipoxygenase from soybean (see fig. 10)[16,17].

At the same time the comparison of synthetic and native LTC-1 was being made, we were completing a second synthetic route to leukotriene A. This one was designed to provide optically active *trans*-5,6-oxide with the 5-*(S)*-chirality. The scheme of synthesis[18] which is outlined in figure 11 and 12 is very effective and allows the direct production of chiral LTA[19]. Reaction of this synthetic LTA with glutathione in methanol containing triethylamine gave LTC-1 directly as the sole coupling product; it was identical

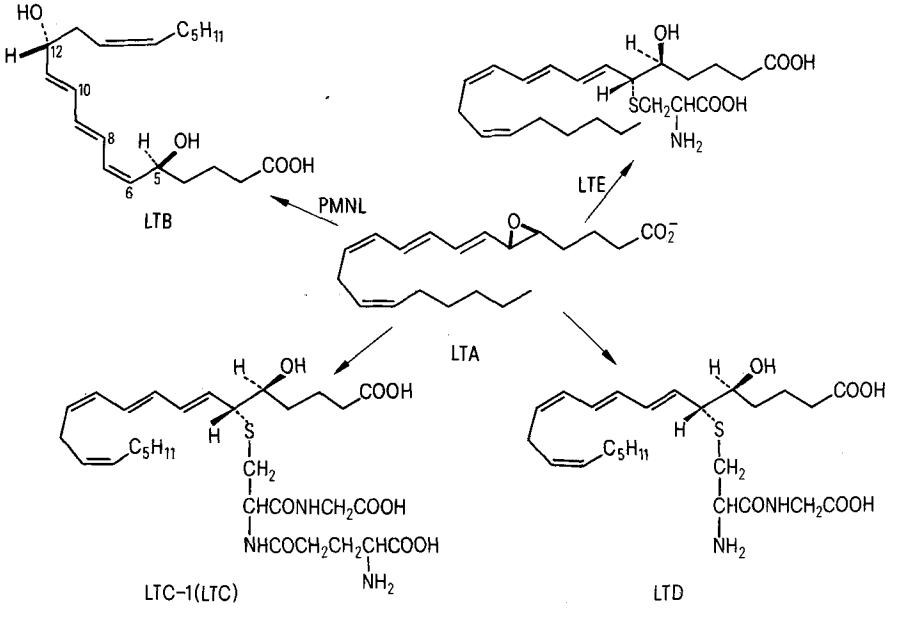


Figure 13. Stereospecific synthesis of Leukotrienes (Corey et al.[18,20,21]).

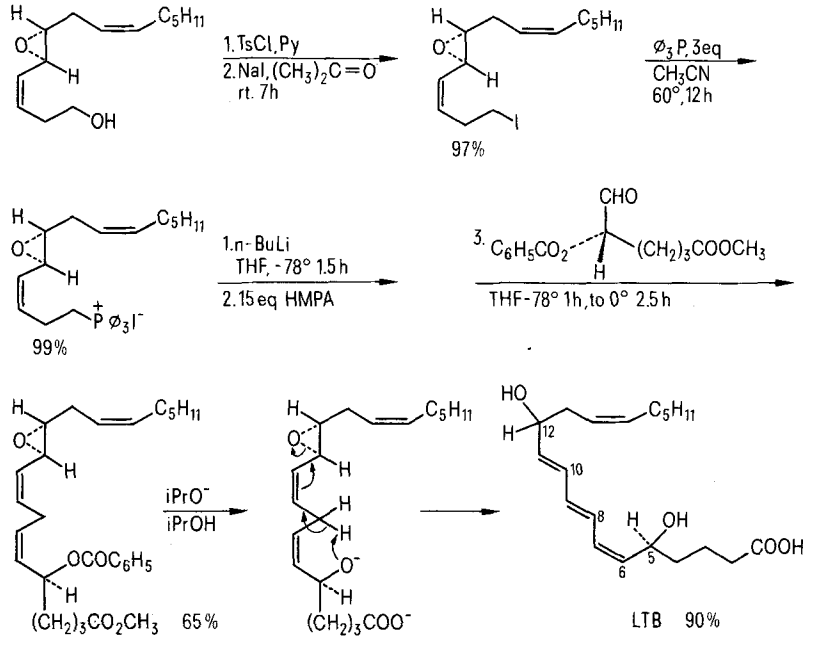


Figure 14a. Synthesis of LTB (Corey et al.[24]).

with naturally derived LTC-1. This synthetic LTA has also been converted to the cysteinyl conjugate (LTE) and the cysteinyl glycine conjugate (LTD). Both are biologically active and both have been shown to be identical with naturally occurring SRSs[20,21]. These interconversions are summarized in figure 13. Further, it was shown that synthetic LTA undergoes enzymic conversion to LTB, the 5,12-dihydroxyeicosanoid which Samuelsson originally isolated from polymorphonuclear leukocytes (PMNs). The correct structure for this 5,12-diol, now termed leukotriene B[23], which has recently been established in our labo-

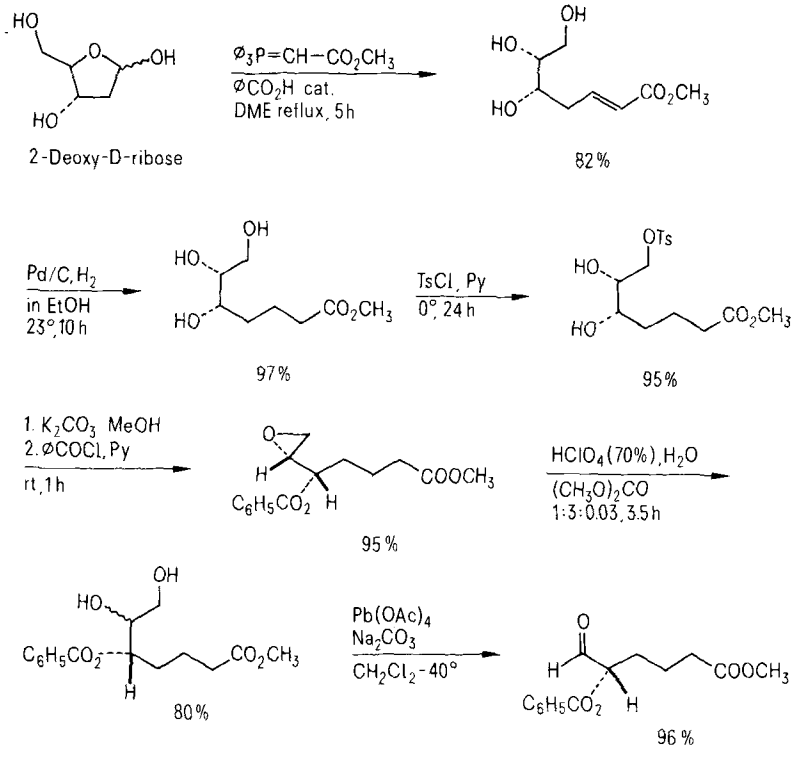


Figure 14b. Synthesis of LTB (Corey et al.[24]).

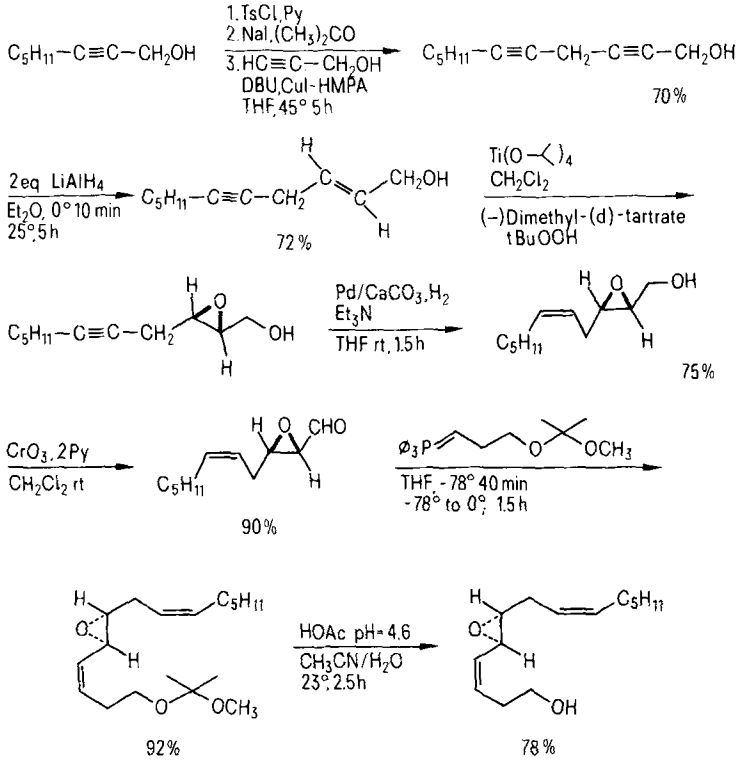


Figure 14c. Synthesis of LTB (Corey et al.[24]).

ratory[24], is shown in figure 13. Leukotriene A has also been isolated from human polymorphonuclear leukocytes and compared with synthetic LTA[25].

Leukotrienes C, D, and E can all be detected in the lung fluids of human asthmatic patients[5c]. Leukotriene D, which is more active than leukotriene C (by a factor of 6 on guinea-pig ileum and of approximately 100 on guinea-pig pulmonary parenchema), occurs in larger amount and, in fact, accounts for most of the activity. Leukotriene D is around 6000 times as active as histamine on a molar basis toward the small airways of guinea-pig lung. Leukotrienes C, D, and E produce their constrictive effects mainly on the small airways and from all indications are major mediators of asthma. Leukotriene C is transformed successively in tissue or serum into leukotrienes D and E. Synthesis of the last two actually predated isolation of the native LTs.

Leukotriene B, initially thought to be without biological activity, turns out to be powerfully bronchoconstricting. It is also a potent chemotactic agent for neutrophils and macrophages (at 1 ng/ml). It is likely that this substance too plays an important role in allergic and inflammatory states. Two syntheses of leukotriene B have been developed[23] which not only have made this substance available in quantity but which have also allowed clarification of the stereochemistry of the conjugated triene unit. An especially simple synthesis utilized as a key step the novel internal elimination depicted in figure 14a (bottom) which illustrates the terminal stage of the synthesis. The synthesis of the ester aldehyde corresponding to the C(1)–C(6) segment is shown in figure 14b and that of the C(7)–C(20) segment is outlined in figure 14c. The trihydroxy ester monotosylate intermediate shown in figure 14b can also serve as an intermediate in the synthesis of leukotriene A itself, being readily convertible by treatment with methoxide in methanol into the epoxy alcohol (fig. 11, bottom) precursor of LTA. Another route to the same epoxy alcohol which has been developed take advantage of the Sharpless method for asymmetric epoxidation of allylic alcohols[26]; this process for the synthesis of the key LTA precursor is outlined in figure 15.

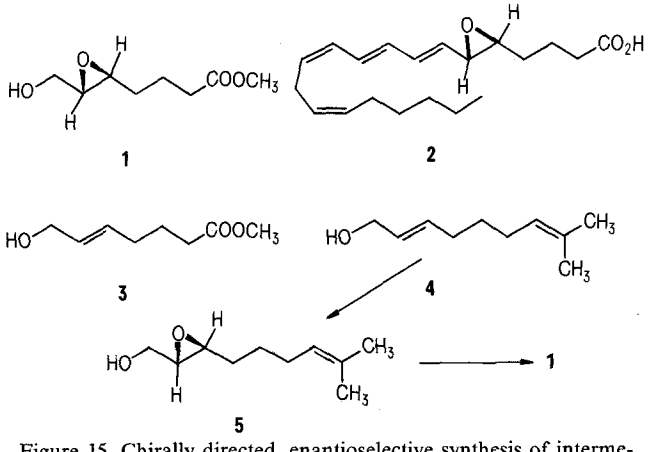


Figure 15. Chirally directed, enantioselective synthesis of intermediate (1) for LTA synthesis (Corey et al.[19]).

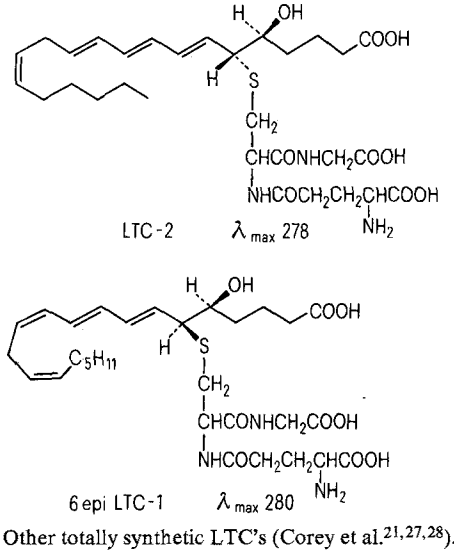


Figure 16. Other totally synthetic LTC's (Corey et al.[21,27,28]).

All the leukotrienes (A–E) are now readily available in pure form by synthesis, and in consequence, biological and medical research on the role of these agents in hypersensitivity, allergy, and inflammation is proceeding at a rapidly accelerating pace.

The establishment of identity of synthetic leukotrienes and native SRSs was far from trivial, mainly because only minute amounts of the naturally occurring SRSs could be obtained (in fact, all of our work at Harvard was done without the benefit of any native SRS and neither PMR nor IR spectra were available). In order to rule out certain stereochemical possibilities for the leukotrienes were therefore synthesized various isomers to compare with naturally derived material. Thus we identified LTC-2 as 11-*trans*-LTC[21,27] and we also showed that the synthetic 6-*epi*-LTC and LTD[28] showed less than 1% of the activity of LTC and LTD and did not correspond to any detectable native SRS material (fig. 16). The formation of 11-*trans*-LTC from LTC appears to occur with great facility in the presence of certain unidentified impurities which can promote the isomerization catalytically. It seems likely that this 11-*cis* → 11-*trans* conversion is a free radical process involving addition elimination of a thiyl (RS·) radical at C(12)[21]. Carefully purified LTC (RP-HPLC) does not readily undergo isomerization.

We now turn to another aspect of these studies, namely the synthesis of the predecessor of the leukotrienes, 5-*(S)*-hydroperoxyeicosa-*trans*-6-*cis*-8,11,14-tetraenoic acid (5-HPETE), and its conversion by a biomimetic process into leukotriene A. Outlined in figure 17a is the scheme successfully employed for the synthesis of (+)-5-HPETE and (+)-5HETE. This

approach has been adapted to the large scale synthesis of either 5-*(S)*- or 5-*(R)*-HETE[29,30] (figure 17b outlines the method of resolution).

An enzymic synthesis of 5-*(S)*-HPETE was also developed. Advantage was taken of the availability of synthetic 5-HETE adn 5-HPETE and a knowledge of their chromatographic behavior to test the ability of various plant lipoxygenases to convert arachidonic acid to 5-HPETE. The first system selected for study was the lipoxygenase of potato since this had been reported to afford an unusually high ratio of 9- to 13-hydroperoxides from linoleic acid. It was a fortunate choice because the potato lipoxygenase did indeed convert arachidonic acid to 5-*(S)*-HPETE![29]

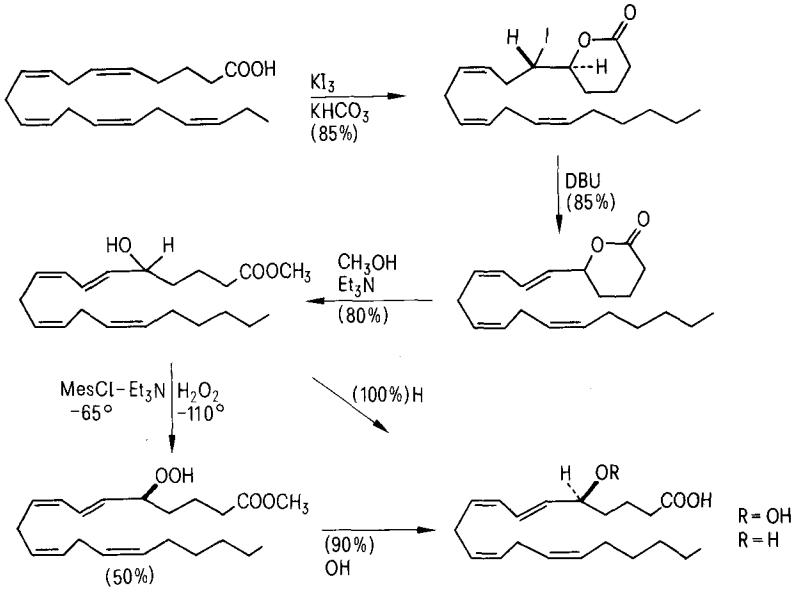


Figure 17a. Chemical synthesis of 5-HETE and 5-HPETE (Corey et al.[29,30]).

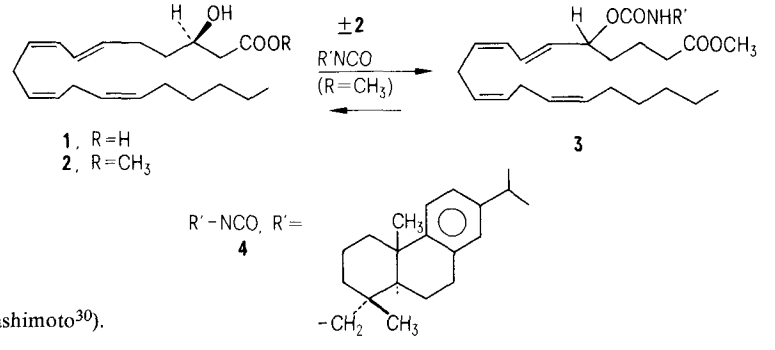


Figure 17b. Resolution of (+)-5-HETE (Corey and Hashimoto[30]).

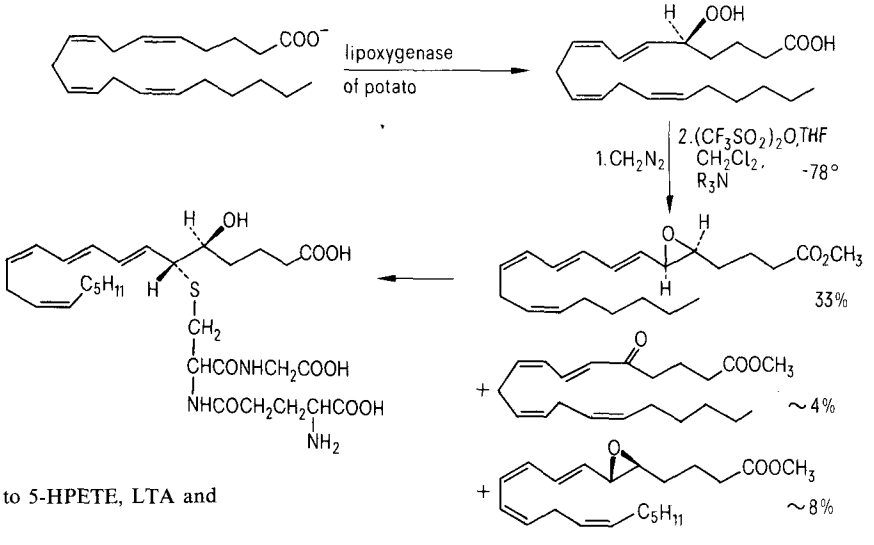


Figure 18. Conversion of arachidonic acid to 5-HPETE, LTA and LTC-1 (Corey et al.[31]).

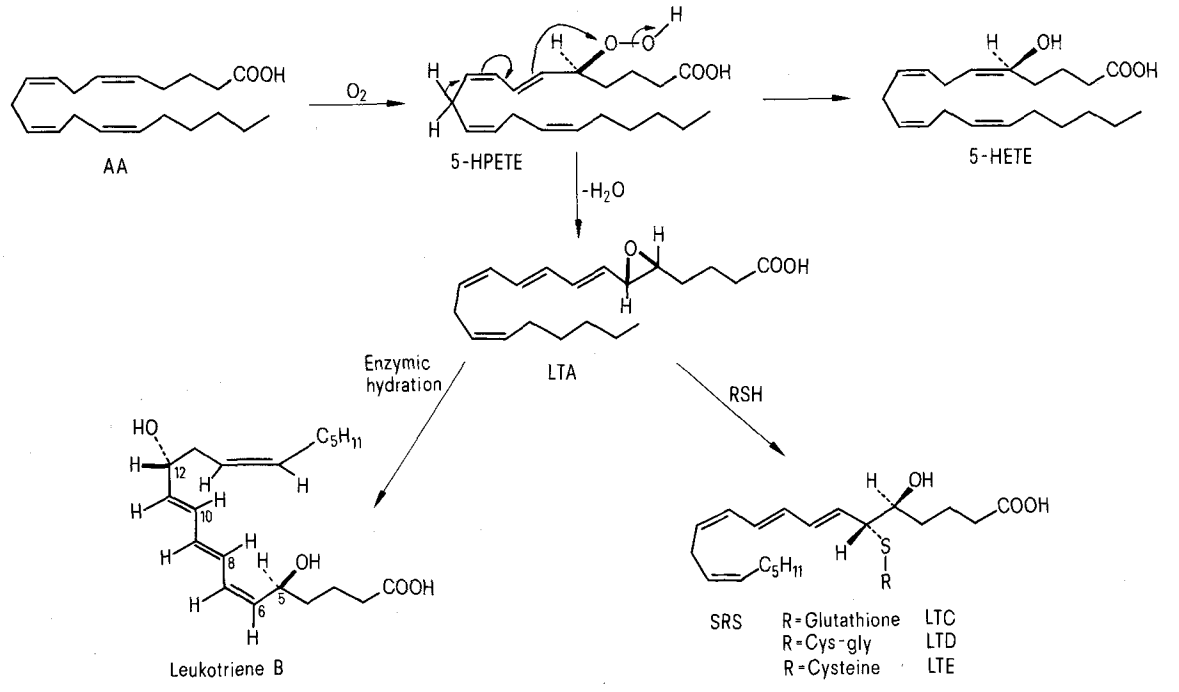


Figure 19. Biosynthesis of leukotrienes.

As shown in figure 18 it was also possible to transform 5-*(S)*-HPETE into LTA by a rational chemical process which followed an original mechanistic hypothesis[31]. Reaction of the LTA so produced with glutathione gave LTC identical in all respects with an authentic sample. This final synthetic conversion brings us full circle to the original concept for leukotriene A structure and biosynthesis from which all of the subsequent chemical work issued. Figure 19 summarizes current knowledge of leukotriene biosynthesis drom arachidonic acid.

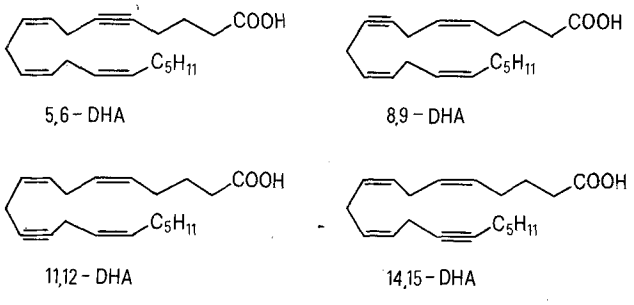


Figure 20. Four monoacetylenic dehydroarachodonic acids (DHA's).

The likely role of leukotrienes in disease states such as asthma, allergy, and inflammation dictates the desirability of finding agents which inhibit this biosynthetic pathway, particularly at the first step, the 5-lipoxygenase (5-LO) catalyzed conversion of arachidonic acid to 5-HPETE. Studies directed at this objective have already yielded promising results using a rational approach. It was conjectured that if an acetylenic dehydroarachidonic acid (DHA; figure 20 shows the 4 possible acetylenic DHAs) could serve as a substrate for a lipoxygenase, delivery of oxygen to the triple bond would result in an unstable vinylic hydroperoxide (estimated O-O bond dissociation enery around 13 kcal/mole) which could decompose to radicals before dissociation from the enzyme. Such homolysis could result in attack on the enzyme at the catalytic site and could cause inactivation. Such a process is outlined for soybean lipoxygenase, a 15-LO for arachidonic acid, with 14,15-DHA as the inactivator in figure 21. In figure 22 is shown a possible mechanism for the lipoxygenase raction and the specific oxidation which has been observed between soybean lipoxygenase, oxygen, and arachidonic acid. The soybean lipoxygenase, i.e., 15-lipoxygenase (15-LO) system was chosen for initial studies because

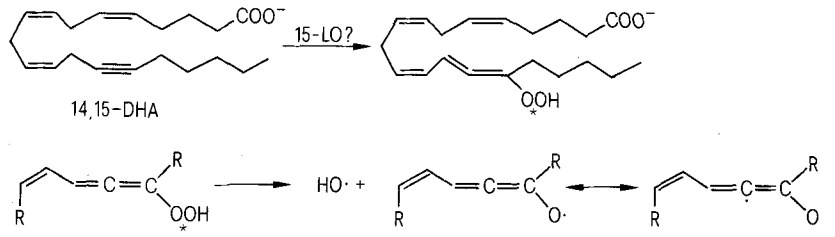


Figure 21. Development of an irreversible inhibitor of soybean lipoxygenase (15-LO). Is 14,15-DHA a substrate for 15-LO? Accelerated homolysis of a vinyl hydroperoxide. O-O Bond energy estimated at 13 kcal/mole (33-20).

of the ready availability, purity, and stability of this enzyme. The following results were obtained[32]:

1. 14,15-DHA irreversibly deactivates soybean 15-LO in a time-dependent manner in the presence of O_2 (but not in absence of O_2).
2. Complete deactivation of the 15-LO by 14,15-DHA occurs with an efficiency of 0.4% (260 14,15-DHA per enzyme molecule).
3. Upon deactivation of the 15-LO by tritiated 14,15-DHA 0.9 molecule of inhibitor becomes bound to the enzyme.
4. 5,6-DHA, 8,9-DHA, and 11,12-DHA + O_2 do not cause irreversible inhibition of the 15-LO even at 100 μM (14,15-DHA inhibition observed below 1 μM) and are not bound covalently to the enzyme.

Similar studies were also conducted with the prostaglandin synthetase (cyclooxygenase) from ram seminal vesicles. A summary of these results is as follows[33]:

1. 11,12-DHA irreversibly deactivates cyclooxygenase in a time-dependent manner in the presence of O_2 but not without O_2.
2. 14,15-DHA also irreversibly deactivates cyclooxygenase, but is much less effective than 11,12-DHA.
3. 5,6-DHA and 8,9-DHA behave like weak competitive inhibitors (substrate analogs); 5,6-DHA serves as a substrate undergoing conversion to 5,6-dehydro PGE_2.
5. In the absence of hematin, a necessary cofactor for PG biosynthesis, the PG cyclooxygenase is *not* inactivated by 11,12-DHA *and* O_2.

Finally, preliminary studies have been made with an unpurified preparation of 5-lipoxygenase from rat basophilic leukemia cells which have produced the following observations[33]:

a) Formation of 5-HETE, LTA, and LTB inhibited by preincubation of the 5-LO from rat basophilic leukemia cells with 5,6-DHA and O_2 in a time-dependent irreversible manner.

b) Inhibition of 5-LO by 5,6-DHA requires not only O_2 but also Ca^{++}. Note conversion of 5-HPETE from arachidonate requires O_2 and Ca^{++}.

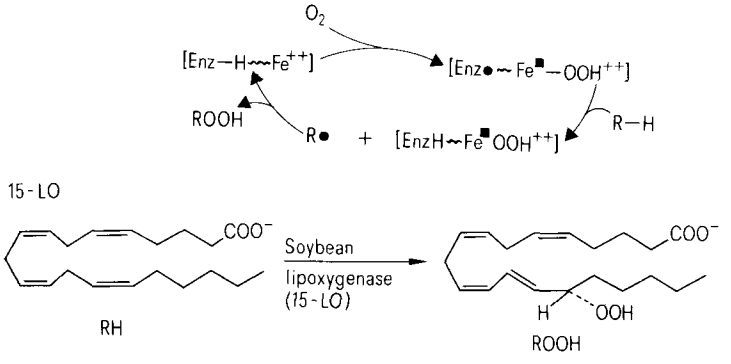
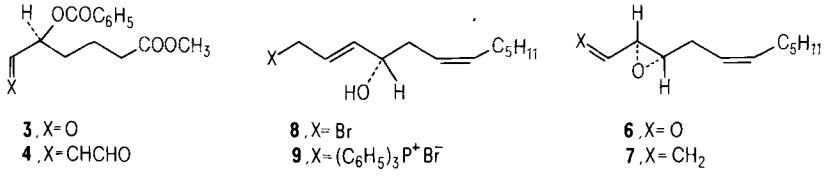


Figure 22. Inhibition of lipoxygenase (LO) reactions.



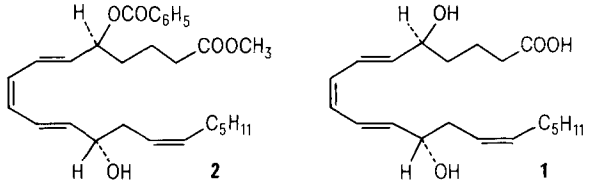


Figure 23. Total synthesis of 5S,12S-DiHETE (12-*epi*-6-*trans*-8-*cis*-LTB) 5S-HETE $\xrightarrow{\text{platelets}}$ 5S,12S-DiHETE $\xleftarrow{\text{PMNS}}$ 12S-HETE 14,15-oxido arachidonic → 14,15-oxido-(±)-12-HETE → (±)-5,12-DiHETE (Corey et al.[36]).

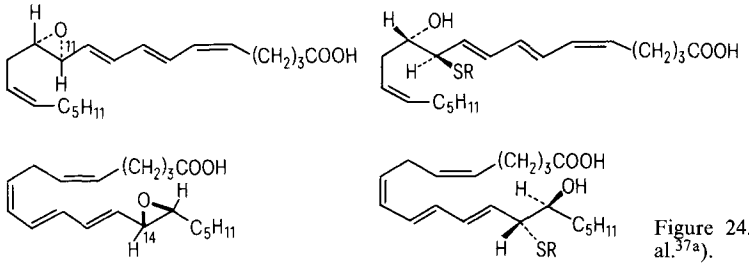


Figure 24. Biosynthetic analogs of LTA, LTC, LTD (Corey et al.[37a]).

It is concluded from these studies[32,33] that replacement of a *cis* HC=CH unit of arachidonic acid by a C≡C unit at the site of lipoxygenation leads to a dehydroarachidonic acid which effectively deactivates the enzyme in the presence of oxygen in a time-dependent irreversible way. Clearly 5,6-DHA effectively blocks the 1st step of the leukotriene pathway.

The 4 monoacetylenic DHAs shown in figure 22 are accessible by efficient synthetic routes[32-34]. Studies on the use of 5,6-DHA in biological systems are in progress. Eicosa-5,8,11,14-tetraynoic acid, a long known competitive inhibitor of the prostaglandin synthetase, does not appear to block the leukotriene pathway.

It has recently been found that a biologically active isomer of LTB is formed from arachidonic acid upon incubation with mixed peripheral blood leukocytes[35]. The proposal that this substance is the product of successive 5- and 12-lipoxygenation reactions, i.e., is a 5-*(S)*,12-*(S)*-diHETE, has been confirmed by the unambiguous total synthesis of the same compound (figure 23, compound **1**) by way of intermediates **2** to **7** in figure 23.[36] The biological role of 5-*(S)*,12-*(S)*-diHETE is not known, but it is of great interest that this substance can antagonize the action of LTB on neutrophils.

In 1980 it was pointed out that in principle compounds analogous to leukotriene A could be formed from 8-, 12-, or 15-HPETE by a mechanism paralleling the biosynthesis of LTA from 5-HPETE[37]. The 3 possible isomers of LTA, designated EPETEs, were synthesized[31b,37] and converted to conjugates with glutathione which are isomeric with leukotriene C; figure 24 displays the structures of 11,12-EPETE and 14,15-EPETE and the corresponding peptide conjugates (RS = glutathionyl). The glutathione conjugates were found to be biologically inactive ($< 10^{-3}$) relative to leukotriene C[38]. Subsequent to this work the formation of hydration products[39] and also a glutathione conjugate[40] of 14,15-EPETE have been observed upon incubation of 15-*(S)*-HPETE with human leukocytes. The biological significance of these findings is unclear, though it shoud be mentioned in this context that 15-HETE and 5,15-di-HETE both appear to inhibit enzymic synthesis of 5-HPETE from arachidonic acid[41]. The 15-EPETE pathway appears to be dominant in the case of eosinophiles, at least for porcine eos[42]. It seems likely that future research in this area will yield exciting new information.

The slow reacting substances LTC, LTD, and LTE have been termed 'mediators in the regulation of the microenvironment' of cells or 'hormones of the microenvironment' and are thought to play a role in maintaining local homeostasis[5c]. They occur in exceedingly low concentrations under physiological conditions and, as a consequence, the study of their regulatory action requires highly sensitive methods of chemical analysis. Toward this end radioimmuno assays have been developed which allow analysis for LTC or for LTC, LTD, and LTE as a group[43], or specifically for LTB. Suitable immunogens were obtained by the chemical coupling of synthetic leukotrienes via the C(1) carboxyl or C(5) hydroxyl to a fraction of the free amino functions of bovine serum albumin[43]. One important advance which has resulted from the availability of the leukotriene radioimmuno assay has been the discovery of a new class of bone marrow-derived mast cells of unique biochemistry. These cells generate large amounts of leukotrienes relative to prostaglandins[44].

The development of antagonists of the leukotrienes (anti-LTs) could be of considerable medical value. For this reason and also to explore the structural factors within the leukotrienes which are essential to the expression of biological activity, a substantial number of carefully chosen analogs of the leukotrienes has been synthesized. Some of the data which have been obtained are shown in tables 4 and 5[38,45]. From these and more recent results a number of important conclusions have emerged with regard to the structural features which are crucial for biological activity. The crucial structural elements include the following: a) lipophilic chain attached to C(6); b) 5-hydroxyl group; c) a single ionized carboxyl, either C(1) or gly. The sulfur atom need not be divalent. Considerable substitution can be tolerated in the peptide moiety of LTD; however, in the case of LTC the free α-amino carboxylic unit is necessary. Although some progress has been made in the development of an anti-LT, the goal of achieving high potency is still elusive.

1 Acknowledgment. It is a pleasure to express admiration, appreciation, and gratitude to each of the collaborators whose names appear in the references to this article. The research has yielded great personal satisfaction as well as scientific excitement. - We are grateful to the U.S. National Science Foundation and the National Institutes of Health for financial support of the research program on leukotrienes at Harvard.

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The biology of schizophrenia*

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For a disease with a life-time prevalence of approximately 1% in contemporary cultures, descriptions of schizophrenia in the past are surprisingly few. One of the earliest probable cases was described by Willis[1] in 1683; accounts of the onset of a progressive personality deterioration in early adult life were offered by Haslam[2] and Pinel[3] in 1809. In the late 19th century the clinical picture of the organic dementias, including general paralysis of the insane, was recognized and interest quickened in the description and classification of the psychoses. From the work of Emil Kraepelin[4] and Eugen Bleuler[5], in the first decade of this century, the concept of the functional psychoses arose; it was held that schizophrenia and manic-depressive psychosis could be distinguished, on psychological grounds, from the organic psychoses.

Thus of 'dementia praecox' Kraepelin[4] wrote 'perception is not usually lessened' (p. 5), 'orientation is not usually disordered' and 'memory is comparatively little affected. The patients are able, when they like, to give a correct detailed account of their past life, and often know accurately to a day how long they have been in the institution' (p. 18). While Kraepelin qualified this view with the reservation that in some terminal states there might be a 'general decay of mental efficiency' and that patients might 'become impoverished in thought, monotonous in their mental activities', Bleuler[5], who refined the concept to the 'group of schizophrenias', was less equivoval: 'In contrast to the organic psychoses we find in schizophrenia ... that sensation, memory, consciousness and motility, are not directly disturbed' and 'memory as

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