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Inhibitors of the lipoxygenase conversion of polyenoic acids

D A Zabolotskii, G I Myagkova, R P Evstigneeva
Lomonosov Moscow Institute of Fine Chemical Technology

ABSTRACT. In this reivew lipoxygenase inhibitors are classified and their inhibitory activity, mode of action, specificity, and chemical synthesis are examined. The future prospects for using them to study enzymatic conversions and the development of antiallergic and antiinflammatory drugs from them are discussed.

The bibliography includes 134 references.

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I. Introduction

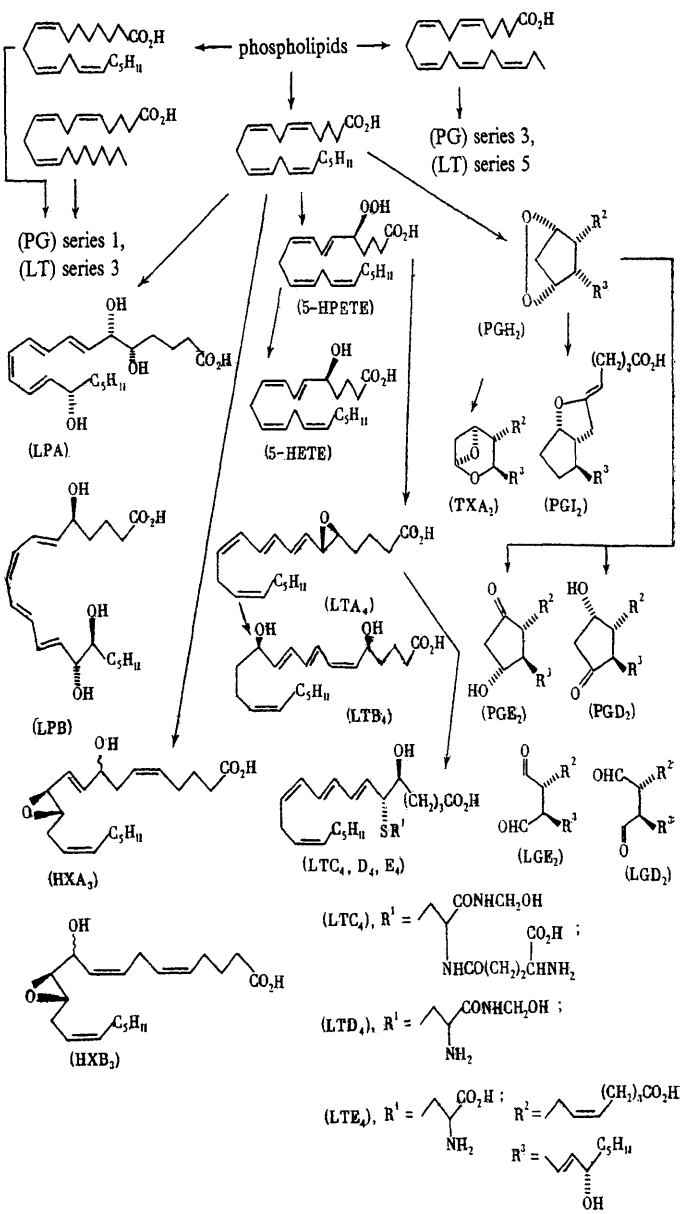
Enzymatic oxidation of eicosapolyenoic acids—arachidonic (5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic), dihomo-γ-linolenic (8*Z*,11*Z*,14*Z*-eicosatrienoic), and timnodonic (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-eicosapentaenoic) acids—is of considerable interest for a number of reasons.

It forms the basis for the biosynthesis of a whole series of biologically active metabolites, such as prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), lipoxins (LP), hepoxylins (HX), various hydroxy acids (HETE), etc. Moreover, this key reaction of lipid metabolism is interesting from the point of view of its mechanism, which appears to be unique and having no known analogues.

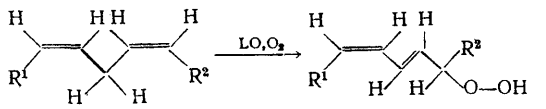
At the present time there are two enzymatic conversion routes of polyunsaturated acids that have been studied to a considerable extent. These are the cyclooxygenase route, in which the biosynthesis of prostaglandins, thromboxanes, prostacyclin (PGI_2), and also levuglandins (LG) takes place through the action of polyenzymatic complexes, and the lipoxygenase route, which results in the formation of acyclic metabolites such as leukotrienes and a number of hydroxy acids, including lipoxins, etc. (Scheme 1).

The mechanism of cyclooxygenase oxidation and cyclooxygenase metabolites have been studied and described in detail in the literature.[1–4] At the same time an active research programme continues on the lipoxygenase metabolism of polyenoic acids, in which the inhibitors of this process play a key role. In the present review the main consideration is given to inhibitors of the biosynthesis of lipoxygenase metabolites and their antagonists in relation to their biological activity and chemical synthesis.

Scheme 1



Lipoxygenases (LO) constitute a group of enzymes containing non-haeme iron that are widely distributed in animals and plants and which catalyse the oxidation of the 1*Z*,4*Z*-pentadiene structure in polyunsaturated fatty acids to a 1-hydroperoxy-2*E*,4*Z*-pentadiene structure:



Lipoxygenase oxidation of polyenoic acids can in principle occur at $2n-2$ carbon positions, where n is the number of *Z*-methylene-separated double bonds. The lipoxygenases known at the present time include 5-LO, which takes part in the biosynthesis of leukotrienes (leucocytes, potato), 8-LO (corals, soybeans), 12-LO (platelets, RBL, PMNL)† and 15-LO (reticulocytes, endothelial cells, soya beans).[5,6]

The interest in lipoxygenase metabolites arises because they play a key role in allergic and inflammatory processes. Thus, peptidoleukotrienes (LTC_4, LTD_4, LTE_4) are powerful stimulators of the contraction of smooth muscle, being 10^2-10^3 times more active than histamine.[7] Leukotriene B_4 is an active chemically toxic agent for polymorphonuclear leucocytes, which also causes an increase in vascular permeability and assists the migration of cells and plasma into extravascular space.[7] An important physiological role is apparently played by lipoxins, which stimulate the elimination of the superoxide anion and regulate the immune activity of lymphocytes[8,9] and by hepoxylins, which are potential natural regulators of insulin release.[10]

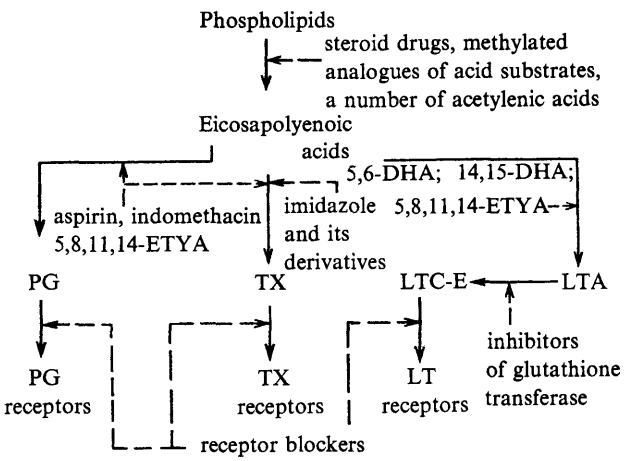
Research into lipoxygenase oxidation of polyenoic acids will contribute to a better understanding of the physiological role of acyclic eicosanoids and the pathogenesis of the disorders caused by them. A promising approach to the resolution of this problem is the use of inhibitors of the enzymatic conversion of acid substrates in order to study its mechanism. The search for new inhibitors of enzymes in the arichidonic acid cascade is also related to the development of antiallergic and antiinflammatory drugs from them and is therefore of direct practical interest.

II. Inhibition of enzymatic oxidative conversion of polyenoic acids

The release and enzymatic conversion of the precursor acids to their metabolites and the occurrence of biological activity of the latter constitute a complex, interrelated multistage process, which can be controlled at different points (Scheme 2).

† RBL = rat basophile leukaemia cells; PMNL = polymorphonuclear leucocytes.

Scheme 2



DHA = dehydroarachidonic acid;
ETYA = eicosatetraynoic acid.

Thus, steroid drugs block the release of eicosapolyenoic acids by inhibiting phospholipase A_2, hence preventing any further stage of their enzymatic conversions.[11,12] Well-known inhibitors of the biosynthesis of cyclic eicosanoids are aspirin and indomethacin.[13,14] Disruption of the balance of acid substrates by these compounds results in an increase in the synthesis of leukotrienes.[15] Imidazole and its derivatives have the ability to suppress selectively the synthesis of TXA_2.[16-18] Inhibitors of the enzymatic conversion of polyunsaturated acids that are of considerable interest are compounds which are close to them in structure, including their acetylene analogues[19-31] and acids modified in various ways on the methylene groups[32-34] and carbon skeleton.[35-39]

The regulation of the biological effects caused by eicosanoids can also be achieved through the use of receptor blocking agents such as analogues of PG, TX, LT, and other metabolites,[40-43] which act as their antagonists.

However, Scheme 2 is far from complete in its representation of the problem of pharmacological regulation of the pathway of arachidonic and other eicosapolyenoic acids. Thus, we have a series of enzymatic reactions in which the analogue of a substrate of one enzyme is at the same time the analogue of the product of another enzyme. Therefore, the same compound can act as an inhibitor of different enzymes in the pathway, exhibiting a variable specificity and activity.

As the enzymatic conversion of acid substrates and their metabolites is, in effect, oxidation with oxygen, many antioxidants that act as its inhibitors have been reported.[44-46]

Compounds which are capable of forming chelate complexes with the iron that forms part of the active site of lipoxygenases constitute an important group.[47-49]

Many nitrogen-containing compounds, such as benzene derivatives with nitrogen-containing substituents, nitrogen monocyclic and condensed heterocycles are also known to be inhibitors of enzymes of the arachidonic acid pathway.[50-52]

Thus, at the present time many inhibitors of the oxidation of eicosapolyunsaturated acids have been obtained with different structures and acting at different points of the enzyme pathway,

from the release of acid precursors to metabolic reactions of the eicosanoids themselves.

Lipoxygenase inhibitors are valuable agents used to study the functioning of these enzymes, to establish their substrate specificity, the structure of the active site, the nature of the catalytic act, etc. In turn, an understanding of these processes makes it possible to design effectively the structures of compounds which could be used as potential antiallergic and antiinflammatory drugs.

III. Different classes of lipoxygenase inhibitors

1. Analogues of polyenoic acid substrates

An important class of inhibitors of the enzymatic oxidation of natural eicosapolyenoic acids is made up by compounds that are similar to them in structure. These analogues of acid substrates can bind to the active site of the enzyme, but they either cannot be converted to biologically active metabolites (because of particular structural features), resulting in competitive inhibition, or they deactivate the active site of the enzyme, eliminating its capacity to convert natural substrates. At the present time various eicosapolyunsaturated compounds, acids containing triple or cumulated double bonds,[53,54] with methylene groups replaced by heteroatoms (sulphur),[32,33] or modified in a particular way on the carbon skeleton,[35–39] have been synthesised and studied as lipoxygenase inhibitors.

Table 1. Acetylene and allene analogues of acid substrates.

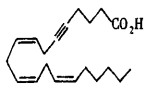
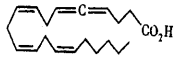
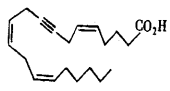
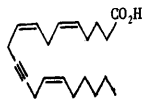
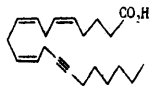
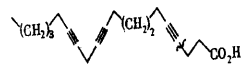
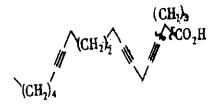
Name	Structural formula	Inhibitory activity	Refs.
5,8,11,14-eicosatetraynoic acid (5,8,11,14-ETYA) (I)		Cyclooxygenase (CO), 5-LO, 12-LO 15-LO	[19—24]
4,7,10,13-eicosatetraynoic acid (4,7,10,13-ETYA) (II)		12-LO	[24, 25]
5,6-dehydroarachidonic acid (5,6-DHA) (III)		5-LO	[53, 54]
4,5-dehydroarachidonic acid (4,5-DHA) (IV)		5-LO	[53, 54]
8,9-dehydroarachidonic acid (8,9-DHA) (V)		weak CO inhibitor (IC_{50} = 50 μM)	[28, 29]
11,12-dehydroarachidonic acid (11,12-DHA) (VI)		CO	[28, 29]
14,15-dehydroarachidonic acid (14,15-DHA) (VII)		15-LO, CO	[28, 29]

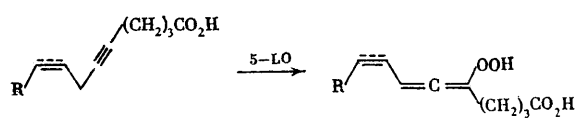
Table 1 (cont'd).

Name	Structural formula	Inhibitory activity	Refs.
5,11,14-eicosatriynoic acid (VIII)		12-LO	[30]
5,8,14-eicosatriynoic acid (IX)		12-LO	[30]
5,8,11,14-heneicosatetraynoic acid ($C_{21:4}$)** (X)		12-LO	[24]
4,7,10,13-heneicosatetraynoic acid ($C_{21:4}$) (XI)		glutathione transferase	[31]
4,7,10,13-nonadecatetraynoic acid ($C_{19:4}$) (XII)		glutathione transferase	[31]
6,9,12,15-docosatetraynoic acid ($C_{22:4}$) (XIII)		12-LO	[25]
7,10,13,16-docosatetraynoic acid ($C_{22:4}$) (XIV)		12-LO	[25]

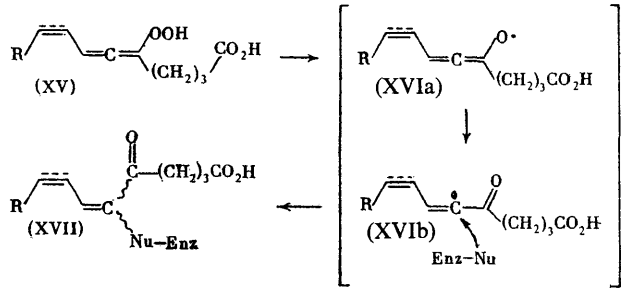
* IC_{50} is concentration of inhibitor producing a 50% loss of enzymatic activity.

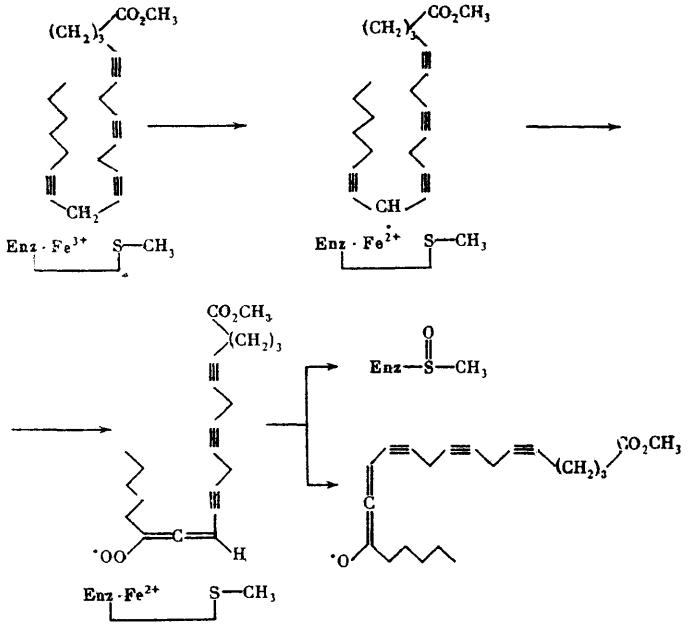
** In $C_{n:m}$ n denotes the number of carbon atoms and m the number of multiple (triple) bonds.

A considerable amount of experimental material on the ability of acetylenic and allenic acids to inhibit enzymes of the arachidonic acid pathway has been collected. The first reports about the inhibitory activity of 5,8,11,14-eicosatetraynoic acid—the acetylene analogue of arachidonic acid—were published in the 1960s.[13] During the following years a considerable amount of work was carried out by different groups of research workers on the synthesis of similar acetylenic fatty acids, some of which as was demonstrated, proved to be selective inhibitors of different lipoxygenases (Table 1).[19–31] Many authors studied the mechanism of inhibition of 5-lipoxygenase by acetylenic acids (5,6-DHA, etc.),[26] which involves the formation of an allenic hydroperoxide as an intermediate:



The unstable allenic hydroperoxide resulting from oxidation of the inhibitor decomposes to form free radicals which irreversibly deactivate the active site of the enzyme. However, different opinions about the exact nature of this step have been advanced. Thus, some authors[7,55,56] maintain that homolysis of the intermediate (XV) results in an active radical product which is attacked by a nucleophile of the active site of 5-lipoxygenase to form a covalent acid–enzyme bond.





Thus, Rapoport et al.[21] regard the deactivation of 15-LO by 5,8,11,14-ETYA as a process that is completely analogous to the deactivation of this enzyme by its reaction product—the hydroperoxy acid—but with the important distinction that deactivation with the acetylenic acid results from each act of substrate conversion.

It should be pointed out that both schemes put forward are based on the formation of the vinyl hydroperoxide (XV), which accords well with the fact that 4,5-dehydroarachidonic acid (4,5-DHA)—the allenic isomer of 5,6-DHA—is a powerful inhibitor of 5-LO.[53,54]

Deactivation of lipoxygenases with acetylenic and allenic acids depends on the time of incubation with the inhibitor and is irreversible, since neither strong dilution of the given system nor subsequent addition of excess substrate (50:1) could regenerate the activity of the enzyme.[21]

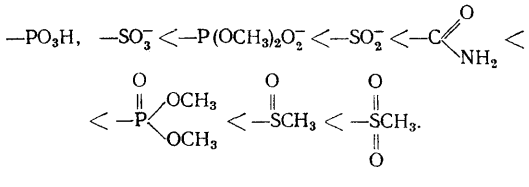
The effect of acetylene analogues of natural fatty acids as inhibitors and their specificity are determined by the number and position of double bonds. In spite of the obvious differences in the steric structure of acid substrates and the polyynoic acids that correspond to them,[50] replacement of particular double bonds leads, as a rule, to inhibition of the enzymatic conversion precisely at those positions. Thus, an obvious disadvantage of 5,8,11,14-ETYA (I) is its lack of specificity since it deactivates both cyclooxygenase and a number of lipoxygenases to approximately the same degree.[30]

At the present time a number of tetraynoic acids have been synthesised with a different configuration of triple bonds to that in natural acids and a different number of carbon atoms, and they exhibit considerable selectivity.[24,25] Thus 4,7,10,13-eicosatetraynoic acid (II) and 5,8,11,14-heneicosatetraynoic acid (X) inhibit the 12-lipoxygenase oxidation of arachidonic acid to 12-HPETE without any significant effect on the cyclooxygenase oxidation to TXB_2 in human platelets.[24] The IC_{50} values relating to 12-lipoxygenase and cycloxygenase were determined experimentally for 4,7,10,13-ETYA, being 7.8 μM and 100 μM respectively.[25] Evidently acids (II) and (X) can serve as selective modulators of 12-lipoxygenase oxidation in platelets and other tissues.

Other polyynoic acids have also been synthesised, in particular 6,9,12,15-docosatetraynoic acid (XIII) and 7,10,13,16-docosatetraynoic acid (XIV), which fairly selectively inhibit 12-LO (IC_{50} CO/IC_{50} LO is 336.8 for (XIII) and 333.3 for (XIV).[25] It is not excluded, however, that the mechanism of the action of acetylenic acids (II), (X), (XIII), and (XIV) is different to that for 5,8,11,14-ETYA. Thus, according to the results of Corey,[31] 4,7,10,13-heneicosatetraynoic acid (XI) and 4,7,10,13-nonadecatetraynoic acid (XII) block the formation of SRS (slow reacting substance) by inhibiting glutathione transferase, namely, at a later stage of biosynthesis.

Thus, these acetylenic and allenic inhibitors act as irreversible inhibitors of the enzymatic conversion of natural polyenoic acids to their metabolites, since they act as suicide substrates, the oxygenation of which is related to an irreversible loss of enzymatic activity.

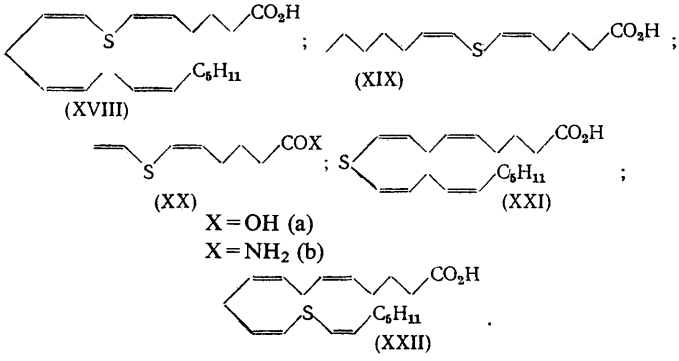
Polyunsaturated acids can be incorporated into the phospholipids of cell membranes, which considerably reduces their activity as inhibitors of lipoxygenase oxidation *in vivo* since phospholipids are not lipoxygenase substrates. A number of analogues of 5,6-DHA (III) containing functional groups other than a carboxyl group have been obtained. The rate of deactivation of 5-LO during aerobic incubation increased in this case according to the following series:



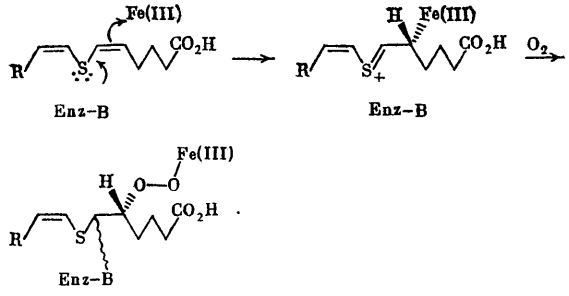
In the opinion of Corey et al.,[26] the inhibition of 5-LO by these 5,6-DHA derivatives indicates that the presence of an anionic group such as carboxyl is not absolutely essential for the binding of acid with enzyme.

Since the mechanism of the catalytic action of lipoxygenases involves the elimination of a hydrogen atom from a bis-allyl methylene group [C(7), C(10), C(13) for arachidonic acid] as the first stage, modification of these positions, making this stage of enzymatic catalysis impossible, opens up extensive possibilities for the development of competitive and non-competitive inhibitors that have the structure of acid substrates with modified methylene groups. This type of modification can be achieved either through the introduction of heteroatoms (sulphur)[32,33] at this position or by replacement of the hydrogen atoms that are prone to elimination with different substituents,[21] and also by converting them into part of ring systems.[38,39,57,58]

In 1985 a series of polyenoic acids containing sulphur atoms at the positions of primary attack by an enzyme of physiological substrates was synthesised by Corey et al.[31–33] and studied as potential inhibitors. It was shown that incubation of 5-LO from RBL-1 with compounds (XVIII)–(XX) leads to the irreversible deactivation of the enzyme, with deactivation showing a dependence on time.



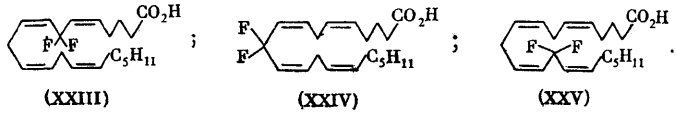
A mechanism for the inhibition of 5-LO by acids (XVIII)–(XX) has been put forward.[32] These compounds do not contain a proton that can be eliminated at the 7-position, but the electron-donating capacity of the thiadivinyl residue, according to Corey et al.,[32] is sufficiently high for direct electron transfer to Fe(III) on the active site of the enzyme to be able to occur, and oxidation can subsequently take place:



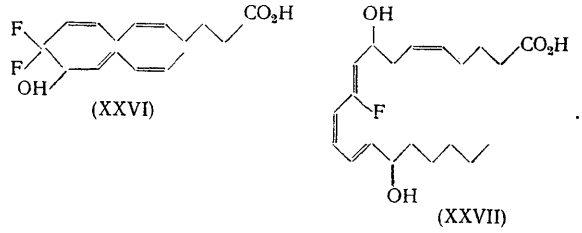
The intermediate formed by oxidation is attacked by the proton-acceptor B^- in the active site, resulting in covalent bonding of the inhibitor[32,33] and is the cause of the irreversible deactivation of the enzyme. The effect of thia-acids (XVIII)–(XX) on cyclooxygenase (isolated from rat testes) was also studied, from which acids (XVIII)–(XX) exhibited properties of competitive inhibitors.[32]

Corey et al.[33] also described the syntheses by 10-thiaarachidonic acid (XXI) and 13-thiaarachidonic acid (XXII). In accordance with the proposed mechanism, it would be reasonable to expect that 13-thia-acid (XXII) would be an irreversible inhibitor of 15-LO. This assumption was confirmed experimentally for 15-LO,[31] which is also fully consistent with the mechanism and substrate specificity of the enzyme.

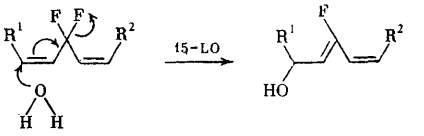
The difluoro analogues of arachidonic acid (XXIII)–(XXV) were also studied as inhibitors that are modified on the bis-allyl methylene group:[34,59]



It would be logical to expect from the similarity of the van der Waals radii of hydrogen and fluorine that compounds (XXIII)–(XXV) would be either substrates or (because of the inability of the enzyme to cause homolytic cleavage of the C–F bond) specific competitive inhibitors of different LOs. 10,10-Difluoroarachidonic acid (XXIV) was studied as a substrate of cyclooxygenase and 15-lipoxygenase of soybeans.[59] In the first case acid (XXIV) was converted to 10,10-difluoro-11-HETE (XXVI) and 10-fluoro-8*S*,15*S*-di-HETE (XXVII). Cyclisation to a PG did not occur. Incubation of the same acid with soybean 15-LO resulted in the formation of 10-fluoro-8*S*,15*S*-di-HETE (XXVII):



A mechanism of general base-catalysed substitution for the formation of HETE (XXVI) and (XXVII) has been proposed, the driving force of which is formation of a stable fluorodiene system:[59]

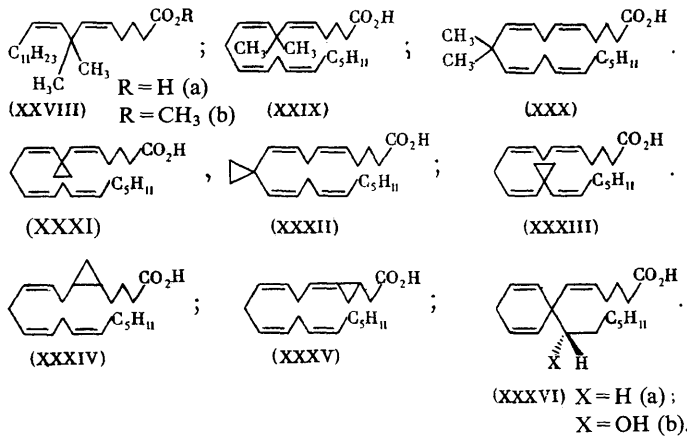


No studies on acids (XXIII) and (XXV) as inhibitors of 5-LO and 15-LO were carried out.[59]

This approach to the development of inhibitors also involves the modification of the carbon skeleton of acid substrates, for which a clear dividing line cannot always be drawn between modification of just methylene groups and more general structural changes.

Many analogues of arachidonic acid have been synthesised, in which the methylene positions, which are subject to attack in the natural acids, are either blocked with alkyl substituents or form part

of cyclic cyclopropane structures, and prove to be competitive lipoxygenase inhibitors [compounds (XXVIII)–(XXXIII)].



Acid (XXVIIIa) at a concentration of 50 μM inhibited 5-LO of human RBL-1 and PMNL cells by 43% and 26% respectively. Its methyl ester was somewhat more active, inhibiting the same enzyme by 74% and 50% respectively.[36] The action of this inhibitor was selective, since at this concentration there was no inhibition of either 12-LO or cyclooxygenase.[36]

gem-Dimethyl derivatives of arachidonic acid (XXIX) and (XXX) also proved to be fairly weak inhibitors of 5-LO (Table 2). However, all of the acids (XXVIIIa)–(XXX) were powerful inhibitors of LT biosynthesis.[23]

Table 2. Inhibitory activity of *gem*-dimethyl analogues of arachidonic acid.[37]

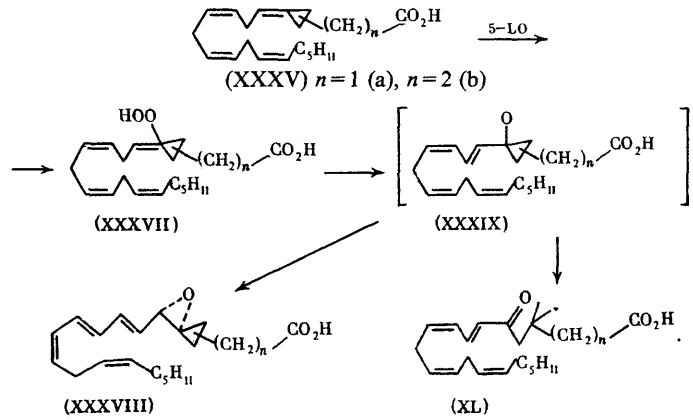
Compound	Inhibition of biosynthesis, % at 10 μM	5-LO inhibition, % at 100 μM	Cyclooxygenase IC_{50}, μM	Phospholipase A_2, %
(XXVIIIa)	100	56	1000	100 (14)
(XXIX)	100 (1–3)	66 (68)	225	100 (14)
(XXX)	98	42	13	100 (14)

Note. IC_{50} values given in brackets.

Thus, it is thought that compounds (XXVIII)–(XXX) block the formation of LT mainly by inhibition of phospholipase A_2 rather than inhibition of 5-LO.[37]

In the opinion of Nicolaou et al.[38,39] by blocking the bis-allyl positions of eicosapolyenoic acids through the introduction of an ethano group in the form of a cyclopropane ring, it is possible not only to achieve the purpose in hand but also to increase the rigidity and stability of the carbon skeleton of arachidonic acid and hence provide a higher biological activity.[38] Studies on the inhibitory activity of ethanoarachidonic acid have been carried out.[39] It was shown that 7-ethanoarachidonic acid (XXXI) is a weak inhibitor of 5-LO (34% inhibition at 100 μM).[60]

A number of methylenecyclopropane analogues of arachidonic acid (XXIV) and (XXXVa) have been synthesised.[57,60] It is assumed that acids (XXXVa) and (XXXVb) can be converted by 5-LO to the HPETE-type hydroperoxide (XXXVII), which can deactivate the enzyme in different ways:[57]



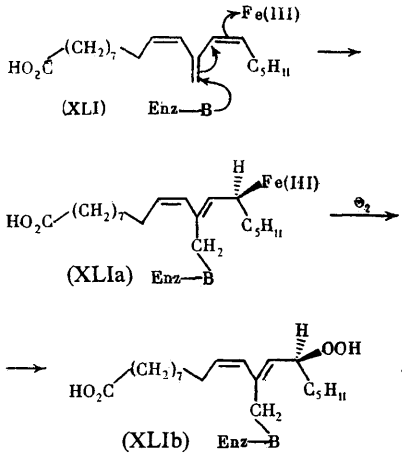
Enzymatic dehydrogenation results in the formation of the reactive oxaspiropentane analogue of LTA_4—(XXXVIII). The additional strain in the carbon skeleton introduced by the cyclopropane ring and the additional cyclopropylcarbinyl stabilisation of the carbonium ion formed by scission of epoxide (XXXVIII) result in the formation of an intermediate at the active site of the enzyme which can bind to nucleophiles contained in it. The alternative conversion of hydroperoxide (XXXVII) to the intermediate (XXXIX) by a homolytic mechanism gives enol (XL) by fragmentation of the cyclopropane ring. Enol (XL) can also irreversibly deactivate the enzyme by binding to a nucleophile.[57] By comparing the ability of compounds (XXXVa) and (XXXVb) to inhibit the synthesis of leukotrienes it would also be possible to determine the preferred conformation of the acid substrate for binding to the enzyme, since these compounds may be regarded as conformers of the C(1)–C(4) carboxyl chain of arachidonic acid that are fixed in a given orientation relative to the 5,6-double bond.[29] Acid (XXXIV) was shown to be inactive as an inhibitor of 5-LO.

By analogy with the *gem*-dimethyl[35,36] and cyclopropane[38,39,57,60] analogues of eicosapolyenoic acids, the 1,1-disubstituted 2,5-cyclohexadienes (XXXVIa) and (XXXVIb) have also been proposed.[58]

Compound (XXXVIa) inhibited 5-LO from RBL-1 (IC_{50} = 120 μM) and inhibited cyclooxygenase to a lesser degree (IC_{50} = 150 μM). By introducing a hydroxyl group on to C(15) it was possible to increase significantly the activity of this structure as an inhibitor of 5-LO (IC_{50} = 6 μM) while its activity towards cyclooxygenase remained unchanged.

In 1986, 12-methylidene-10*Z*,13*Z*-nonadecadienoic acid (XLI), an irreversible inhibitor of soybean 15-lipoxygenase, was synthesised.[31,61] Incubation of acid (XLI) with the enzyme resulted in a time-dependent deactivation. The kinetic characteristics were similar to those in the incubation of 13-thiaarachidonic acid (XXII) and soya bean 15-LO.[61]

The following mechanism of deactivation has been proposed:



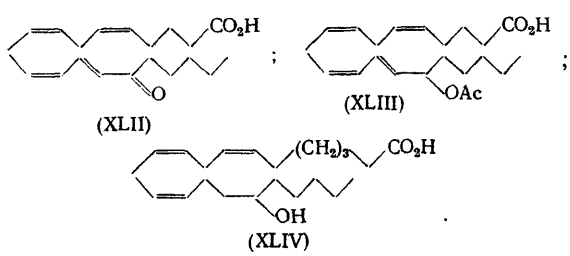
In Corey's opinion[31,61] the activity of this inhibitor is also due to the absence of oxygen that can be eliminated while at the same time there is the ability to be activated on the active site to form intermediates that attack and form covalent bonds with certain functional groups of the active site.

2. Analogues of lipoxygenase metabolites

In addition to the development of various modified acid substrates, a rational approach to the question of obtaining lipoxygenase inhibitors is the use of analogues of lipoxygenase metabolites (leukotrienes, hydroxy acids, etc.).

Thus, in 1980 Vanderhoek et al.[63] reported that 15-hydroxyeicosatetraenoic acid (15-HETE) acts as an inhibitor of 5-LO [62] and 12-LO.[63] These findings served as a basis for the development of a whole class of lipoxygenase inhibitors.

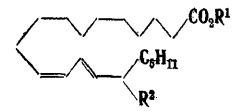
With the aid of three-dimensional computer models, a number of 15-HETE analogues were selected, synthesised, and tested for biological activity:



All modifications directly to the 15-hydroxy group resulted in a reduction of activity. Thus, the 15-keto (XLII) and 15-acetoxy (XLIII) analogues had an IC_{50} (RBL-1, 5-LO) of 26 and 17 μM respectively compared to 7.3 μM for 15-HETE. Extension of the carbon chain by 2 atoms [compound (XLIV)] also reduced the activity.

It was established that 15-HETE analogues that contain a 5*Z*,8*Z*-diene system inhibit 5-LO, by acting as alternative substrates.[64] Research was then initiated on inhibitor compounds that were devoid of this structure. The prototype of this series — 15-hydroxy-11*Z*,13*E*-eicosadienoic acid (15-HEDE) — is a comparatively weak inhibitor (IC_{50} = 26 μM).[64]

Table 3. Inhibitory activity of 15-HEDE derivatives.[64]

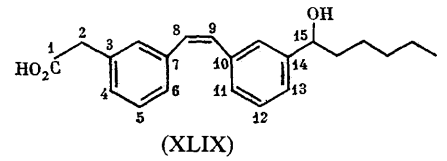


Compound	R^1	R^2	IC_{50}, μM
(XLV)15 *S*	OH	OH	26
(XLVI)15 *S*	OH	OAc	76
(XLVII)	OH	=O	55
(XLVIII)	OH	H	73

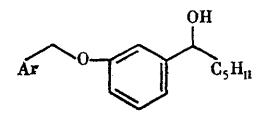
The stereochemistry of the 15-hydroxy group does not appear to be important for inhibition since the natural *S*-isomer and racemic 15-HEDE [64] showed no difference in activity (Table 3). Acetylation and oxidation of the hydroxy group reduced the activity by a factor of 2–3.[64]

Thus, research into 15-HEDE analogues did not yield any active inhibitors of 5-LO, but it confirmed the possibility that the inhibitory activity of compounds lacking the 1*Z*,4*Z*-pentadiene structure that is typical for lipoxygenase substrates would be retained.[60] It was shown that although there are certain structural features in the analogues of 15-HETE and 15-HEDE (the presence of a free carboxyl group, the length of the carboxyl- and ω-chains, the absence in the latter of polar groups, etc.), no single part of the molecule plays a decisive role in determining inhibitory activity.[64] These findings indicate, according to Haviv et al.,[64] that there are many sites for binding of these inhibitors to an enzyme.

By using three-dimensional computer models of 15-HETE, it was possible to establish the potential application of analogues in which the 11*Z*,13*E*-double bonds were replaced by a benzene ring:[65]



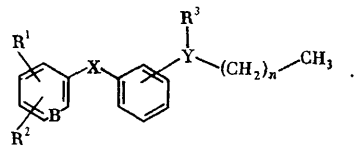
(XLIX)



(L)

Replacement of the 5- or 8-double bond [compounds (XLIX) and (L) respectively], which involves the formation of a C(3)–C(7) bridge (XLIX) and the introduction of an ether bond at C(8)–C(9) (L) with retention of a good isosteric conformity with 15-HETE is

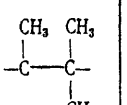
less apparent.[65] On the basis of the stereochemical arguments presented by Musser et al.,[65] 54 compounds with the general structure (LI) were synthesised:



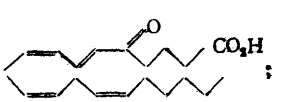
(LI)

All of the compounds with structure (LI) were tested as inhibitors of 5-LO from rat neutrophiles (Table 4). The most active inhibitors were compounds (LIa) and (LIb) (IC_{50} of 0.12 and 0.13 μM respectively).

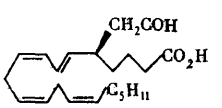
Table 4. Inhibitors (LIa) and (LIb).[65]

Compound	R^1, R^2	B	X	Y	R^3	*h*
(LIa)		N	CH_2O	$-CH_2CH_2-$	OH	3
(LIb)		N	CH_2O		OH	3

The inhibition of 5-LO by the following 5-HETE analogues has been reported:[66]



(LII)



(LIII)

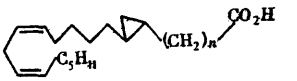
Both compounds are inhibitors of 5-LO from guinea pig PMNL (the second being very weak), with IC_{50} of 20 μM (LII) and 100 μM (LIII).

A number of analogues of LTA_4 which can inhibit 5-LO have been synthesised.[66–69] The carbon analogue of LTA_4—(LIVa)—is an active inhibitor of 5-LO (IC_{50} = 3 μM).[68] Other cyclopropane analogues which differ according to the length of the carbon chain have been reported.[7,66] The position of the cyclopropane ring affects the inhibitory activity of the compounds much less than the presence and position of multiple bonds.[66] The IC_{50} values of compounds (LV) and (LVI) were 20–30 μM, while saturation of the double bonds results in an almost inactive product (LVII) (IC_{50} = 100 μM).



(LIV)
X = CH_2 (a)
X = S (b)
X = NH (c)

;



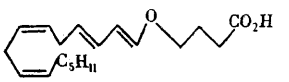
(LV), *n* = 2;
(LVI), *n* = 4.



(LVII)

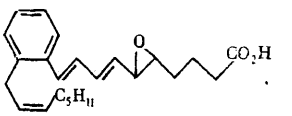
The thio analogue of LTA_4—(LIVb)—is a weak inhibitor of 5-LO (IC_{50} = 65 μM).[66] The synthesis of the aziridine (LIVc) has been reported but its biological activity is not mentioned.[69]

In addition, (LVIII) and (LIX)—two LTA_4 analogues with a modified carbon skeleton—have been synthesised.



(LVIII)

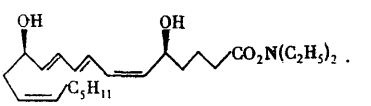
;



(LIX)

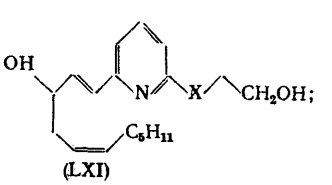
It is thought that their activity is due to the impossibility of opening the epoxide ring because of the modified structure, which excludes their subsequent conversion to LTB_4 and the peptidoleukotrienes LTC_4, LTT_4, and LTE_4.[70]

LTB_4 analogues that act as inhibitors of 5-LO have not been prepared up to the present time. Antagonists of LTB_4 have been reported. The LTB_4 diethylamide (LX) inhibits the degranulation of neutrophiles caused by LTB_4 at concentrations of 10^{-6}–10^{-7} M.[7]

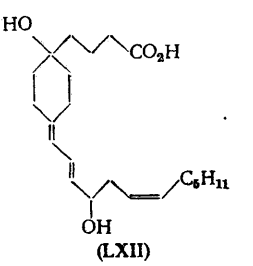


(LX)

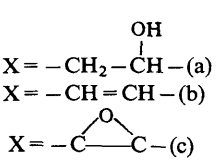
A number of stereoisomers of LTB_4, such as 15*S*,12*S*-di-HETE, proved to be capable of inhibiting 5-LO from PMNL.[71] A number of ring-containing analogues of LTB_4 have been reported; (LXI) [72] and (LXII)[73] act in competition to LTB_4.



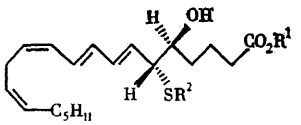
(LXI)



(LXII)



Only a few peptidoleukotriene analogues that inhibit 5-LO are known.[74] However, the absence of a common method for determining the activity of 5-LO led Japanese research workers[74] to the conclusion that the activity of compounds with the general structure (LXIII) may in fact be due to their inhibition of glutathione transferase.



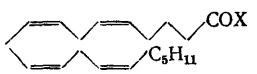
(LXIII) $R^1 = H, CH_3$; $R^2 = CH_2C_6H_5, C_3H_7$, iso-C_4H_9.

3. Compounds which form chelate complexes with Fe(III)

The enzyme mechanism of the lipoxygenase reaction is associated with the presence of iron [Fe(III)] at the active site of the enzyme.[67,75] These findings formed the theoretical basis for the synthesis of different eicosapolyenoic acid derivatives and compounds of a different structure that can form chelate complexes with Fe(III), hence deactivating the enzyme.

An additional argument to support the key role of iron in the act of catalysis is the theory put forward by Corey[76,77] concerning the formation of an organoiron intermediate with the substrate at the active site of the enzyme.

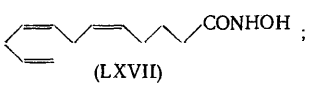
The first compounds to be studied as inhibitors of this type were hydroxamates of arachidonic acid, which are ligands capable of coordinating with Fe(III) ions ($K_{assoc} = 10^{-12}$). The K_m values were consistent with competitive inhibition.[78] However, not one of these compounds (LXIV)–(LXVI) acts as a substrate of 5-LO, although they are some of the most powerful inhibitors of it. Thus, the EC_{50}‡ values for these compounds are 2.2, 0.3 and 0.2 μM respectively:[78]



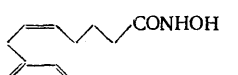
(LXIV), X=NHOH; (LXV), X=N(CH$_3$) OH,
(LXVI), X = N (t-C$_4$H$_9$)OH.

It is the hydroxamate groups which are thought to be responsible for bonding with the ferric ions participating in the catalytic cycle, so that the presence of the complete carbon skeleton of eicosapolyenoic acids is not absolutely essential for inhibitors of this type.[78,79]

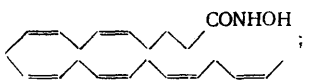
This is supported by the fact that compounds (LXVIII)–(LXX) (obtained by total synthesis) are very powerful inhibitors of 5-LO (EC_{50} = 1.9 μM).[78]



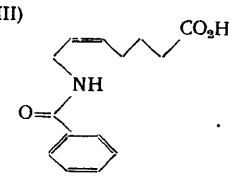
(LXVII)



(LXVIII)



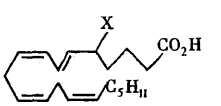
(LXIX)



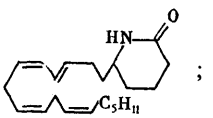
(LXX)

‡ EC_{50} is the effective concentration of inhibitor that produces a 50% loss of enzyme activity.

The 5-hydroxyamates of 6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid were subsequently synthesised and tested as inhibitors of 5-LO:[80,81]



(LXXI), X = CONHOH;
(LXXII), X = CH_2CONHOH;
(LXXIII), X = NNHCONH$_2$;
(LXXIV), X = NNHCSNH$_2$;
(LXXV), X = SH.

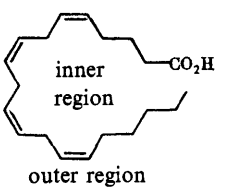


(LXXVI)

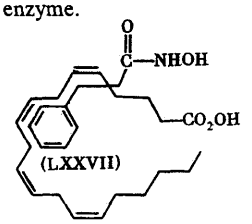
Thus, compounds (LXXI) and (LXXII) proved to be powerful inhibitors of 5-LO in a supernatant of homogenised RBL-1 cells (IC_{50} = 1.4 and 0.19 μM respectively).[80] Hydroxamate (LXXII) is over ten times more active than the arachidonic acid hydroxamate (LXIV) (IC_{50} = 0.19 and 2.2 μM respectively).[80,81] Thus, transfer of the hydroxamate functional group to the C(5) position considerably increases the ability to chelate with ferric ions. The effect of replacing the hydroxamate functional group by others was also studied, taking into account the possibility that they could interact with the active site of the enzyme in addition to chelation with Fe(III).[81] The most active of these proved to be the semicarbazide (LXXIII), thiosemicarbazide (LXXIV), the thiol (LXXV) (IC_{50} = 7.0, 7.9, and 7.6 μM respectively). The cyclic lactam (LXXVI) also proved to be an active inhibitor, being three times more active (IC_{50} = 10.0 μM) than its linear analogue.[81]

However, although the compounds reported by Corey et al.[78,80,81] are extremely active inhibitors of 5-LO, it is unlikely that they could be of therapeutic use.[79] As analogues of arachidonic acid they would tend to undergo chemical oxidation and would be rapidly degraded *in vivo*. Therefore, there is considerable interest in more stable hydroxamates with quite a simple structure,[79] which nevertheless retain the high activity which was demonstrated for the arachidonic acid analogues described above.

The approach of Summers et al.[79] is based on a selection of compounds that can effectively bind to the active site, which may be regarded as consisting of non-haeme Fe(III), a hydrophobic region, and a section for bonding of the carboxyl group of the substrate,[82] because of the correspondence of their structure to the conformation of arachidonic acid bound to the enzyme.

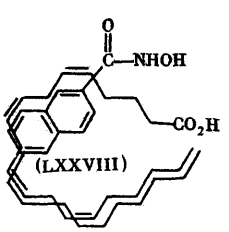


(A)



(LXXVII)

(B)



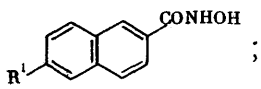
(LXXVIII)

(C)

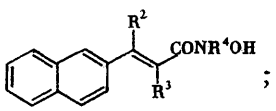
The hypothetic conformation (A) of arachidonic acid at the active site[22,79] is shown above together with the "superposition" of simpler aromatic hydroxamates on it in (B) and (C). It is clear that in case (C) the "superposition" is considerably more precise, which is in agreement with the experimental results.[79] In selecting inhibitors, the authors have attempted, other conditions being equal, to give preference to those compounds and their isomers which occupy the volume in the inner region to a lesser extent.

ω-Phenylalkyl- and ω-naphthylalkyl-hydroxamates were studied as inhibitors of this type (sources of enzyme activity—supernatant RBL-1), and the latter were, as a rule, more active,[79] which is consistent with a greater affinity of the enzyme towards more lipophilic compounds. The introduction of a methylene group between the benzene ring and the carboxyl group increased the activity of the compounds. A study of the effect of substituents in the aromatic systems showed that derivatives with electron-withdrawing groups are more active. Any modification of the hydroxamate function (apart from methylation) reduce the activity of the inhibitors.[79]

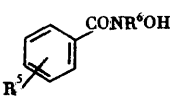
Thus, it was shown that even compounds with a simple structure that contain the hydroxamate group can be powerful inhibitors of 5-LO:



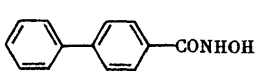
(LXXIX), $R^1 = O(CH_2)_3CH_3$;
(LXXX), $R^1 = O(CH_2)_2CH{=}CH(CH_2)_4CH_3(Z)$;



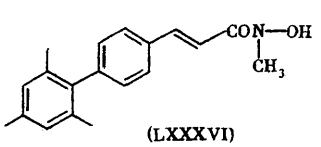
(LXXXI), $R^2 = CH_3$, $R^3 = H$, $R^4 = CH_3$,
(LXXXII), $R^2 = H$, $R^3 = H$, $R^4 = Ph$;



(LXXXIII), $R^5 = p$-(1-naphthyl);
$R^5 = p$-(2,4,6-trimethylphenyl);



(LXXXV)

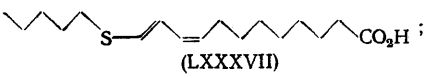


(LXXXVI)

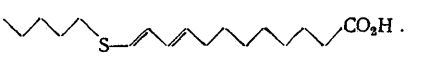
Compound	(LXXIX)	(LXXX)	(LXXXI)	(LXXXII)	(LXXXIII)	(LXXXIV)	(LXXXV)	(LXXXVI)
IC_{50}, μM	6.5	2.9	0.12	0.052	0.18	0.29	0.41	0.022

Compound (LXXXVI) is one of the most active inhibitors of 5-LO to be studied.[78,79]

Sulphur-containing polyunsaturated acids can also be grouped with inhibitors that chelate with iron.[48] In the opinion of Funk et al.,[48] the effect of these compounds is derived from a similarity in their structure to substrates of LO as well as their ability to chelate with Fe(III):



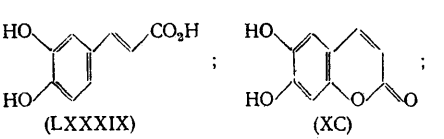
(LXXXVII)



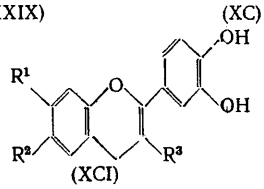
(LXXXVIII)

13-Thia-9*Z*,11*E*-octadecadienoic acid (LXXXVII) and 13-thia-9*E*,11*E*-octadecadienoic acid (LXXXVIII) were tested as inhibitors of soybean 15-LO and they exhibited the properties of competitive inhibitors.[83]

The ability of caffeic acid (LXXXIX) and its derivatives,[84] esculetin (XC),[85] and certain flavonoids (XCI) [86] to form chelate complexes with Fe(III) is known, their activity as inhibitors of 5-LO being related to this:



(LXXXIX) (XC)



(XCI)

cirsilol $R^1 = H$, $R^2 = OCH_3$, $R^3 = OCH_3$ (a);
pedalitin $R^1 = H$, $R^2 = OH$, $R^3 = OCH_3$ (b);
quercetin $R^1 = OH$, $R^2 = H$, $R^3 = OH$ (c).

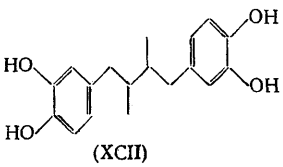
The mechanism of complexation of flavonoids with Fe(III) is unclear, it is also possible that they act through a dual mechanism—as chelating agents of Fe(III) and as antioxidants, which they behave as at the same time (see below).[7,86]

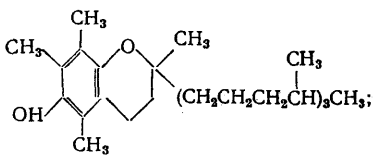
It should be mentioned, however, that 5-LO is definitely not the only iron-containing enzyme of the arachidonic acid pathway (cyclooxygenase, 12-LO, and 15-LO also contain it).[7,31,75,87] Hence there is no reason to expect high specificity from inhibitors of this type.

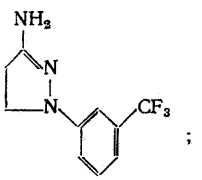
4. Antioxidants

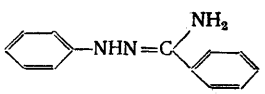
Since the lipoxygenase reaction is, in essence, the oxidation of acid substrates with oxygen, it was natural that many antioxidants should prove to be inhibitors.

Virtually all known antioxidants inhibit 5-LO to a given extent, these include sterically hindered phenols [nordihydroguaiaretic acid (NDGA)] (XCII), α-tocopherol (XCIII), flavonoids (XCI),[88] etc.

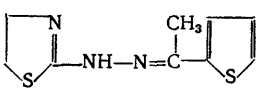

(XCII)

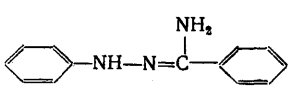

(XCIII)


(XCIV)


(XCV)

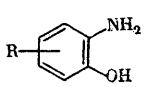
A separate group is made up by the hydrazone antioxidants BW-755C (XCIV) [89] and CBS-1114 (XCV) [90] and hydrazones (XCVII) and (XCVIII):[91]


(XCVI)

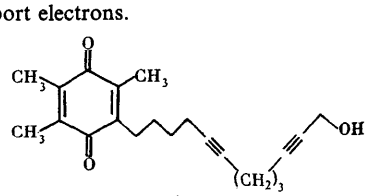

(XCVII)

A characteristic feature of these inhibitors is the absence of selectivity towards LO. Thus, compounds (XCVII) and (XCVIII) inhibit 5-LO, 12-LO, and cyclooxygenase to virtually the same extent.[91]

o-Aminophenols with the general structure (XCVIII) have also been reported[92] as antioxidant inhibitors of 5-LO. However, a dual mechanism, similar to that for flavonoids, cannot be excluded for their mode of action [chelation with Fe(III) and inhibition of oxidation].


(XCVIII)

A special group of antioxidants is made up by the so-called Takeda quinones,[93] which are similar to ubiquinones in structure. There is most interest in quinone AA-861 (XCIX), which has the highest selectivity in this series and inhibits 5-LO (IC_{50} = 1 μM) without having any significant effect on cyclooxygenase and 12-LO at concentrations up to 10 μM (when 5-LO retains only 3% activity). This compound has passed clinical trials as a potential drug for the treatment of bronchial asthma.[7] Its mode of action has not been established, although ubiquinone is known for its ability to transport electrons.


(XCIX)

A correct interpretation of the role of antioxidants as potential pharmacological drugs should evidently be made on the basis of recently published reports concerning the role of the so-called active forms of oxygen—AOS (active oxygen species) (superoxide anions, peroxides, hydroxyl and ferryl radicals, etc.)[94] in diseases such as cancer, cardiovascular disorders, allergic and inflammatory processes. Thus, it is thought, for example, that the ferryl radical (the hypothetical intermediate in lipoxygenase catalysis) damages the active sites of a number of enzymes, lipid membranes, DNA, etc. Although the mechanism of these processes is not known, AOS can apparently give rise to rearrangement of regions of the membrane, affect the transport of Ca^{2+} and the activation of phospholipases, and cause the release of eicosanoids, which in turn can produce a whole series of biochemical changes.

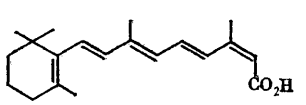
5. Certain other inhibitors

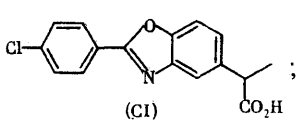
There are many compounds that inhibit LO (mainly 5-LO) for which the mechanism is not known and there is no reasonable theory for combining them into classes. There are also many individual reports about inhibitors which do not belong to any of the groups described above.

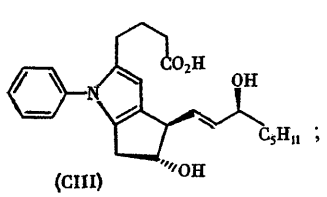
Thus, the ability of retinoids to inhibit 5-LO selectively has been established,[95] the most active being 13*Z*-retinoic acid (C).

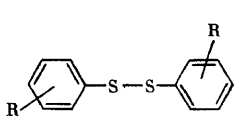
Benoxaprofen (CI), a non-steroid antiinflammatory drug used for the treatment of psoriasis, is a non-selective inhibitor of 5-LO whose mode of action is unclear.[96]

Aryl disulphides (CII), reported by research workers from Merck as a new class of selective inhibitors of 5-LO, are able to reduce the level of hydroperoxides needed for enzyme activation.[75,96,97]

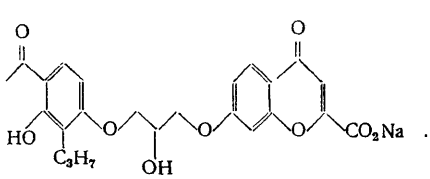

(C)


(CI)


(CIII)


(CII)

Compound U-60,257 (CIII) is an inhibitor which acts simultaneously at several points of the arachidonic acid pathway. Thus, it was described originally as an inhibitor of glutathione transferase. However, it was subsequently shown to have the ability to deactivate 5-LO and have the properties of a peptidoleukotriene antagonist.[98] Another active antagonist, SRS-A-FLP-55712 (CIV), which had already been adopted for medical use and was widely used for biochemical studies of the effects caused by LTC_4,[99] also proved to be an inhibitor of 5-LO (IC_{50} = 20 μM).[100]


(CIV)

An interesting example of an inhibitor that acts as a suicide substrate is phenylhydrazine (CV), which irreversibly deactivates soybean 15-LO.[101] It was established that deactivation is dependent on phenyldiazine (CVI) formed by autoxidation of phenylhydrazine:

$$\underset{\text{(CV)}}{\text{PhNHNH}_2} \xrightarrow[M^{n+}]{O_2} \underset{\text{(CVI)}}{\text{RhN=NH}} + H_2O_2, \quad (1)$$

$$\underset{\text{(CVI)}}{\text{PhN=NH}} + \text{Fe (III)} \rightarrow \text{Ph}^{\cdot} + N_2 + \text{Fe (II)}. \quad (2)$$

The Fe(III)-enzyme is sensitive to phenyldiazine formed non-enzymatically according to Eqn. (1). However, deactivation is related not only to the conversion of the enzyme to a Fe(II)-containing inactive form, since excess 13*S*-HPOD does not cause the enzyme to be reactivated and the Fe(II)-enzyme is also irreversibly deactivated by phenylhydrazine.[101]

The activity exhibited by the haloethane 1,1,1-trifluoro-2-bromoethane is difficult to explain in terms of its structure. This compound (widely used in anaesthesia) suppresses the synthesis of leukotrienes and hydroxy acids by 5-LO in leucocytes.[102] Inhibition is reversible (removal of the inhibitor reactivates the enzyme) and depends on the dose. It was also established that this haloethane blocks the release of arachidonic acid from phospholipids and the synthesis of LTC_4 by glutathione transferase.[102] Its mode of action remains unclear, but it is evident that this compound acts at the second or even third stages of the enzymatic pathway.

Thus, the suppression of lipoxygenase activity may occur as a result of the effect of very different factors, which have still in part not received a rational explanation. Thus, it was established that the selective suppression of the 5-lipoxygenase activity of alveolar macrophages occurs during smoking.[103] Selective suppression only occurred for 5-lipoxygenase products (LT and 5-HETE) while TXB_2 and PGE_2 were formed in unchanged quantities. The mechanism of inhibition has not been established but it is thought to be related to the effect of free radicals generated by smoking on the active site of 5-LO.[103] However, this does not explain the selectivity of inhibition.

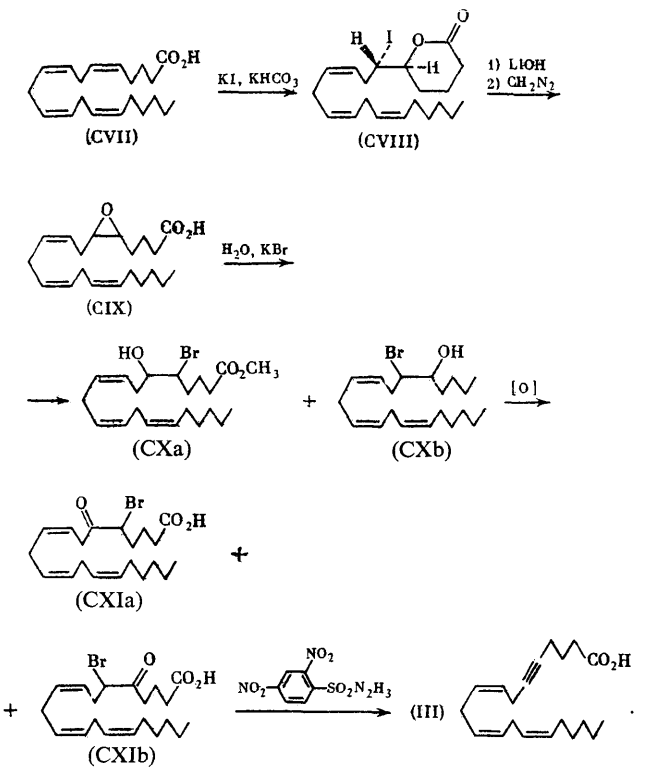
IV. The chemical synthesis of lipoxygenase inhibitors

The majority of the inhibitors of lipoxygenase oxidation of polyenoic acids examined are polyunsaturated systems containing double (separated by methylene groups, conjugated or cumulated) and triple bonds. Their synthesis is related, on the one hand, to the formation of a given carbon skeleton and, on the other hand, to the introduction (including during the build-up stage) of double bonds of a given configuration and triple bonds.

The following methods have been used to produce triple bonds in inhibitor molecules: 1) from the epoxide of the corresponding polyenoic acid;[67,104] 2) by condensing iodo- or bromo-allene with suitable lithium derivatives;[27,105] 3) by the reaction of metal acetylides with halogen derivatives.[106,107]

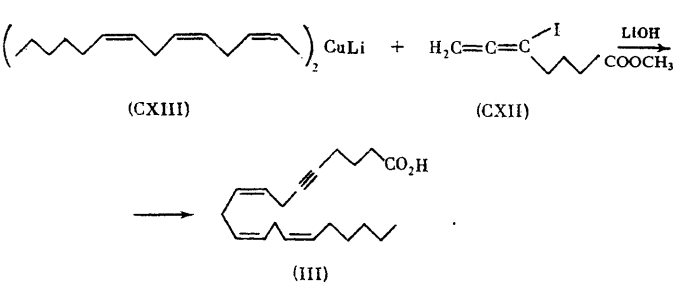
The application of these three approaches to the synthesis of acetylenic inhibitors can be conveniently examined in the case of 5,6-dehydroarachidonic acid.

Thus, the first synthesis of 5,6-DHA from arachidonic acid (CVII) was carried out by converting it to a lactone and then to epoxide (CIX) by the action of lithium hydroxide,[68] Epoxide (CIX) was converted to a mixture of isomeric bromohydrins (CXa, b) followed by a Jones oxidation to form a mixture of bromoketones (CXIa, b). Reaction of the isomeric mixture (CXIa, b) with 2,4-dinitrobenzenesulphonyl hydrazone yielded the methyl ester of 5,6-DHA (IIIa):[67]

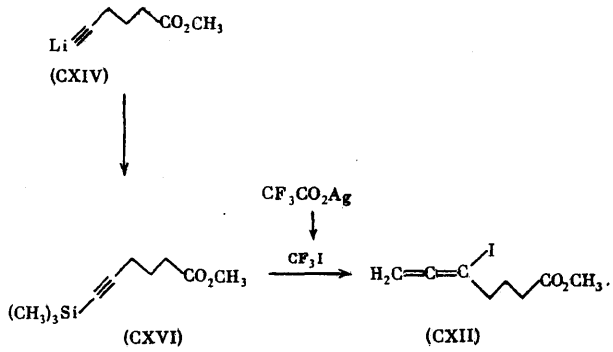


8,9-, 11,12-, and 14,15-DHA were obtained similarly via the respective epoxides.[67,108]

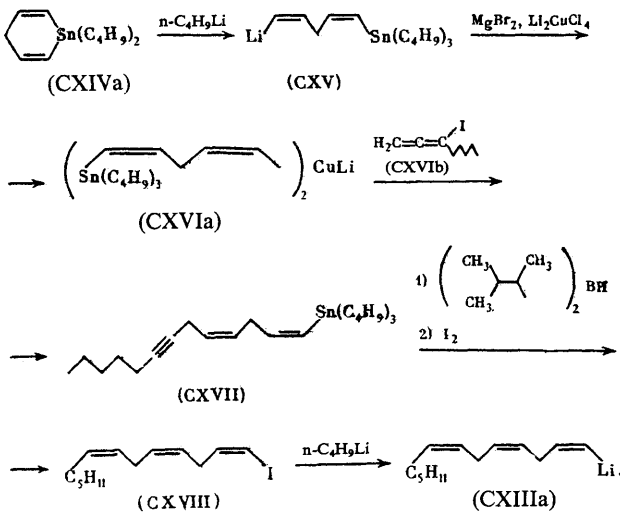
5,6-Dehydroarachidonic acid (III) may also be obtained by a complete synthesis, the key components of which are methyl 5-iodo-5,6-heptadienoate (CXII) and the lithium derivative of tridecatriene (CXIII):[27]



Iodoallene (CXII) is obtained by silylation of the lithium derivative of methyl 5-hexynoate (CXIV) with trimethylsilyl chloride in THF–HMPT followed by a reaction with silver trifluoroacetate in methylene chloride:

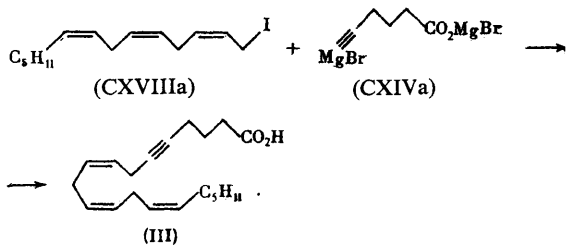


The synthesis of 1-lithio-1,4,7-tridecatriene (CXIII) is carried out by ring opening of 1,1,-di-n-butyl-1-stanna-2,5-cyclohexadiene (CXIVa) with n-butyllithium followed by extension of the carbon chain through the reaction of 1-lithio-5-tributylstannyl-1,4-pentadiene cuprate (CXVIa) with 3-iodo-1,2-octadiene (CXVIb), obtained in a similar manner to iodoallene (CXII):

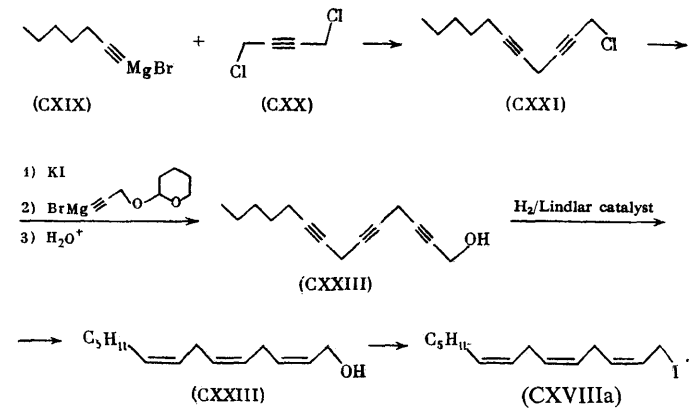


The triple bond in (CXVII) formed as a result is reduced with disiamylborane to a *cis*-double bond.[27]

The synthesis of 5,6-DHA from acetylenic compounds was achieved by cross-combining propargyl- and allyl-type halides (tosylates) with ω-acetylenic compounds.[107] 1-Iodo-2*Z*,5*Z*,8*Z*-tetradecatriene (CXVIIIa) and 5-hexynoic acid (CXIVa) were selected as the key synthons:

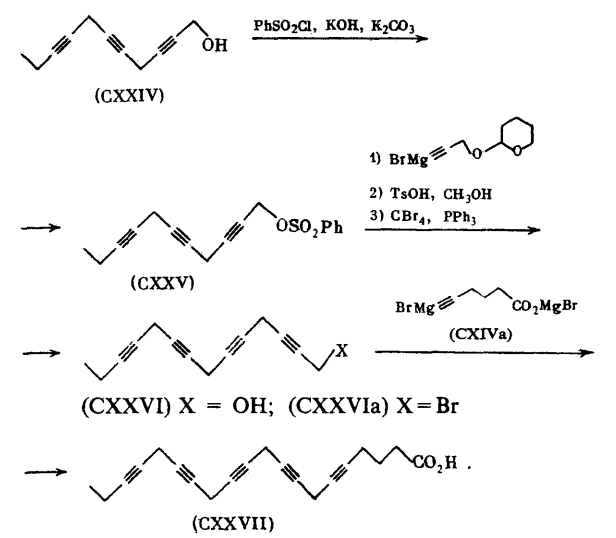


The triene iodide was synthesised by starting from 1-heptyne (CXIX), which was condensed with 1,4-dichlorobutyne (CXX) to give 1-chloro-2,5-undecadiyne (CXXXI):



Iodide (CXXIa), obtained from the diyne chloride (CXXI), was condensed with protected propargyl alcohol followed by removal of the protecting group and hydrogenation on a Lindlar catalyst. The triene alcohol (CXXIII) obtained in this manner was converted to the corresponding iodide (CXVIII) via the mesylate.

Another example of the successful application of a method for the preparation of eicosapolyynoic acids from acetylenic compounds is provided by a novel synthesis of 5,8,11,14,17-eicosapentaynoic acid (CXXVII)—a potential LO inhibitor.[108] The starting material for this was 2,5,8-undecatriynol (CXXIV),[109] conversion of which to the benzenesulphonate (CXXV) and condensation with the magnesium bromide derivative of protected propargyl alcohol gave 2,5,8,11-tetradecatetraynol (CXXVI). Subsequent replacement of the hydroxyl group in this compound by bromine gave 1-bromo-2,5,8,11-tetradecatetrayne (CXXVIa). The latter condensed with 5-hexynoic acid to form the required acid (CXXVII):



This type of approach to the synthesis of the pentaynoic acid (CXXVII) was also known previously,[110] but the use of propargyl halides to form polyacetylenic structures (in particular for the preparation of 5,8-nonadiynoic acid)[110] resulted in relatively low yields. Therefore, the tetrayne bromide (CXXVIa) and 5-hexynoic acid (CXIV) were selected by the authors as key components. The use of the benzenesulphonate (CXXV) instead of the corresponding bromide[111] made it possible to conduct the reaction under milder conditions (20 °C) and led to an increase in yield by 10–20%.

The double bonds in inhibitor molecules (both *cis*- and *trans*-) have been formed, as a rule, by means of the Wittig reaction.[35,36,38,39,48,82] Moreover, as has already been demonstrated, *Z*-double bonds may be obtained by the reduction of triple bonds on a Lindlar catalyst,[34,59,107] disiamylborane,[27] R1 or R2 nickel[112] (the use of the latter has only been reported for systems containing no more than two triple bonds).

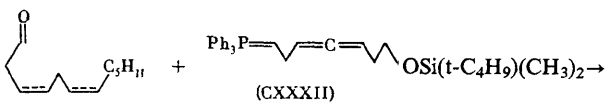
A significant disadvantage of the Wittig reaction is its low stereoselectivity.

The stereochemical result of the reaction is affected by the presence of lithium salts, which causes an increase in the proportion of *trans*-alkenes in the same reactions when the synthesis is conducted in non-polar solvents.[113] On the other hand, by using HMPT in the Wittig reaction it is possible to increase the proportion of the *cis*-isomer from 85 : 15 (*cis* : *trans*) to virtually pure *Z*.[54]

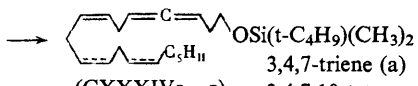
The Wittig reaction may be accompanied by side reactions, for example, isomerisation of the neighbouring double bond.[114] By using phosphonates (Wittig–Horner reaction) it is possible to improve the stereoselectivity of condensation slightly in favour of the formation of *trans*-alkenes. Attempts to increase the proportion of *Z*-products proved largely unsuccessful.[113,115,116]

Despite all these disadvantages the Wittig reaction remains the main method for forming double bonds in modified acid inhibitors (mainly the *Z*-configuration).[38,39,54]

Thus, the synthesis of 4,5-eicosaallenic acids is based on the stereoselective Wittig condensation of the allenic ylide (CXXXII) with the respective aldehyde (CXXXIIIa–c), the products of which are the nonadecasilyl ethers (CXXXIVa–c):[54]



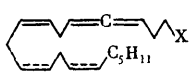
(CXXXIIIa–c) (CXXXII)



(CXXXIVa–c)

3,4,7-triene (a)
3,4,7,10-tetraene (b)
3,4,7,10,13-pentaene (c).

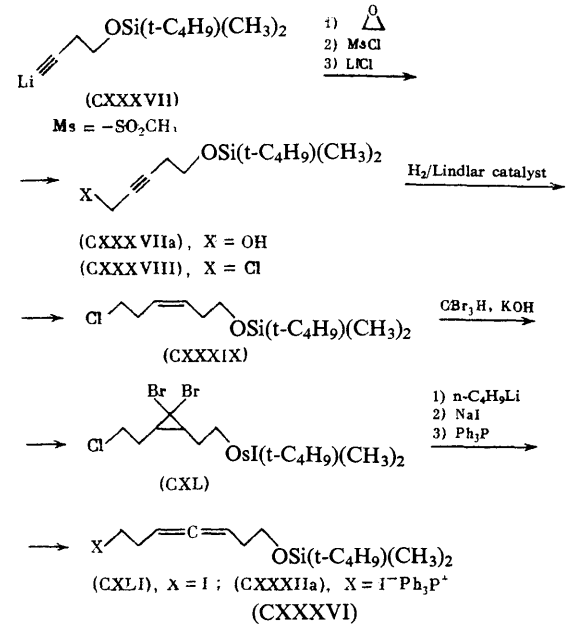
The subsequent conversion of the silyl ethers to bromides (CXXXVa–c) and the reaction of the Grignard reagents obtained from them with carbon dioxide gave 4,5-alleneicosanoic acids (IVa–c):



(CXXXVa–c) X = Br
3,4,7-triene (a)
3,4,7,10-tetraene (b)
3,4,7,10,13-pentaene (c)

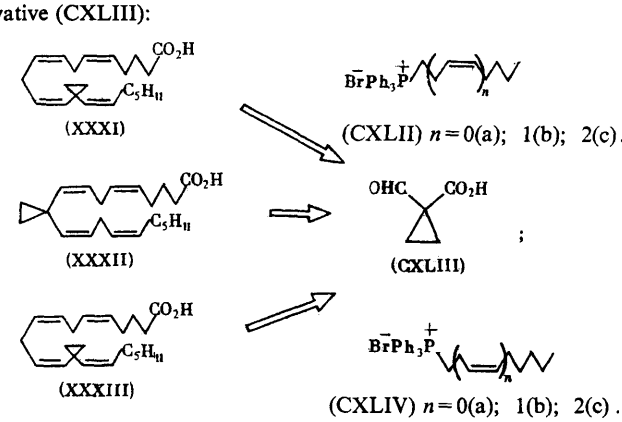
(IVa–c) X = CO_2H
8*Z*-eicosa-4,5,8-triene (a);
8*Z*,11*Z*-eicosa-4,5,8,11-tetraene (b);
8*Z*,11*Z*,14*Z*-eicosa-4,5,8,11,14-pentaene (c).

A common intermediate in the synthesis of allenic acids (IVa–c) is [7-(t-butyldimethylsilyloxy)-hepta-3,4-dien-1-yl]triphenylphosphonium iodide (CXXXVI), which was obtained from the lithium derivative of 3-butynyl t-butyldimethylsilyl ether (CXXXVII) by alkylation of the latter with ethylene oxide to form the monoprotected diol (CXXXVIIa), which was then converted to the chloride via the mesylate. Catalytic reduction of the triple bond of the chloride gave 6-chlorohexen-3*Z*-yl t-butyldimethylsilyl ether (CXXXIX), which was then converted to the corresponding dibromocyclopropane (CXL) by reaction with bromoform and potassium hydroxide in the presence of a phase-transfer catalyst. Conversion of the cyclopropane (CXL) to 1-chlorohepta-3,4-dien-6-yl t-butyldimethylsilyl ether (CXLI) by reaction with n-butyllithium and the subsequent replacement of chlorine by iodine and quaternisation led to the required component (CXXXVI):



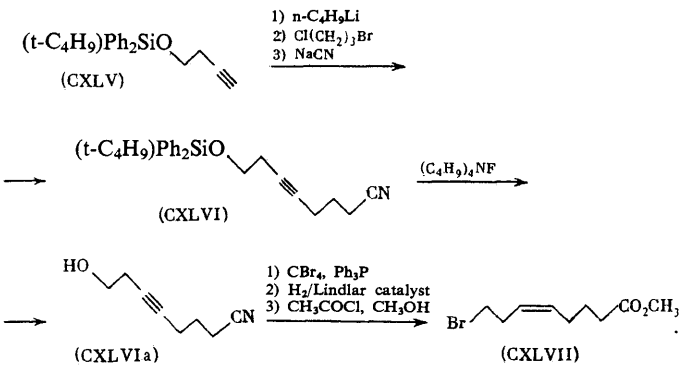
(CXXXVII)
Ms = $-SO_2CH_3$
(CXXXVIIa), X = OH
(CXXXVIII), X = Cl
(CXXXIX)
(CXL)
(CXLI), X = I ; (CXXXIIIa), X = $I^-Ph_3P^+$
(CXXXVI)

For a number of syntheses of polyenoic lipoxygenase inhibitors construction of the carbon skeleton using condensations of terminal acetylenic compounds with propargyl (allyl) halides (followed by selective reduction) as well as the formation of double bonds by the Wittig reaction is typical. This approach was used in the synthesis of the so-called ethanoarachidonic acids (XXXI)–(XXXIII).[38,39] Retrosynthetic analysis led Nicolaou et al.[38,39] to the phosphonium bromides (CXLIIa–c), (CXLIVa–c), and the cyclopropane derivative (CXLIII):

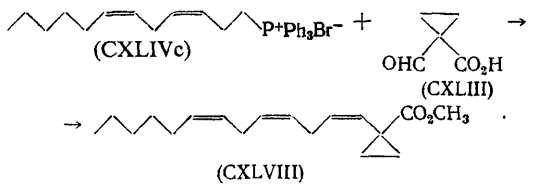


(XXXI) (CXLII) *n* = 0(a); 1(b); 2(c).
(XXXII) (CXLIII);
(XXXIII) (CXLIV) *n* = 0(a); 1(b); 2(c).

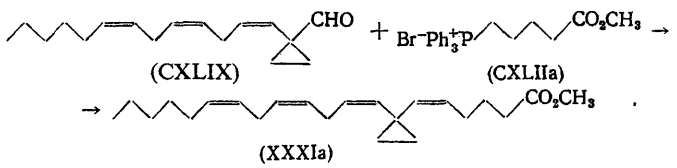
The synthesis of phosphonium salt (CXLIIb) was carried out by alkylation of the lithium derivative (CXLV) with chlorobromopropane followed by replacement of bromine by a cyano group, giving nitrile (CXLVI), which after desilylation, bromination, and reduction was converted to methyl ester (CXLVII)—the direct precursor of the required compound:



Phosphonium salts (CXLIVa–c), formed in a similar manner from the corresponding terminal alcohols, were used for subsequent condensation with the cyclopropane derivative (CXLIII):

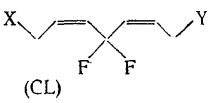


Subsequent conversion of methyl ester (CXLVIII) to an aldehyde and its condensation with phosphonium salt (CXLIIa) gave ethanoarachidonic acid (XXXI):



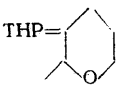
As has already been indicated, a versatile approach to the synthesis of methylene-separated polyene and polyenyne structures is a method which has already become established and the basic principles of which were previously developed by Osbond.[117] This method is based on the successive build-up of the carbon skeleton by condensation of magnesium halide derivatives of terminal acetylenic compounds with propargyl halides followed by reduction of the complete polyyne system or its individual parts. However, in a number of cases this method proves to be unacceptable despite the formal correspondence of the structure to what is required.

Thus, the synthesis of fluorine-containing analogues of arachidonic acid (XXII)–(XXV) is based on a substantially different approach to the formation of a methylene-separated polyene structure.[34,59] The starting point in this approach is formation of the difluoroallyl system (CL), which is carried out by means of a chain reaction involving an acetylene anion containing substituents X and CF_2ClBr:[34]



$X = OTHP,\ Y = OSi(t\text{-}C_4H_9)(CH_3)_2$

$$\underset{\text{(CLI)}}{THPOCH_2C{\equiv}CLi} \xrightarrow[THF,\ -80\ ^\circ C]{CF_2ClBr} \underset{\text{(CLII), 52\%}}{THPOCH_2C{\equiv}CCF_2Br} + \underset{\text{(CLIII), 25\%}}{THPOCH_2C{=}CBr}. \quad (3)$$

THP= 

The following mechanism for an ionic chain reaction has been proposed:[34,39]

$$\text{Initiation } Nu^- + BrCF_2Cl \rightarrow NuBr + CF_2Cl^-,$$

$$\text{Chain growth} \begin{cases} CF_2Cl^- \rightarrow :CF_2 + Cl^-, \\ Nu^- + :CF_2 \rightarrow NuCF_2^-, \\ NuCF_2^- + BrCF_2Cl \rightarrow NuCF_2Br + CF_2Cl^-, \end{cases}$$

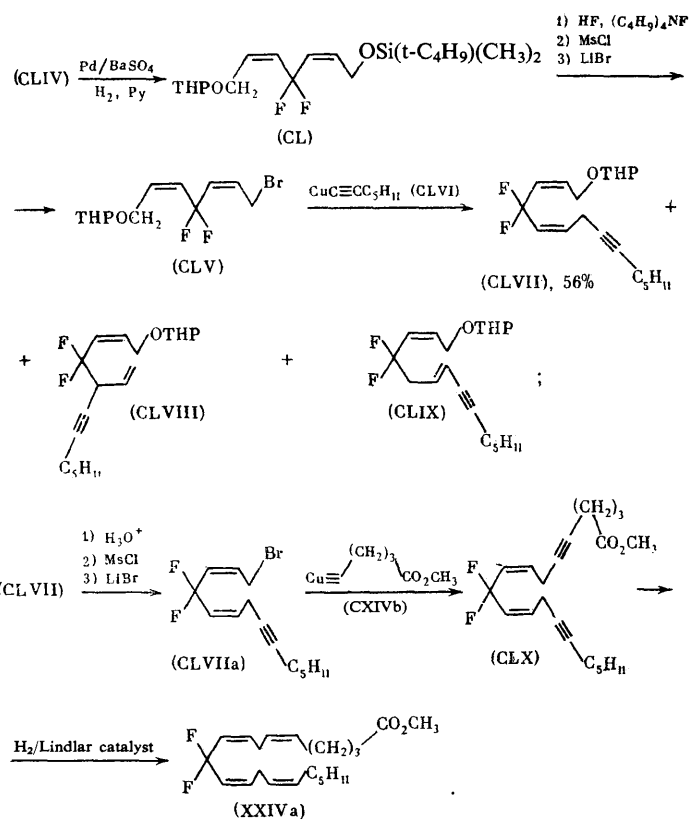
which is supported by the formation of bromide (CLIII) in reaction (3). By subsequently converting difluorobromide (CLII) to the corresponding iodide, it was possible to carry out a second condensation with acetylide anion as in (4), leading to the formation of the asymmetric difluorodiyne (CLIV):

$$\underset{\text{(CLIIa)}}{THPOCH_2C{\equiv}CCF_2I} + \underset{\text{(CLIa)}}{LiC{\equiv}CCH_2OSi(t\text{-}C_4H_9)(CH_3)_2} \rightarrow$$

$$\rightarrow \underset{\text{(CLIV)}}{THPOCH_2C{\equiv}CCF_2C{\equiv}CCH_2OSi(t\text{-}C_4H_9)(CH_3)_2}. \quad (4)$$

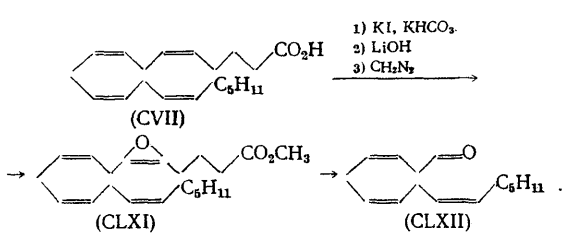
Reduction of difluorodiyne (CLIV) over $Pd-BaSO_4$ in pyridine yielded the key intermediate—difluoro-*Z*-diene (CL). After this there was the problem of forming two different carbon bonds with the respective substituents. This only proved possible with the use of cuprate reagents. Thus, condensation of the allyl bromide (CLV) with the heptynyl cuprate (CLVI) gave difluorodienyne (CLVII) (56% yield). There are different opinions in the literature about the possibility of using a diyne bromide (CLIVa) in a condensation.[34,118] The condensation is accompanied by the formation of branched (CLVIII) and conjugated (CLIX) enynes. Subsequent condensation with the cuprate of methyl hexynoate led to the dienyne (CLX),

which was hydrogenated on a Lindlar catalyst to give the required acid (XXIVa):[34]

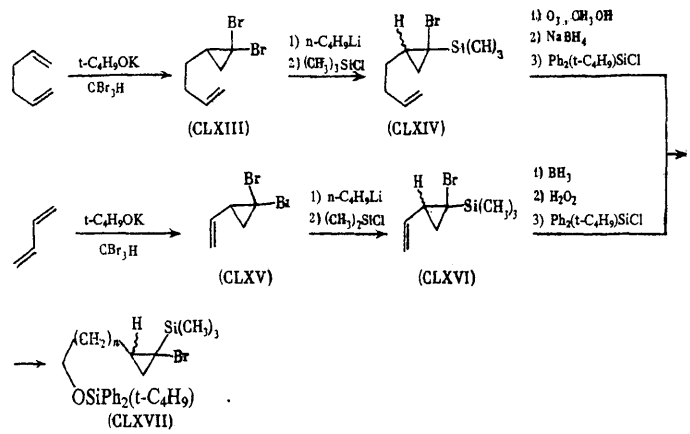


A widely used method for the synthesis of derivatives of arachidonic acid involves its direct conversion to the required compounds or the formation from it of separate residues of the final structures.

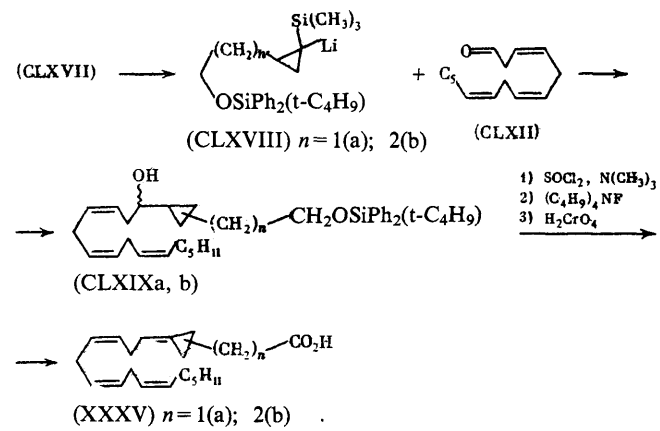
Thus the synthesis of methylenecyclopropane analogues of arachidonic acid[57] was achieved by iodolactonisation of the latter with subsequent conversion to an epoxide (in a similar manner to the approach of Corey et al.[67]) and its oxidation to an aldehyde:



The starting compounds in the synthesis of the methylenecyclopropane residue were 1,3-butadiene and 1,4-heptadiene:



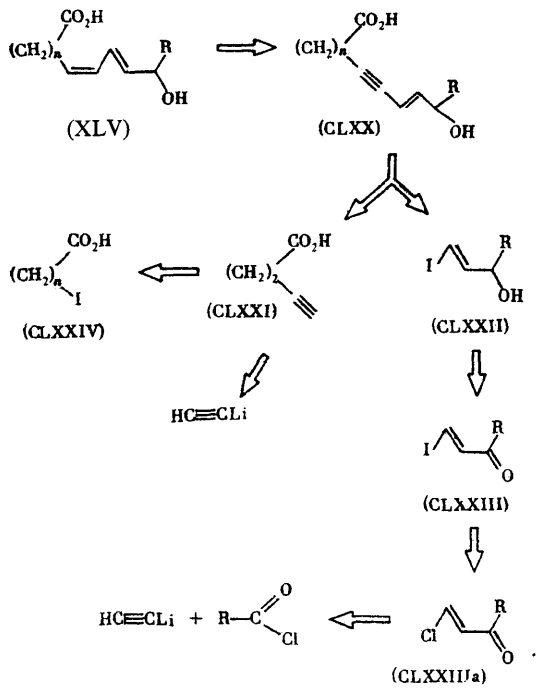
Metallation of (CLXVII) and reaction with aldehyde (CLXII) gave a mixture of four diastereomers (CLXVIIIa, b) which after chromatographic separation were converted to the silanes (CLXIXa, b). Desilylation and oxidation of the latter yielded the required acids (XXXVa) and (XXXVb):



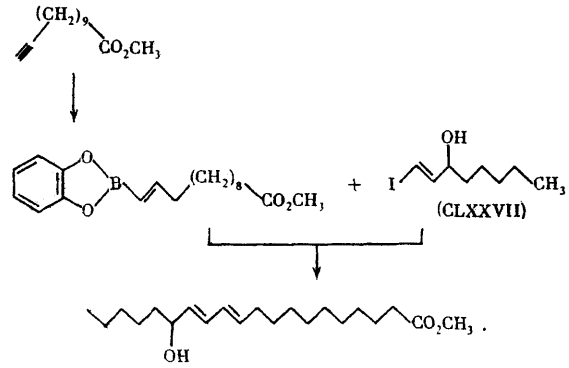
The synthesis of inhibitor analogues of lipoxygenase metabolites is carried out using similar synthetic methods.

Retrosynthetic analysis of 15-hydroxy-11*Z*-13*E*-dienoic acid (XLV) [64] shows that a reasonable method of building up this structure involves the condensation of ω-acetylenic acid (CLXXI)

with hydroxyvinyl halides to form the hydroxyenyne intermediates (CLXX), which are then reduced to the final (*Z*,*E*)-dienes:



Condensation was carried out by using tetrakis(triphenylphosphine)palladium(0) and cuprous iodide in triethylamine. By modifying the conditions it was possible to obtain the (*E*,*E*)-dienes, which were synthesised using 11*E*-carbomethoxy-1-undecylpyrocatechinborane (CLXXVI) and 3-hydroxy-1*E*-octenyl iodide (CLXXVII):



A detailed examination of the methods for synthesising inhibitors and antagonists with a different structure to that of the analogues of acid substrates and lipoxygenase metabolites (monocyclic and condensed heterocycles, phenol derivatives, etc.) is beyond the scope of the present review. These topics are covered in several publications.[119–124]

V. Conclusion

It is clear from the information given in this review that a large number of effective lipoxygenase inhibitors and antagonists of the corresponding metabolites with different chemical structures have been developed up to the present time. However, only a few of them have been used as antiallergic and antiinflammatory drugs;[7,50,93,125] without exception, all of these compounds in terms of their structure are far removed from lipoxygenase substrates and metabolites.

Heterocyclic lipoxygenase inhibitors acting as antioxidants, chelating agents of Fe(III) and via other, often undetermined mechanisms[7,88,90,91,129] have become quite widespread. A number of compounds that have been isolated from plants and microorganisms belong to this group.[126–128]

The synthesis of analogues of the precursor acids of eicosanoids is a promising trend for the development of compounds with antilipoxygenase activity, even though they are generally inactive *in vivo* and cannot be used as drugs. Compounds of this type can be used for a detailed study of the mechanism of conversion of eicosapolyenoic acids by LO and for obtaining information about the nature of groups forming part of the active site.[25,31,32,54]

Special mention should be made concerning inhibitors that do not have direct structural similarities with natural lipoxygenase substrates but with a good isosteric correspondence to the active site of the enzyme, which have been developed with the aid of three-dimensional computer models.[64,65,79]

Antagonists of lipoxygenase metabolites constitute an important independent group of antilipoxygenase compounds whose activity is determined by their ability to block the receptor interactions of eicosanoids.[40–43,72,73,112,123]

Thus, the problem of pharmacological regulation of the arachidonic acid pathway and its lipoxygenase metabolism in particular is an extremely complex, multilevel question and may be resolved only by the continuation of studies in all the existing areas of research into fundamentally new antilipoxygenase agents and factors as well as combining the separate research efforts on the basis of a common approach.

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