A series of stereospecifically labeled polyunsaturated fatty acids were prepared by biosynthesis from [8-Dna-3H]- and [10-Dna-3H]-stearic acids. The labeled stearic acids were synthesized by a novel scheme employing readily available alkyne and aldehyde starting materials. The stereocchemical purity of the prochiral tritium labels was judged to be >99%, as determined by analysis of the octadec-1-yn-8-(R)- and 8(S)-ol intermediates in the synthesis.

Previously, the labeled arachidonic acids were used to investigate the stereoselectivity of hydrogen abstraction in the biosynthesis of leukotriene epoxides. We have now investigated the selectivity of hydrogen abstraction in a chemical synthesis of 14,15-leukotriene (LT) A4 from mixtures of [3-14C]- and either [10-Dna-3H]- or [10-Dma-3H]-15(S)-HPETE methyl esters. Reaction with either chirally labeled precursor led to 70-95% retention of 3H relative to 14C in the 15(S)-LTA4 and 10-Z-14,15-LTA4 products after purification by high performance liquid chromatography. The 15-diene obtained from this reaction was consistently enriched in 3H relative to 14C after isolation and purification. Evidence was obtained to indicate that the majority of the 3H in the products was retained in its original location and configuration. These results indicate that the biomimetic chemical reaction is stereo-random with respect to hydrogen loss from carbon 10 and that, in contrast to the reaction as it occurs in leukocytes and platelets, in the chemical model the reaction begins by decomposition of the hydroperoxide group, with hydrogen loss from carbon 10 occurring as a late or final step.

Studies on leukotriene biosynthesis in leukocytes and platelets have revealed that, during the course of leukotriene A4 and 14,15-leukotriene A4 formation from their respective hydroperoxide precursors 5(S)-HPETE1 and 15(S)-HPETE, stereoselective hydrogen removal occurs from the prochiral center2 at carbon 10 (1-3). While the hydrogen removed from carbon 10 in these two cases has the opposite absolute configurations, being pro-R in the case of LTA4 and pro-S in the case of 14,15-LTA4, it is of interest that in each case the hydrogen which is removed occupies the same relative configuration with respect to the parent hydroperoxide group. It has also been shown for LTA4 biosynthesis that a large primary isotope effect is associated with substitution of 3H into the pro-R position at carbon 10 of 5(S)-HPETE (1, 2). These findings are reminiscent of results obtained in studies on the soybean, platelet, and corn lipoygenase enzymes (4-6). Stereospecific hydrogen abstraction has also been demonstrated for the reactions catalyzed by the cyclooxygenase and RBL cell 5-lipoxynagenase (7, 8). In each case, stereoselective hydrogen removal precedes oxygen insertion and, when 3H is substituted for hydrogen, an isotope effect can be detected. The similarity between the findings obtained for the platelet 12-lipoxynagenase and the formation of 14,15-LTA4 recently led us to propose that biosynthesis of 14,15-LTA4 from 15(S)-HPETE is catalyzed by an enzyme with many mechanistic features in common with the 12-lipoxygenase (3). While the results on leukotriene biosynthesis are clearly consistent with an enzymatic mechanism of formation, it is possible that similar findings might obtain in the nonenzymatic formation of leukotriene epoxides from HPETE precursors. The possibility exists that the observed stereospecificity of hydrogen removal and the accompanying isotope

---

1 The abbreviations used are: 15(S)-HPETE, 15(S)-hydroperoxy-5,8,11Z-13-E-eicosatetraenoic acid; 14,15-LTA4, trans-14(S),15(S)-5Z,8Z,10Z-12-E-eicosatetraenoic acid; 15(dizene, 15-oxo-5,8,11Z-13-E-eicosatetraenoic acid; SP- and RP HPLC, straight-phase and reversed-phase high performance liquid chromatography; GC-MS, gas-liquid chromatography-mass spectrometry; TLC, thin layer chromatography; THF, tetrahydrofuran.

2 The 10-carbon is prochiral in the sense that replacement of either of the two hydrogen atoms attached to it with a new point ligand of different priority results in a chiral assembly. The pro-R (or Dn) hydrogen is that which, when arbitrarily accorded a priority higher than that of the other enantiotopic hydrogen (e.g. replacement with tritium) but not higher than that of the other remaining ligands, results in an assignment of R configuration according to the R/S system. Similarly, the pro-S (or Ln) hydrogen is that which yields an S configuration by application of the same rule. By convention, the pro-R and pro-S nomenclature refers to the configuration in the parent arachidonic acid. This is because introduction of the 15(S)-hydroperoxy group in the formation of 15(S)-HPETE from arachidonic acid results in a reversal of configuration according to strict application of the priority system.
effect might actually be general features of the leukotriene epoxide-forming reaction mechanism and not necessarily reflections of enzymatic control of the reaction. This notion is plausible because the pre-existing chirality of the precursor 5(S)- or 15(S)-hydroperoxide group could conceivably direct the chiral loss of hydrogen from carbon 10, even in a non-enzyme-catalyzed process. Two different nonenzymatic reactions are known to lead to leukotriene epoxide formation from HPETEs. The first of these is the chemical biomimetic reaction, in which a HPETE as the methyl ester is derivatized as the trflate or mesylate in the presence of a highly hindered non-nucleophilic base, with formation of leukotriene epoxide as the major product (9-12). Hydroxy-epoxy derivatives have also been used as precursors for this type of reaction (13). In addition, leukotriene epoxides can also be formed from their corresponding hydroperoxides by autoxidative processes (14, 15).

In order to test the idea that the stereoselectivity and isotope effects observed in the cellular formation of LTA4 and 15,15-LTA4 from 5(S)- and 15(S)-HPETEs really do result from enzymatic control of the reaction, [10-L-3H]- and [10-D-3H]arachidonic acids were prepared and used to investigate the mechanism involved in the two nonenzymatic routes of leukotriene epoxide formation. The present report discusses the stereochemical fidelity with respect to hydrogen loss at carbon 10 in the chemical biomimetic formation of 14,15-LTA4 from 15(S)-HPETE. An accompanying report describes the findings for the autoxidative formation of HPETEs and leukotriene epoxides (15). In neither case is the same pattern of hydrogen loss and isotope effect observed as is found with incubation of these stereolabeled materials with leukocytes and platelets. From these results, we conclude that the key steps in leukotriene epoxide formation by leukocytes and platelets are in fact enzyme catalyzed.

**EXPERIMENTAL PROCEDURES**

The synthetic scheme used to prepare the [10-D-3H]- and [10-L-3H]arachidonic acids and 15(S)-HPETEs is shown in Fig. 1.

**RESULTS**

**Stereochemical Purity of Starting Materials—**In the resolution step of the chemical synthesis, Fig. 1, octadec-6-ynylamine, and the R and S enantiomers were separated by HPLC (see Miniprint). The final preparations of R and S enantiomers were 99.3 and 100% pure, respectively (see Miniprint, Fig. S4). Acetylenic migration to give the terminal alkylkyls did not change the stereochemical purity. Tosylation and treatment with LiAlH4 converted the R and S alcohols to [8-S-3H]- and [8-R-3H]-octadeceylene, respectively. The final preparations of [10-D-3H]- and [10-L-3H]arachidonic acids were shown previously to retain very high stereochemical purity as judged by the stereoselective abstraction of the tritium labels in the enzymatic conversion to HPETEs and leukotrienes (1, 3).

**Epoxide Reaction Employing Unlabeled 15(S)-HPETE Methyl Ester—**The leukotriene epoxide chemical reaction using 15(S)-HPETE methyl ester, performed as described under "Experimental Procedures," resulted in the formation of three main products. These were identified, in complete accord with published work, as methyl trans-14(S),15(S)-oxido-5,8,12-E-eicosatetraenoate (14,15-LTA4 methyl ester), methyl trans-14(S),15(S)-oxido-5,8,10Z-12-E-eicosatetraenoate (10Z-14,15-LTA4, methyl ester), and methyl 15-oxo-5,8,11Z-13-E-eicosatetraenoate (15-dieneonone methyl ester). Small amounts of conjugated pentaene, presumably formed as an elimination product, were observed in some reactions. This side product showed λmax (hexane) at 376, 357, and 319 nm and eluted just after the solvent front on SP-HPLC. In experiments employing radioactive substrates, no other radioactive reaction products could be detected, and no 15(S)-HPETE methyl ester or 15(S)-HETE methyl ester were recovered. Following extractive isolation, the crude yield of the three main products assayed from λmax at 280 nm totaled 40-50%.

SP-HPLC analysis demonstrated the presence of two main peaks absorbing in the UV at 280 nm, as shown for the case of an experiment involving the 15(S)-HPETE methyl ester derived from the pro-S-labeled arachidonic acid, Fig. 2. The first eluting peak was 14,15-LTA4, methyl ester, which after SP-HPLC was nearly, but not completely, pure. The second peak consisted of 10Z,14,15-LTA4, methyl ester and the 15-dieneonone methyl ester, which was marginally resolved from the epoxide, chromatographing in the back half of the second UV absorbing peak. In later experiments, using a newer chromatographic column, the 15-diene and 10Z,14,15-LTA4 could be resolved to about a 10% trough. In either case, the three reaction products were purified to homogeneity, Fig. 3, by recycling them over SP-HPLC or, as described below, by RP-HPLC.

Resolution of these products by SP-HPLC revealed that 14,15-LTA4, methyl ester was the major product in all experiments. When the 10Z,14,15-LTA4, methyl ester and the 15-dieneonone methyl ester in the second UV absorbing peak were resolved, the molar distribution of the three products of the reaction could be calculated as follows: 49 ± 8% 14,15-LTA4, methyl ester, 25 ± 11% 10Z,14,15-LTA4, methyl ester, and 26 ± 6% 15-dieneonone methyl ester (mean ± S.D., n = 6), or a ratio of about 2:1:1. As reported by Corey and Barton (12), we observed that recovery of 14,15-LTA4 methyl ester from SP-HPLC was highly dependent on the length of time spent on the chromatographic column. Using a solvent system composed of 1% triethylamine in hexane and varying the column flow rate from 2.0 to 1.0 to 0.5 ml/min resulted in ratios of the first, 14,15-LTA4, peak to the second peak, comprised of 10Z,14,15-LTA4, and the stable 15-diene, of 2.6, 2.2, and 1.6, respectively. A flow rate of 1.0 ml/min was selected as offering the best compromise between resolution and yield. The final isolated yield of analytically pure 14,15-LTA4, methyl ester in these experiments after SP-HPLC purification ranged from 8 to 18%, with corresponding recoveries of the other two products.

**Epoxide Reactions Employing 15(S)-HPETE Methyl Esters Prepared from [3-14C,10-L-3H]- and [3-14C,10-D-3H]Arachidonic Acids—**The biomimetic epoxide reaction was conducted using 15(S)-HPETE methyl ester (3.6 mg, 150,000 dpm 14C, 40,000 dpm 3H) derived from pro-S-[3H]arachidonic acid and the resulting products separated by SP-HPLC. The eluate was monitored with UV detection at 280 nm, and aliquots of individual fractions were counted by liquid scintillation counting. The three main products appeared as four main radioactive and UV absorbing peaks, with the 15-diene-onone present as a shoulder on the backside of the peak corre-
On the Chemical Conversion of HPETE to Leukotriene Epoxide

1. Synthetic scheme used to prepare stereospecifically labeled [8-D_2-^3_H] and [10-D_2-^3_H] stearic acids and [10-L_2-^3_H] and [10-L_2-^3_H] arachidonic acid and 15(S)-HPETEs. The tosylation-LiAlH_4 reduction sequence is associated with inversion of configuration at the asymmetric center. Absolute stereochemistry and enantiomeric purity were assigned from the propargyl intermediate and the terminal alkynol (intermediates 2 and 3 in the scheme). For experimental details and pertinent spectroscopic data see Miniprint Supplement. The 15(S) HPETEs used in the experiment were formed from the arachidonic acids by reaction with the soybean lipoxygenase followed by extraction and esterification in CH_2N_2.

2. SP-HPLC separation of the reaction products obtained from the biomimetic chemical reaction performed on [3-14C,10-L_2-^3_H] 15(S)-HPETE methyl ester. UV chromatogram of the reaction products at 280 nm and radiochromatogram of the products (from a separate injection). Column: Waters Spherisorb, 10 μm, silicic acid, 1% triethylamine in UV grade hexane, 1.0 ml/min, 25 °C, 200 p.s.i. 0.25-mI fractions. The three major products were collected and further purified by additional cycles of SP-HPLC or RP-HPLC (see Fig. 4).

3. SP-HPLC separation of the reaction products obtained from the biomimetic chemical reaction performed on [3-14C,10-L_2-^3_H] 15(S)-HPETE methyl ester. UV chromatogram of the reaction products at 280 nm and radiochromatogram of the products (from a separate injection). Column: Waters Spherisorb, 10 μm, silicic acid, 1% triethylamine in UV grade hexane, 1.0 ml/min, 25 °C, 200 p.s.i. 0.25-mI fractions. The three major products were collected and further purified by additional cycles of SP-HPLC or RP-HPLC (see Fig. 4).

In a second experiment involving the pro-S-labeled material, the 10Z-14,15-LTA_4 methyl ester isolated by SP-HPLC was heated at 55 °C in benzene under argon for 12 h. This resulted in quantitative conversion to the more stable non-allylic conjugated tetraene epoxide, methyl trans-14(S),15(S)-oxido-5,9,11,Z-E-eicosatetraenoate. The latter compound could be chromatographed on RP-HPLC without breakdown or hydrolysis, Fig. 4. This step also served to remove traces of 15-dienone from the conjugated tetraene epoxide. Control experiments employing the 10Z-14,15-LTA_4 obtained from [1-14C,5,6,8,9,11,12,14,15-^3_H] 15(S)-HPETE methyl ester demonstrated that there was no significant change in ratio of ^3 H/14C involved in the intramolecular (1, 7) hydrogen rearrangement reaction associated with the thermal conversion to conjugated tetraene.

The results of liquid scintillation counting to high accuracy of the chromatographically pure products obtained from the leukotriene epoxide reaction using 15(S)-HPETE methyl ester derived from [10-L_2-^3_H] arachidonic acid are shown in Table I. The retention of ^3 H relative to 14C in the 14,15-LTA_4 and 10Z-14,15-LTA_4 products ranged from 77 to 85%, while the 15-dienone methyl ester underwent an enrichment in ^3 H relative to 14C, with 109 to 129% ^3 H/14C relative to starting 15(S)-HPETE methyl ester. These results indicate a modest apparent loss of ^3 H in the formation of the epoxide reaction products. On a molar basis, the ^3 H enrichment observed in the 15-dienone can account only for about 30% of the ^3 H loss in the epoxide products. It is likely that the remainder of the residual ^3 H is accounted for by that found in the aqueous phase after extraction of the reaction products with organic solvent.

The workup and analysis of experiments conducted using 15(S)-HPETE methyl ester obtained from pro-R-[^3_H] arachidonic acid were similar to that described for the pro-S experiments. 14,15-LTA_4 methyl ester was purified by SP-HPLC. These experiments led to the result that the 14,15-LTA_4 methyl ester retained from 89 to 95% of the ^3 H to 14C specific activities found in the starting material.

Because the combination of initial SP-HPLC purification, followed by conversion to conjugated tetraene and RP-HPLC purification, was considered a particularly powerful purification scheme, the 10Z-14,15-LTA_4 methyl ester in both cases was subjected to this sequence. The rigorously purified conjugated tetraene epoxide methyl ester obtained in this manner had from 70 to 74% of the starting 15(S)-HPETE methyl ester ratio of ^3 H to 14C-specific activities. The 15-dienone...
 isolated from reaction with 15(S)-HPETE methyl ester prepared from pro-R-labeled arachidonic acid was also purified by sequential SP HPLC and RP HPLC. In addition to HPLC, the 15-dienone was found homogeneous by UV spectroscopy. Liquid scintillation counting showed 111 to 113% of the ^1H/^13C ratio of specific activities as found for the starting 15(S)-HPETE methyl ester. Sample conditions exactly as in Fig. 2.

**purity of products** — The purity of all products was assayed by SP-HPLC with UV detection and by UV spectroscopy (Figs. 3 and 5). Liquid scintillation of individual SP- and RP-HPLC fractions was also performed. In some instances, epoxide samples were subject to ^1H Fourier transform NMR at 90 MHz. In other cases, an aliquot of the SP-HPLC-purified epoxides was treated with dimethylethylamine-pH 3 water to effect conversion to hydrolysis products, and the purity of these was assessed by RP-HPLC with UV detection.

**discussion**

**reaction mechanism in the biomimetic chemical reaction** — The present results clearly demonstrate that the cellular and chemical formation of leukotriene epoxides from their HPETE precursors are mechanistically distinct. The cellular biosynthesis of LTA_4, or 14,15-LTA_4, from 5- or 15-HPETE is accompanied by stereoselective hydrogen loss from carbon 10 and also by a substantial primary isotope effect, detected by progressive tritium enrichment in unused substrate as the reaction proceeds. In addition, a smaller secondary isotope effect can also be detected when experiments are performed using substrate containing ^3H label geminal to the site of stereoselective hydrogen abstraction. In contrast, the data presented in Table I indicate that hydrogen loss from carbon...
absorbances shown do not reflect the actual molar absorptivities.

D, cation at C-13, followed by loss of a proton from carbon 10 as progressively would be consistent with hydrogen loss from carbon triene biosynthesis. In leukotriene biosynthesis, hydrogen loss from carbon 10 does not occur as the initial step in the chemical reaction mechanism as it does in leukotriene biosynthesis. In leukotriene biosynthesis, hydrogen abstraction from carbon 10 precedes or coincides with the attainment of the activation energy required for the reaction (1-3). In all likelihood, hydrogen removal is in fact the activation energy-requiring process. Hence, the $^1H$-labeled substrate reaches the transition state at a slower rate than the $^{13}C$ standard, thus leaving the unconverted substrate pool progressively $^1H$-enriched as the reaction progresses. In contrast, in the chemical reaction, the data presented in Table I would be consistent with hydrogen loss from carbon 10 occurring after the required activation energy along the reaction coordinate has been attained. This would be the case if the chemical reaction were initiated by elimination of triflic anion accompanied by concomitant formation of an allylic carbocation at C-13, followed by loss of a proton from carbon 10 as a late or ultimate step. Such a scheme, involving formation of a carbonium ion intermediate via electrophilic attack of the peroxo triflate, with final loss of a proton from C-10, is summarized in Fig. 6. In this case, once having surpassed the transition state in the rate-determining step, the carbonium ion intermediate must either undergo obligate loss of a proton from C-10 to yield the leukotriene epoxides or, alternatively, decompose to a different product. In the event of stereospecific substitution with tritium at C-10, the geminal proton to C-10 bond is broken more rapidly than the tritium to C-10 bond, resulting in net $^3H$ retention in the epoxide products, in excess of 50%. This thus accounts for $^3H$ retentions on the order of 70-95% in the leukotriene epoxides in the present experiments. It should be noted that such a finding is not entirely surprising. Previously, Corey et al. (16) reported an analogous reaction involving the triterpene 11α,12α-oxidotaraxerol in which it was postulated that oxirane formation from a hydroperoxide precursor might occur with electrophilic attack of the hydroperoxide group on an allylic double bond to yield a carbocation intermediate, followed by a subsequent rearrangement and proton loss. Thus, the biomimetic chemical formation of leukotriene epoxides is not truly "biomimetic," because although both the cellular enzymatic and chemical processes result in conversion of a hydroperoxide to a leukotriene epoxide, they proceed by entirely different mechanisms.

One additional experimental finding that must be incorporated into the proposed mechanism for the chemical biomimetic reaction relates to the observed $^3H/^4C$ enrichment in the 15-dienone reaction product. While this product clearly can and undoubtedly does in part originate from 15(S)-HPETE methyl ester as substrate indicates that loss of hydrogen from carbon 10 is a late step in the rate-determining step in the chemical reaction mechanism as it does in leukotriene biosynthesis. In leukotriene biosynthesis, hydrogen abstraction from carbon 10 precedes or coincides with the attainment of the activation energy required for the reaction (1-3). In all likelihood, hydrogen removal is in fact the activation energy-requiring process. Hence, the $^1H$-labeled substrate reaches the transition state at a slower rate than the $^{13}C$ standard, thus leaving the unconverted substrate pool progressively $^1H$-enriched as the reaction progresses. In contrast, in the chemical reaction, the data presented in Table I would be consistent with hydrogen loss from carbon 10 occurring after the required activation energy along the reaction coordinate has been attained. This would be the case if the chemical reaction were initiated by elimination of triflic anion accompanied by concomitant formation of an allylic carbocation at C-13, followed by loss of a proton from carbon 10 as a late or ultimate step. Such a scheme, involving formation of a carbonium ion intermediate via electrophilic attack of the peroxo triflate, with final loss of a proton from C-10, is summarized in Fig. 6. In this case, once having surpassed the transition state in the rate-determining step, the carbonium ion intermediate must either undergo obligate loss of a proton from C-10 to yield the leukotriene epoxides or, alternatively, decompose to a different product. In the event of stereospecific substitution with tritium at C-10, the geminal proton to C-10 bond is broken more rapidly than the tritium to C-10 bond, resulting in net $^3H$ retention in the epoxide products, in excess of 50%. This thus accounts for $^3H$ retentions on the order of 70-95% in the leukotriene epoxides in the present experiments. It should be noted that such a finding is not entirely surprising. Previously, Corey et al. (16) reported an analogous reaction involving the triterpene 11α,12α-oxidotaraxerol in which it was postulated that oxirane formation from a hydroperoxide precursor might occur with electrophilic attack of the hydroperoxide group on an allylic double bond to yield a carbocation intermediate, followed by a subsequent rearrangement and proton loss. Thus, the biomimetic chemical formation of leukotriene epoxides is not truly "biomimetic," because although both the cellular enzymatic and chemical processes result in conversion of a hydroperoxide to a leukotriene epoxide, they proceed by entirely different mechanisms.

One additional experimental finding that must be incorporated into the proposed mechanism for the chemical biomimetic reaction relates to the observed $^3H/^4C$ enrichment in the 15-dienone reaction product. While this product clearly can and undoubtedly does in part originate from 15(S)-HPETE methyl ester as substrate indicates that loss of hydrogen from carbon 10 is a late step in the rate-determining step in the chemical reaction mechanism as it does in leukotriene biosynthesis. In leukotriene biosynthesis, hydrogen abstraction from carbon 10 precedes or coincides with the attainment of the activation energy required for the reaction (1-3). In all likelihood, hydrogen removal is in fact the activation energy-requiring process. Hence, the $^1H$-labeled substrate reaches the transition state at a slower rate than the $^{13}C$ standard, thus leaving the unconverted substrate pool progressively $^1H$-enriched as the reaction progresses. In contrast, in the chemical reaction, the data presented in Table I would be consistent with hydrogen loss from carbon 10 occurring after the required activation energy along the reaction coordinate has been attained. This would be the case if the chemical reaction were initiated by elimination of triflic anion accompanied by concomitant formation of an allylic carbocation at C-13, followed by loss of a proton from carbon 10 as a late or ultimate step. Such a scheme, involving formation of a carbonium ion intermediate via electrophilic attack of the peroxo triflate, with final loss of a proton from C-10, is
HPETE by simple, 1,2-elimination, the finding of \(^{3}H/^{14}C\) enrichment in the dienone indicates that the situation in the present reaction is probably more complex. This is because there is no required loss of hydrogen from carbon 10 in conversion of 15(S)-HPETE methyl ester to 15-dieneone methyl ester. Thus, in the case of simple, 1,2-elimination, there should be no change in \(^{3}H/^{14}C\) ratio. Tritium enrichment in the 15-dieneone likely indicates that this reaction by-product can also be formed from intermediate(a) generated after collapse of the hydroperoxy triflate but prior to the expulsion of hydrogen from C-10 that occurs as a late if not ultimate step in the reaction process. The fact that some \(^{3}H\) is apparently lost from the epoxide reaction products is consistent with either actual loss of \(^{3}H\) from the carboxylation intermediate or, alternatively, with decomposition to some other reaction product which would be tritium containing and possibly, tritium-enriched.

An alternate explanation for the above findings which was excluded related to the unlikely possibility that tritium migration to other sites within the product molecules during the course of the reaction was responsible for the observed retention of \(^{3}H\) in excess of 50\%, following reaction with stereolabeled substrate. This possibility was dismissed by the findings that 1) reaction conducted using [5,6,8,9,12,14,15-\(^{3}H\)] 15(S)-HPETE methyl ester led to complete retention of all eight deuteriums in their original positions in the epoxide-derived hydrolysis products when the latter were analyzed by gas chromatography-mass spectrometry, and 2) reductive ozonolysis of the 8,15-dihydroxy acids derived from 14,15-LTA\(_{2}\) as their methyl ester acetates and also analysis of the 15-dieneone by sodium borohydride reduction, saponification, and reaction with soybean lipoygenase, which established that the \(^{3}H\) in these products was in its original position and configuration (Miniprint).

The present study indicates that the chemical conversion of 15(S)-HPETE to 14,15-LTA\(_{2}\), and presumably also the analogous conversion of 5(S)-HPETE to LTA\(_{2}\), occurs by a mechanism which is different from that involved in the biosynthesis of these compounds. The chemical conversion of hydroperoxide to leukotriene epoxide would thus not appear to be a useful model of the enzyme-catalyzed reaction. Studies described in the companion paper concerning the autoxidative formation of 14,15-LTA\(_{2}\) from 15(S)-HPETE reveal that process to be stereorandom with respect to hydrogen loss from carbon 10 and to proceed with hydrogen loss as an early or initial step. The chemical, autoxidative, and cellular formation of leukotriene epoxides can all three be clearly distinguished by this type of analysis, which thus establishes the premise that the latter process, as it occurs in platelets and leukocytes, is an enzymatic process.

Acknowledgment—The skillful technical assistance of John Law-son in recording some of the mass spectra is gratefully acknowledged.

REFERENCES

1. Maas, R. L., Ingram, C. D., Taber, D. F., Oates, J. A., and Brash, A. R. (1982) J. Biol. Chem. 257, 13515–13519
2. Panossian, A., Hamberg, M., and Samuelsson, B. (1982) FEBS Lett. 150, 511–513
3. Maas, R. L., and Brash, A. R. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2884–2888
4. Hamberg, M., and Samuelsson, B. (1967) J. Biol. Chem. 242, 5329–5335
5. Hamberg, M., and Hamberg, G. (1980) Biochem. Biophys. Res. Commun. 95, 1090–1097
6. Egmond, M. R., Vliegenthart, J. F. G., and Boldingh, J. (1972) Biochem. Biophys. Res. Commun. 48, 1055–1060
7. Hamberg, M., and Samuelsson, B. (1980) Biochim. Biophys. Acta 617, 545–547
8. Corey, E. J., and Lansbury, P. T., Jr. (1983) J. Am. Chem. Soc. 105, 4093–4094
9. Corey, E. J., Barton, A. E., and Clark, D. A. (1980) J. Am. Chem. Soc. 102, 4278–4279
10. Corey, E. J., Marfat, A., and Goto, G. (1980) J. Am. Chem. Soc. 102, 6697–6608
11. Atrache, V., Pai, J-K., Sok, D-E., and Sih, C. J. (1981) Tetrahe- dron Lett. 22, 3443–3446
12. Corey, E. J., and Barton, A. E. (1982) Tetrahedron Lett. 23, 2351–2354
13. Corey, E. J., Mehrotra, M. M., and Cashman, J. R. (1983) Tetrahedron Lett. 24, 4917–4920
14. Sok, D-E., Chung, T., and Sih, C. J. (1983) Biochim. Biophys. Res. Commun. 110, 273–279
15. Brash, A. R., Porter, A. T., and Maas, R. L. (1984) J. Biol. Chem. 260, 4210–4216
16. Agata, I., Corey, E. J., Hortmann, A. G., Klein, J., Proskow, S., and Ursprung, J. J. (1965) J. Org. Chem. 30, 1688–1710
17. Schroepfer, G. J., Jr., and Bioch, K. (1965) J. Biol. Chem. 240, 54–63
18. Hamberg, M., and Samuelsson, B. (1967) J. Biol. Chem. 242, 5336–5343
19. Egmond, M. R., Vliegenthart, J. F. G., and Boldingh, J. (1973) Tetrahe- dron Lett. 24, 546–547
20. Gottstein, W. J., and Chesney, L. C. (1965) J. Org. Chem. 30, 2072–2073
21. Taber, D. F. (1982) J. Org. Chem. 47, 1351–1352
22. Corey, E. J., and Hashimoto, S-i. (1981) Tetrahedron Lett. 22, 299–302
23. Hamberg, M. (1971) Anal. Biochem. 43, 515–526
24. Hammarström, S. (1975) Methods Enzymol. 35, 326–334
25. Genzer, W. J., and Schlein, H. N. (1955) J. Am. Chem. Soc. 77, 4846–4849
26. Brown, C. A. (1974) J. Org Chem 39, 3913–3918
27. Brown, C. A., and Yamashita, A. (1975) J. Am. Chem. Soc. 97, 891–892
28. Brown, C. A., and Yamashita, A. (1976) J. C. S. Chem. Commun. 959–960
29. Midland, M. M., Haltermann, R. L., Brown, C. A., and Yamachi, A. (1981) Tetrahedron Lett. 22, 4171–4172
30. Midland, M. M., and Lee, P. E. (1981) J. Org. Chem. 46, 3933–3934
31. Woodgate, P. D., Mayer, K. K., and Djerassi, C. (1972) J. Am. Chem. Soc. 94, 3115–3124
32. Brown, H. C. (1975) Organic Synthesis via Boranes, pp. 28–29, John Wiley and Sons, New York
33. Zweifel, G., Ayangar, N. R., and Brown, H. C. (1963) J. Am. Chem. Soc. 85, 2072–2075
34. Zweifel, G., Clark, G. M., and Polston, N. L. (1971) J. Am. Chem. Soc. 93, 3395–3399
35. Zweifel, G., and Bucqlund, S. J. (1977) J. Am. Chem. Soc. 99, 3184–3185
36. Gellerman, J. L., and Schlenk, H. (1979) Biochim. Biophys. Acta 573, 23–30
37. Hamberg, M. (1980) Biochim. Biophys. Acta 618, 386–398
38. Jubiz, W., Räddmark, O., Lindgren, J. A., Malmsten, C., and Samuelsson, B. (1981) Biochem. Biophys. Res. Commun. 99, 976–982
39. Van Os, C. P. A., Rijke-Schilder, G. P. M., Van Halbeek, H., Verhage, J., and Vliegenthart, J. F. G. (1981) Biochim. Bio-phys. Acta 683, 177–193
The Chemical Conversion of HPETE to Leukotriene Epoxide

Preparation of Tetrafluoro-Labeled Labeled Arachidonic Acid

The use of stereoregularly labeled fatty acids to investigate an enzymatic reaction mechanism was initially facilitated by Schreiber and Blatt (17), who demonstrated stereoselective production of the practical 9- and 10-D-hydroxy-arachidonic acids via the desaturation of 13,14-D-trans-arachidonic acid (16:1,13-14-9H) and (16:1,13-14-1H) acids were prepared from 9-D- and 10-D-hydroxy-16:1 acid via reactivity as the cyclohexane salt, acetylation, and acetic coupling, followed by the same acetylation, LAD-1 AcO, sequence employed by Schreiber and Blatt (17). These reactions were then converted to their respective 13H-labeled diastereoisomers by desaturation with TBAH in benzene and selective elution by column chromatography (18).

Flanking acylation and saponification this approach in the investigation of the stereochemistry (19, 20) and cyclohexane resins (26, 27) of 9-D, 10-D, and 16:1,13-14-1H acid were prepared from 9-D- and 10-D-hydroxy-16:1 acid via reactivity as the cyclohexane salt, acetylation, and acetic coupling, followed by the same acetylation, LAD-1 AcO, sequence employed by Schreiber and Blatt (17). These reactions were then converted to their respective 13H-labeled diastereoisomers by desaturation with TBAH in benzene and selective elution by column chromatography (18).

To the reaction mixture was added 1.2 ml of 1.55 n-butyl lithium in hexane (16.46 mmol, 1.14 eq, without an ice-bath). After 5 min, underlithium 18.78 mmol was added and the mixture was allowed to react for 30 min. TLC showed 16% of hexane B (80%) and 14% with no added base (70% in hexane B) (18). For each reaction, after 5 min, the mixture was added to a 15 ml separatory funnel and then all were formed as an orange upper phase. Additional 1.0 ml was added and the mixture was refluxed with a few ml of Hex. The reaction mixture was extracted with 3 x 5 ml CHCl3, dried over Na2SO4, filtered, and evaporated to yield 1.56 g of pure (S)-2-(4-hydroxy-2-napthyl)alanine (100% yield). The product was stored in 9 ml CH2Cl2 at -20°C.

Spectra are shown in Figs. 1 and 2.

The reaction mixture was added to a 15 ml separatory funnel and all were formed as an orange upper phase. Additional 1.0 ml was added and the mixture was refluxed with a few ml of Hex. The reaction mixture was extracted with 3 x 5 ml CHCl3, dried over Na2SO4, filtered, and evaporated to yield 1.56 g of pure (S)-2-(4-hydroxy-2-napthyl)alanine (100% yield). The product was stored in 9 ml CH2Cl2 at -20°C.

Spectra are shown in Figs. 1 and 2.

Preparation of 2-Chloro-3-R-HPETE

The reaction mixture was added to a 15 ml separatory funnel and all were formed as an orange upper phase. Additional 1.0 ml was added and the mixture was refluxed with a few ml of Hex. The reaction mixture was extracted with 3 x 5 ml CHCl3, dried over Na2SO4, filtered, and evaporated to yield 1.56 g of pure (S)-2-(4-hydroxy-2-napthyl)alanine (100% yield). The product was stored in 9 ml CH2Cl2 at -20°C.

Spectra are shown in Figs. 1 and 2.

Preparation of 2-Chloro-3-R-HPETE

The reaction mixture was added to a 15 ml separatory funnel and all were formed as an orange upper phase. Additional 1.0 ml was added and the mixture was refluxed with a few ml of Hex. The reaction mixture was extracted with 3 x 5 ml CHCl3, dried over Na2SO4, filtered, and evaporated to yield 1.56 g of pure (S)-2-(4-hydroxy-2-napthyl)alanine (100% yield). The product was stored in 9 ml CH2Cl2 at -20°C.

Spectra are shown in Figs. 1 and 2.

Preparation of 2-Chloro-3-R-HPETE

The reaction mixture was added to a 15 ml separatory funnel and all were formed as an orange upper phase. Additional 1.0 ml was added and the mixture was refluxed with a few ml of Hex. The reaction mixture was extracted with 3 x 5 ml CHCl3, dried over Na2SO4, filtered, and evaporated to yield 1.56 g of pure (S)-2-(4-hydroxy-2-napthyl)alanine (100% yield). The product was stored in 9 ml CH2Cl2 at -20°C.

Spectra are shown in Figs. 1 and 2.

Preparation of 2-Chloro-3-R-HPETE

The reaction mixture was added to a 15 ml separatory funnel and all were formed as an orange upper phase. Additional 1.0 ml was added and the mixture was refluxed with a few ml of Hex. The reaction mixture was extracted with 3 x 5 ml CHCl3, dried over Na2SO4, filtered, and evaporated to yield 1.56 g of pure (S)-2-(4-hydroxy-2-napthyl)alanine (100% yield). The product was stored in 9 ml CH2Cl2 at -20°C.

Spectra are shown in Figs. 1 and 2.
On the Chemical Conversion of HPETE to Leukotriene Epoxide

Preparation of 5-KAPA

The above mixture was transferred to a 3 mL reaction vessel to which were added 500 mg of tetrachloroethane, 10 mL of benzene, and NHCl. The mixture was then refluxed on a water bath at 90°C. After 2 h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (100 g) using a gradient of 10% ethyl acetate in hexane to 100% ethyl acetate. The major fraction was collected and concentrated under reduced pressure, and the residue was used for further studies.

Preparation of 10-HETE

The above mixture was transferred to a 3 mL reaction vessel to which were added 500 mg of tetrachloroethane, 10 mL of benzene, and NHCl. The mixture was then refluxed on a water bath at 90°C. After 2 h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (100 g) using a gradient of 10% ethyl acetate in hexane to 100% ethyl acetate. The major fraction was collected and concentrated under reduced pressure, and the residue was used for further studies.

Preparation of 11,12-EET

The above mixture was transferred to a 3 mL reaction vessel to which were added 500 mg of tetrachloroethane, 10 mL of benzene, and NHCl. The mixture was then refluxed on a water bath at 90°C. After 2 h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (100 g) using a gradient of 10% ethyl acetate in hexane to 100% ethyl acetate. The major fraction was collected and concentrated under reduced pressure, and the residue was used for further studies.

Preparation of 15-HPETE

The above mixture was transferred to a 3 mL reaction vessel to which were added 500 mg of tetrachloroethane, 10 mL of benzene, and NHCl. The mixture was then refluxed on a water bath at 90°C. After 2 h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (100 g) using a gradient of 10% ethyl acetate in hexane to 100% ethyl acetate. The major fraction was collected and concentrated under reduced pressure, and the residue was used for further studies.

Preparation of 12-HETE

The above mixture was transferred to a 3 mL reaction vessel to which were added 500 mg of tetrachloroethane, 10 mL of benzene, and NHCl. The mixture was then refluxed on a water bath at 90°C. After 2 h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (100 g) using a gradient of 10% ethyl acetate in hexane to 100% ethyl acetate. The major fraction was collected and concentrated under reduced pressure, and the residue was used for further studies.

Preparation of 13-HETE

The above mixture was transferred to a 3 mL reaction vessel to which were added 500 mg of tetrachloroethane, 10 mL of benzene, and NHCl. The mixture was then refluxed on a water bath at 90°C. After 2 h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (100 g) using a gradient of 10% ethyl acetate in hexane to 100% ethyl acetate. The major fraction was collected and concentrated under reduced pressure, and the residue was used for further studies.

Preparation of 14-HETE

The above mixture was transferred to a 3 mL reaction vessel to which were added 500 mg of tetrachloroethane, 10 mL of benzene, and NHCl. The mixture was then refluxed on a water bath at 90°C. After 2 h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (100 g) using a gradient of 10% ethyl acetate in hexane to 100% ethyl acetate. The major fraction was collected and concentrated under reduced pressure, and the residue was used for further studies.

Preparation of 15-HETE

The above mixture was transferred to a 3 mL reaction vessel to which were added 500 mg of tetrachloroethane, 10 mL of benzene, and NHCl. The mixture was then refluxed on a water bath at 90°C. After 2 h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (100 g) using a gradient of 10% ethyl acetate in hexane to 100% ethyl acetate. The major fraction was collected and concentrated under reduced pressure, and the residue was used for further studies.
On the Chemical Conversion of HPETE to Leukotriene Epoxide

CH_{3}CH(OH)CH_{2}CH(OH)CH_{2}CH(=O)H, in a volume of 50 ml at 18.2 to 6.00 mm, 26.5 or 7.60 mm, 10 mg. After initial mitotane 
conditioning, appropriate fractions were condensed, diluted with 50 ml of CHCl_{3}, and concentrated to a small volume. This
extract was with 3 equal vol of CHCl_{3} which were condensed, washed with MgSO_{4} dried under N_{2} and
filtered through Celite. The filtrate was vacuum dried. The residue was purified by the addition of the
chemical extract and the same derivative was obtained by 10 mmol of CHCl_{3} which was obtained
in the manner described. The yield of the reaction with the derivative was 93%.

Following completion of the reaction preparative TLC of the crude reaction mixture was treated with
methanol and water to precipitate 1H.TK to obtain 49.5 mmol of a crude reaction mixture. The crude reaction
mixture was then separated into 1H.TK and 15R-TK was recovered by RP-HPLC. All aliquots were treated to the
preparation of 1H.TK. Careful choice of the 1H.TK content of the crude reaction mixture allowed for
the isolation of the desired derivatives. The reaction mixture was then subjected to RP-HPLC and the
UV absorption at 214 nm. The fraction containing 1H.TK was collected and concentrated to
a small volume. The residue was then subjected to RP-HPLC and the yield of the reaction was 93%.

Degradation Reaction of 15-Desoxo-15,12,13,14-Tetrahydroleukotriene Eueoxide (15DTE) with 15-Desoxo-15,12,13,14-Tetrahydroleukotriene Eueoxide 15DTE was obtained by RP-HPLC. The 15DTE was subjected to RP-HPLC and the yield was 93%.

Data in Table 1 indicate that, as expected, 15DTE was the most reactive of the various compounds. The data
prior to the development for the reaction mixture described above was obtained from the
preparation of 1H.TK. The absence of 1H.TK and 15DTE was confirmed by UV and NMR spectroscopy.

Following completion of the reaction with 1H.TK and isolation of 15DTE, the reaction mixture was treated
with methanol and water to precipitate 1H.TK to obtain 49.5 mmol of a crude reaction mixture. The crude reaction
mixture was then subjected to RP-HPLC and the UV absorption at 214 nm. The fraction containing 1H.TK
was collected and concentrated to a small volume. The residue was then subjected to RP-HPLC and the yield of
the reaction was 93%.

Degradation Reaction of 15-Desoxo-15,12,13,14-Tetrahydroleukotriene Eueoxide (15DTE) with 15-Desoxo-15,12,13,14-Tetrahydroleukotriene Eueoxide 15DTE was obtained by RP-HPLC. The 15DTE was subjected to RP-HPLC and the yield was 93%.

Data in Table 1 indicate that, as expected, 15DTE was the most reactive of the various compounds. The data
prior to the development for the reaction mixture described above was obtained from the
preparation of 1H.TK. The absence of 1H.TK and 15DTE was confirmed by UV and NMR spectroscopy.

Following completion of the reaction with 1H.TK and isolation of 15DTE, the reaction mixture was treated
with methanol and water to precipitate 1H.TK to obtain 49.5 mmol of a crude reaction mixture. The crude reaction
mixture was then subjected to RP-HPLC and the UV absorption at 214 nm. The fraction containing 1H.TK
was collected and concentrated to a small volume. The residue was then subjected to RP-HPLC and the yield of
the reaction was 93%.

Degradation Reaction of 15-Desoxo-15,12,13,14-Tetrahydroleukotriene Eueoxide (15DTE) with 15-Desoxo-15,12,13,14-Tetrahydroleukotriene Eueoxide 15DTE was obtained by RP-HPLC. The 15DTE was subjected to RP-HPLC and the yield was 93%.

Data in Table 1 indicate that, as expected, 15DTE was the most reactive of the various compounds. The data
prior to the development for the reaction mixture described above was obtained from the
preparation of 1H.TK. The absence of 1H.TK and 15DTE was confirmed by UV and NMR spectroscopy.

Following completion of the reaction with 1H.TK and isolation of 15DTE, the reaction mixture was treated
with methanol and water to precipitate 1H.TK to obtain 49.5 mmol of a crude reaction mixture. The crude reaction
mixture was then subjected to RP-HPLC and the UV absorption at 214 nm. The fraction containing 1H.TK
was collected and concentrated to a small volume. The residue was then subjected to RP-HPLC and the yield of
the reaction was 93%.
The Chemical Conversion of HPETE to Leukotriene Epoxide

In order to ascertain whether or not any secondary isotope effects were associated with the hydrolysis of 15-14CI and 16-14CI methyl esters and with the rearrangement of 15-2,14-15-LTA4 methyl ester to its conjugated tetraene, the following experiments were performed. A portion of purified 15-2,14-15-LTA4 methyl ester was subjected to acid hydrolysis and the 8,15-dihydroxy esters which were formed were purified by RP-HPLC and quantified by high accuracy. The results of this test indicated that there was only a small change in the ratio of 15-2 to 14-2 15-LTA4 methyl ester to its conjugated tetraene. A portion of purified 15-2,14-15-LTA4 methyl ester was converted to its stable conjugated tetraene epoxide by heating in benzene under argon at 95°C for 5 h and the conjugated tetraene epoxide was purified by RP-HPLC. The results of this experiment indicated that there was again only a small change in the ratio of specific activities of 15-2 and 14-2 involved in this rearrangement reaction. Thus, no correction was needed in the ratio of 15-2 to 14-2 in the experiments in which the 15-2,14-15-LTA4 methyl ester was determined as its conjugated tetraene.

Conversion of 13,14-diene Methyl Ester (15/14) to 8,15(14)-dihydroxy Methyl Ester (15/14) (Fig. 2)—The 13,14-diene methyl ester ca. 15 mg was dissolved in 1.5 ml of absolute EtOH at 5°C containing 1.5 mg/ml NaBH₄, 1 Molar quantitative reduction proceeds over ca. 1 h at 5°C in EtOH, monitored continuously in the UV (λ max 293 nm changing to 295 nm). Excess NaBH₄ was decomposed with glacial HOAc, Me₂SO was added, and the 8,15(14)-dihydroxy methyl ester as obtained was extracted 3 times into CH₂Cl₂. Purification was carried out using 1 N HClO₄/Me₂SO at RT for 7 h and under argon. The reaction solution was neutralized with KOAc and the ODE was evaporated under N₂. The crude 8,15(14)-dihydroxy was added directly at 20 mg/ml 10% Na borate buffer pH 8.7 and borane (propargylamine 0.53 mg/ml Sigma Type IV) was added. The formation of conjugated triene corresponded to the ESI-hydration (MS/MS); a dihydroxy (me) was obtained in a UV cuvette, was complete in 6 min, and was terminated by reduction to the DHETE by addition of excess NaBH₄, acidification to pH 3 with glacial HOAc and extraction with CH₂Cl₂. The 8,15-dihydroxy esters were purified by sequential SE-HPLC followed by RP-HPLC and taken on LC.
On the Chemical Conversion of HPETE to Leukotriene Epoxide

Figure 4. RP-HPLC showing resolution and purity of carbamate isomer 1 and isomer 2, monitoring the substituted aromatic chromophore at 217 nm. HPLC conditions: Waters µPorasil, 5.9 x 250 mm, isopropyl alcohol: hexane, 1:495, v/v, at 1.0 ml/min.

Figure 5. Mass spectrum (70 eV) of octadec-1-yn-8-ol, recorded on the HP 5988 GC/MS. GC column: 3% SP2100, C-value: 16.80.

Figure 6. IR spectrum of octadec-1-yn-8-ol in CCl₄ (capillary). The monosubstituted alkyne absorption at 2167 cm⁻¹ is apparent.

Figure 7. Total ion current trace and mass spectrum (70 eV) of [8-D₂,⁻²H] 1-octadecyne. The mass spectrum shown corresponds to the unlabeled component. The small peak eluting at scan no. 20 was identified as 1-octadecyne.

Figure 8. RP-HPLC of the fatty acids obtained by incubation of [8-¹⁴C]-stearic acid with Helicobacter pyloritis. HPLC conditions: Waters SpheriPrep 3µ C₁₈ column. MeOH: H₂O: HOAc: THF: 75:25:0.01 at 2 ml/min for fractions 1-100; 80:20:0.01 at 4 ml/min for fractions 111-150; 100% MeOH at 4 ml/min for 151-170. Fractions 1-60: 2 ml; 61-170: 4 ml.
On the Chemical Conversion of HPETE to Leukotriene Epoxide

Figure 15. Degradative reactions performed on methyl 13-decanoate [3,4,5] after the isomeric epoxide reaction performed on 10-L-[1,2-3H]HPETE methyl ester, in order to establish retention of $^{3}$H in its original position and configuration at carbon-10 in the 10-diene reaction products. This product was subjected as shown in the scheme; (a) methylation, (b) ozonolysis, (c) reaction with the selenium (IV) ozonide, (d) NBD$_2$ reduction of the peroxide group to alcohol, and (e) esterification with CH$_2$CO$_2$H. Under these conditions, the trisubstituted acetylated product was observed to have lost (218.2->216.1) a 1.5% of its original $^{3}$H. This agrees with the result of 1.5% $^{3}$H loss observed in the 11-,15-DIENETE when the original 10-D-[1,2-3H]HPETE derived from [3,4,5] 10-L-[1,2-3H]13-decanoate was incubated directly with the selenium ozonide.

Figure 12. Mass spectrum (70 eV, Riber) of the acetylated reductive ozonolysis fragment corresponding to the carbonyl terminus of 8,15-dihydroxy-9,11,13-methyl eicosatrienoate. The fragment thus corresponds to carbons 1-9 of the parent compound. For GC properties, see Table III.

Figure 11. Mass spectrum of the acetylated reductive ozonolysis fragment of 8,15-dihydroxy-9,11,13-methyl eicosatrienoate as the diacetate.