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STEREOCHEMISTRY OF LEUKOTRIENE C-1

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SUMMARY

Leukotriene C-1, a "Slow Reacting Substance" (SRS), has been shown to possess the molecular structure depicted by V (5(S)-hydroxy-6(R)-S-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid) by its identity with a totally synthetic product of known structure and stereochemistry.

INTRODUCTION

Previous investigations of the chemical nature of "Slow Reacting Substance" (SRS) (1-3) have led to the isolation of two biologically active substances designated as leukotriene C-1 (LTC-1) and leukotriene C-2 (LTC-2) (4,5), and to the formulation of the biologically more active LTC-1 as a 5-hydroxy-7,9,11,14-eicosatetraenoic acid linked at C-6 to the sulfur of a cysteine containing substituent (4). This substituent was recently identified as glutathione (6). Described herein is the complete structural elucidation of LTC-1 which has been achieved by comparison of this substance as isolated from mouse mast cell tumor with substances of known structure and stereochemistry produced by unambiguous total synthesis.

MATERIALS AND METHODS

Synthetic V (LTC-1). The optically active aldehyde ester I was synthesized by a multistep process, described in detail

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elsewhere (7), starting from D-ribose. Carbons 3-7 of I originate from carbons 1-5, respectively, of D-ribose. All chemical intermediates were fully characterized by infrared, proton magnetic resonance and mass spectroscopy using chromatographically purified and homogeneous samples. Starting from I, $[\alpha]_D^{25}$ +68.6°. (c = 0.31 in chloroform), chain extension by Wollenberg (8) and Wittig (9) methods provided methyl 5(S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoate (II), $[\alpha]_D^{25}$ -21.9° (c = 0.32 in cyclohexane). The Δ^9-isomeric epoxy tetraene III, 5(S)-trans-5,6-oxido-7-trans-9,11,14-cis-eicosatetraenoate, was also synthesized from I using 2-carbon silyl imine (10) and 11-carbon Wittig (9) chain extension. The racemic mixture of the 5,6-cis- and 5,6-trans-epoxides IV was prepared as previously described (9).

All experiments with and storage of polyunsaturated intermediates such as II-VI were conducted under argon using 4-hydroxy-2,2,6,6-tetramethylpiperidinooxy free radical as antioxidant (9).

Synthetic LTC-1 was formed as the major product, and only glutathione-eicosanoid conjugate (9) by reaction of epoxide II with 3 equiv. of glutathione, 12 equiv. of triethylamine in concentrated methanol solution at 23° for 6 hr. followed by isolation and treatment of the resulting coupling product with 0.1 M potassium carbonate in water at 0° for 2 hr to cleave the mono ester.

Reaction of the 7-trans-9,11,14-cis-tetraene ester oxide III with glutathione followed by mild base cleavage produced as major product a new eicosanoid-glutathione conjugate, formulated as VI, which was distinctly different from LTC-1 or LTC-2 by reverse phase HPLC analysis (RP-HPLC) being more rapidly eluted. The racemic 5,6-cis- and trans-oxide mixture IV (9) reacts with glutathione to afford after lithium hydroxide mono ester cleavage at 0° for 30 min, a mixture of 4 glutathione C_{20}-conjugates, as expected and as shown by RP-HPLC, one component of which corresponds to LTC-1.

HPLC analyses of synthetic leukotrienes at Harvard were carried out using a 300 x 4 mm μ-Porasil-C_{18} reverse phase column (Waters Associates) using methanol/water 65:35 v/v containing 0.1% acetic acid buffered to pH 5.6 with ammonium hydroxide for elution. Samples were injected as aqueous solutions buffered to pH 5.5 - 5.6. Since there was a possibility of small variations in retention times in different runs, co-injection with a standard of synthetic LTC-1, or appropriate component was routinely employed for calibration.

Leukotriene C-1 from mouse mast cell tumor was generated and purified as previously described (4).

HPLC at Karolinska Institutet was performed on either C_{18} Polygosil (10 x 500 mm) or C_{18} Nucleosil (4.6 x 250 mm) using methanol/water, 7:3 v/v or 65:35 v/v plus either 0.01% acetic acid or 0.02% acetic acid adjusted to pH 5.4 with ammonium hydroxide (4).

Bioassay was performed on the isolated guinea pig ileum (4) in Tyrode's buffer containing atropine sulfate (1 μM) and mepyramine maleate (1 μM). FPL 55712 (0.1 μg/ml) added at maximal contraction caused an immediate relaxation of the ileum. Biological and synthetic compounds were added as 2.5 μM solutions in methanol/water, 7:3 plus 0.01% acetic acid assuming ε^{280} = 40,000. Maximal contractions were plotted vs ^{10}log dose.

Conversion by lipoxygenase. To synthetic or naturally derived leukotriene C-1 (2.5 nmol), dissolved in 1 ml Tyrode's buffer, was added 10 μg of soybean lipoxygenase (Sigma Chemical Company, Type I). Ultraviolet spectra were recorded before addition of enzyme and after 30 min at 20°C, using a Cary 219 instrument.

RESULTS

Reaction of glutathione with synthetic epoxy ester II under conditions conducive to S_N2 displacement affords a single product which upon very mild base treatment undergoes cleavage of the single methyl ester function to give C_{20}-glutathione conjugate V.

The structure and stereochemistry of V follow unambiguously from the method of synthesis (7). The course of the reaction of II with a variety of cysteine derivatives and sulfhydryl compounds has also been established unambiguously as attachment of sulfur to C-6 (7), thereby allowing only V as the structure of the glutathione conjugate.

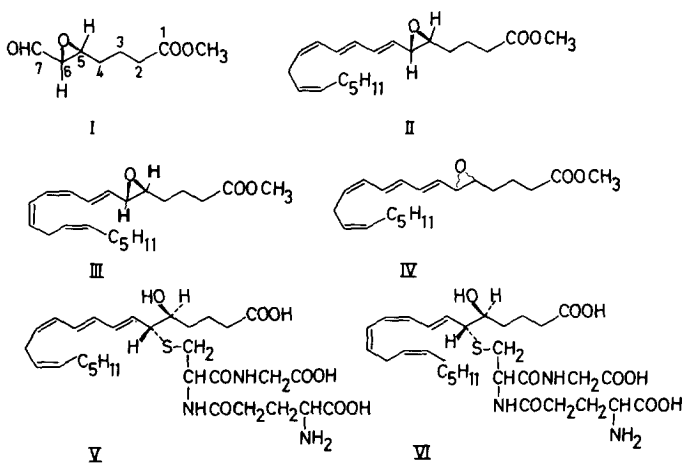


Fig. 1 Structures of synthetic intermediates and C_{20}-conjugates.

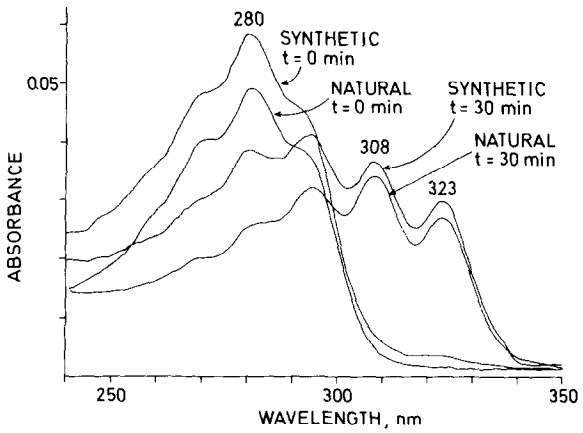
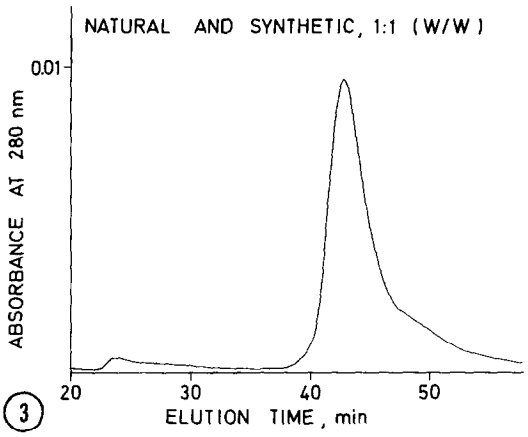


Fig. 2 Ultraviolet spectra of synthetic V and natural leukotriene C-1 before (t=0 min) and after treatment with soybean lipoxygenase (t=30 min). Spectra were recorded in Tyrode's buffer.

Fig. 2 shows ultraviolet spectra of V and of leukotriene C-1 in Tyrode's buffer (t = 0 min). Both spectra showed a λ_{max} at 280 nm and shoulders at 270 and 292 nm. Fig. 2 also shows the ultraviolet spectra of synthetic V and of natural leukotriene C-1 after 30 min incubations with soybean lipoxygenase. This enzyme converts leukotriene C to a conjugated tetraene. The reaction is observed as a bathochromic shift of the ultraviolet spectrum (4). The synthetic and the natural materials reacted to a similar extent and gave chromophores which showed a λ_{max} at 308 nm and shoulders at 295 and 323 nm.

Equal amounts of V and natural leukotriene C-1 were chromatographed together on RP-HPLC (Fig. 3). A single peak was obtained with the same shape and elution time as either V or LTC-1 when chromatographed separately.

Synthetic V and natural LTC-1 elicited similar slow sustained contractions of the isolated guinea pig ileum (Fig. 4). The responses were inhibited by the SRS antagonist, FPL 55712. Furthermore, the dose-response curves for the synthetic and natural materials were essentially the same (Fig. 4).



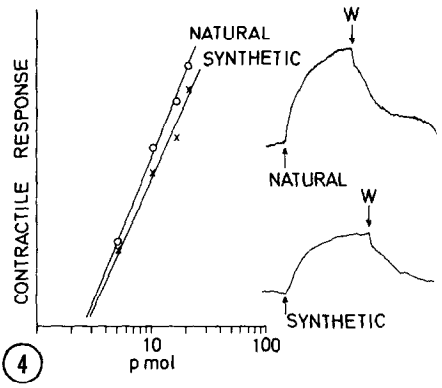


Fig. 3 Cochromatography of synthetic V and natural leukotriene C-1 on reverse phase high pressure liquid chromatogrpahy (Nucleosil C_{18}, 5 µ; methanol/water 65:35 (v/v) + 0.01% acetic acid, 1 ml/min).

Fig. 4 Bioassay on guinea pig ileum of synthetic V and natural leukotriene C-1.

Similar comparisons using the reaction products of glutathione with the 7-trans-9,11,14-cis-tetraene-epoxide III show that the final product (VI) is different from leukotriene C-1. Furthermore, studies with the mixture of cis-5,6-oxide and trans-5,6-oxide IV show that the two products resulting from reaction of glutathione with the cis-5,6-oxide and one of the products following from the reaction with trans-5,6-oxide also differ from LTC-1.

DISCUSSION

The structure of a slow reacting substance (SRS) from mouse mast cell tumor (leukotriene C (5)) was recently reported (4,6). Leukotriene C-1 is a derivative of 5-hydroxy-7,9,11,14-eicosatetraenoic acid in which the sulfur of glutathione is attached as a thioether at C-6.

Concerning the stereochemistry of the molecule, the 11,14-diene in LTC-1 should retain the cis geometry of the precursor (arachidonic) acid since it is unaffected by the reactions lea-

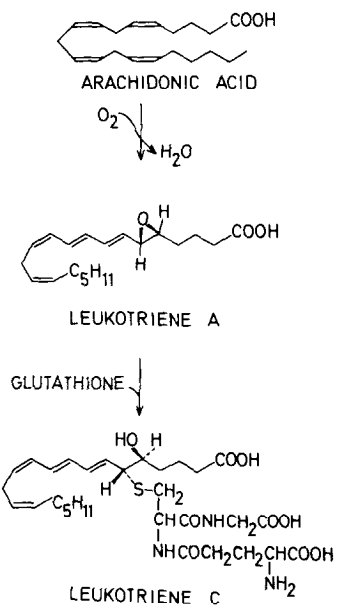


Fig. 5 Proposed biosynthetic pathway of leukotriene C-1.

ding to leukotrienes A and C in the proposed biosynthetic pathway (Fig. 5). Furthermore, LTC-1 is a substrate for soybean lipoxygenase which requires a 1,4-cis-pentadiene structure (4).

In the course of establishing the complete stereochemistry of LTC-1, methyl 5(S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoate (II) was transformed chemically into V. This derivative was indistinguishable from LTC-1 when compared by ultraviolet and RP-HPLC measurements, showed identical biological activity in the guinea pig ileum assay, and the same reactivity towards lipoxygenase. These observations strongly indicated that LTC-1 contained a 7,9-trans-diene. Synthetic leukotriene VI which has a 7-trans-9-cis-diene differed from LTC-1 in RP-HPLC, biological activity and reactivity with lipoxygenase. Although corresponding 7,9-cis-diene and 7-cis-9-trans-diene containing isomers of V have not been synthesized and compared with LTC-1 it seems reasonable to assume that they would be distinguishable from V employing the criteria mentioned above. Additional support

for the proposed configuration of the 7,9-diene in LTC-1 is provided by the recent finding that 5(S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid (IIa, II is the methyl ester) is converted to 5(S),12(R)-dihydroxy-6,8,10,14-eicosatetraenoic acid (leukotriene B) (11). These results indicate that the proposed biological precursor of LTB and LTC-1, leukotriene A, has the structure of IIa (containing a 7,9-trans-diene).

The C-6 adducts of glutathione and racemic cis- and trans-5,6-oxide IV consist of four disastereoisomers with different configurations at C-5 and C-6. The two products from the cis-5,6-oxide and one product from the trans-5,6-oxide were different from LTC-1 as judged by RP-HPLC, biological activity and conversion by soybean lipoxygenase. This strongly indicates that the absolute configuration at C-5 and C-6 of LTC-1 and V are the same. The complete structure of LTC-1, 5(S)-hydroxy-6(R)-S-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid is in agreement with the proposed biosynthetic pathway (Fig. 5) in which 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid and 5(S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid are intermediates.

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