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SYNTHESIS OF 5S-HYDROXY-14,15 LTA_4 A BIOGENIC PRECURSOR TO THE LIPOXINS

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Summary: A synthesis of 5S-hydroxy-14,15-LTA_4 **10** an intermediate in the biosynthesis of the lipoxins is described.

Since the first report of their isolation in 1984,[1,2] from the incubation of 15-HPETE with human leukocytes, the lipoxins have been the subject of a considerable amount of research.[3-12] Previous reports from these laboratories have dealt with the identification

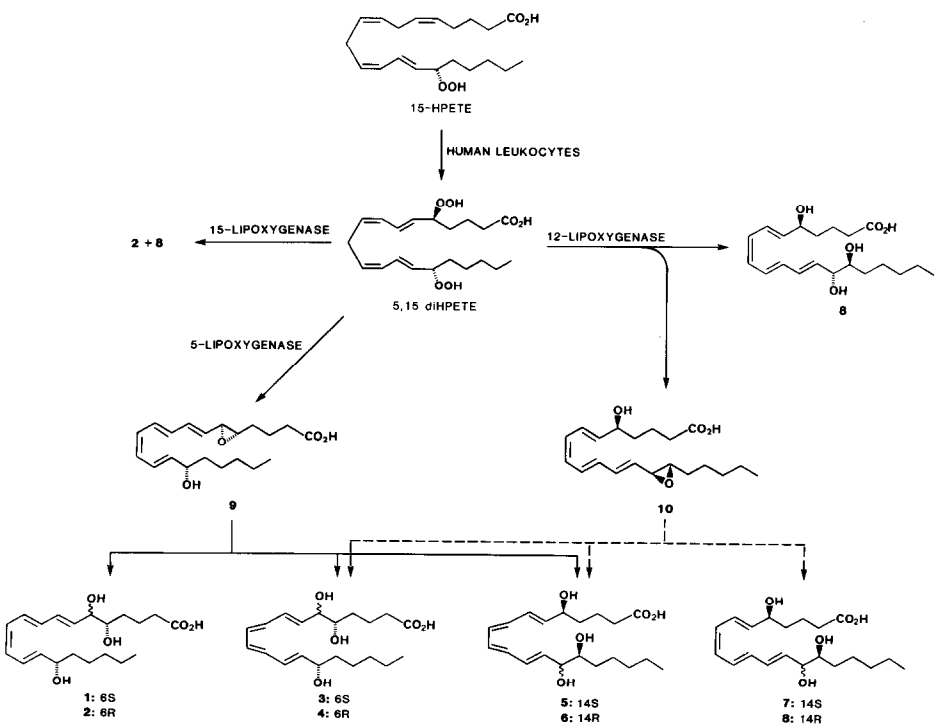
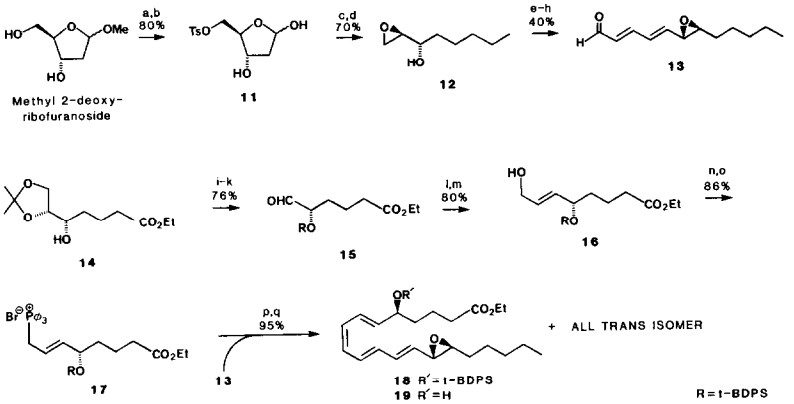


FIGURE 1: BIOSYNTHETIC ROUTES TO LIPOXINS

of six lipoxin isomers produced from this incubation and the elucidation of their biosynthesis. Specifically we have shown the 15-HPETE is transformed by human leukocytes into the lipoxin isomers 1 through 6 and that the 5,6 epoxytetraene 9 was the key biosynthetic precursor to these lipoxins.[8] The epoxytetraene 9 is in turn derived from 5,15 diHPETE. Subsequent to this work a seventh isomer has been found from this experiment by these laboratories and others,[10] that being the 5S,14R,15S-8-cis-lipoxin B isomer 8 (Figure 1).

Since the 12-lipoxygenase enzyme is a component of many cellular systems, we sought to investigate the action of this enzyme on 5,15-diHPETE to determine if this was a viable route for the biosynthesis of lipoxins. The incubation of 5,15-diHPETE, with purified 12-lipoxygenase also produces various lipoxin isomers (Figure 2).[13] However, it quickly became evident that this incubation produced a different distribution of lipoxin isomers than the 5-lipoxygenase enzyme (Figure 1). The major product of this incubation was the 5S,14R,15S-8-cis-lipoxin B isomer 8. However, the question remained as to the origin of these lipoxin isomers produced in this incubation. Two possible pathways for the biosynthesis of the lipoxin isomers found are outlined in Figure 1. While lipoxygenation of 5,15-diHPETE by 15-lipoxygenase had been previously shown to yield the lipoxin B isomer 8,[8] it was felt that the 14,15-tetraene epoxide 10 was an intermediate which could account for the formation of the other lipoxin isomers generated in this experiment.

Herein we describe the synthesis of the 14,15-tetraene epoxide 10 and the results of its aqueous hydrolysis and a biosynthetic proposal for its role in the production of lipoxins from 5,15-diHPETE by porcine 12-lipoxygenase.



a) TsCl/py b) 80% HOAc • △ c) Ph_3P=CH_3 d) H_2/Pd•C e) NaOEt/EtOH f) CrO_3•py/Celite g) OHC-≈-PPh_3
h) I_2(cat)hv i) t-$BuPh_2SiCl/Et_3N$ j) TFA/H_2O/THF k) $Pb(OAc)_4/CH_2Cl_2$ l) OHC-≈-PPh_3 m) $NaBH_4$•$CeCl_3$
n) CBr_4, Diphos o) PPh_3/CH_3CN p) LiHMDS q) Bu_4NF/HOAc

SCHEME 1: SYNTHESIS OF TETRAENE EPOXIDE 10

To prepare the bottom epoxide bearing portion of the target, methyl 2-deoxy-ribofuranoside was chosen as the starting material. Derivatization of the primary hydroxyl group as its sulfonate ester followed by hydrolysis of the glycoside gave the lactol 11. Treatment of the lactol 11 with propylidenetriphenylphosphorane resulted in concomitant

formation of the olefin and terminal epoxide to give after catalytic hydrogenation (Pd-C) the epoxide 12. Treatment of the epoxide 12 with sodium ethoxide gave the internal epoxide. Oxidation of the primary hydroxyl group of this intermediate and condensation of the resultant aldehyde with 3-formylpropenylidenetriphenylphosphorane gave after photo-isomerization with a catalytic amount of iodine the diene aldehyde 13 representing the bottom portion of the tetraene epoxide 10.

The top portion of the target was also derived from the 2-deoxy-D-ribose via the known alcohol 14.[14] Protection of the hydroxyl group as its silyl ether followed by hydrolysis of the acetonide and cleavage of the resultant diol with $Pb(OAc)_4$ gave the aldehyde 15. Treatment of the aldehyde 15 with formylmethylidenetriphenylphosphorane gave the homologated α,Ç unsaturated aldehyde which was reduced by treatment with sodium borohydride/cerium chloride to give the alcohol 16. The alcohol 16 was converted into the corresponding phosphonium salt 17 by treatment with carbon tetrabromide/DIPHOS then allowing the resulting bromide to react with triphenylphosphine.

Treatment of the phosphonium salt 17 in THF with 0.95 equivalent of lithium hexamethyldisilazide at -100° for one min. then addition of the aldehyde 13 (1.0 equivalent) gave after warming to -40° for 2 hours the desired protected tetraene epoxide 18 and its all trans isomer in equal amounts.

All initial attempts to remove the silyl protecting group with nBu_4NF met with dismal failure, resulting consistantly in the complete loss of recognizable material. This was reasoned to be at least partially due to the alkoxide generated upon removal of the silyl protecting group, therefore it was decided to add a small amount of acetic acid to the reaction mixture to quench the alkoxide, despite the exquisite acid lability of the tetraene epoxide moiety.[15] This strategy proved successful yielding a mixture of the alcohol ester 19 and the corresponding δ-lactone.[16]

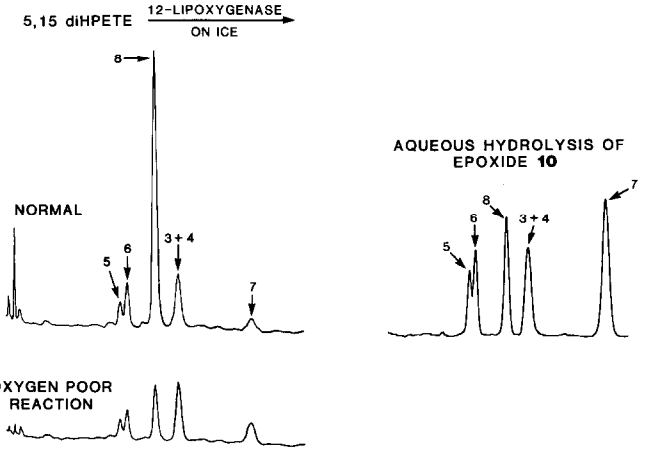


FIGURE 2: HPLC OF LIPOXIN ISOMERS
Waters Novapak Column 57.5:21.25:21.25:0.01
Water/Acetonitrile/Methanol/Acetic acid, 1.2 ml/min

Sponification of the ester 19 and/or lactone with methanolic sodium hydroxide followed by aqueous hydrolysis of the tetraene epoxide 10 at various pH's consistantly gave

comparable amounts of the six lipoxin isomers complementary to the six generated by aqueous hydrolysis of the 5,6-tetraene epoxide **9** (Figure 2). The ratios of the various isomers varied only slightly with pH. Since similar amounts of all six isomers are produced by aqueous hydrolysis of the epoxide **10** the 5S,14R,15S-8-cis-lipoxin B isomer **8** must also be produced by another pathway to account for it being the major product of the incubation. Indeed the lion's share of this isomer appears to be produced via the lipoxygenation pathway as demonstrated by oxygen exclusion dramatically reducing the relative amount of this compound produced (Figure 2).

In conclusion, the tetraene epoxide **10**, a biochemical precursor to the lipoxins was prepared in optically and stereochemically pure form. The aqueous hydrolysis of this epoxide in concert with oxygen exclusion experiments demonstrated that the lipoxin isomers generated by the incubation of 5,15-diHPETE with 12-lipoxygenase arise from both this epoxide and lipoxygenation of the diHPETE. The former route producing the six lipoxin isomers **3**→**8** and later giving the 8 cis lipoxin B isomer **8**.

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15. The half life of the epoxide **10** is estimated to be 2 min. in pH 7.4 buffer.
16. All intermediates were characterized by ^{1}H-NMR, I.R. and U.V. spectroscopy and $[\alpha]_D$.

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