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SYNTHESIS OF THREE NEW DEHYDROARACHIDONIC ACID DERIVATIVES AND THEIR OXIDATION BY SOYBEAN LIPOXYGENASE

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Summary: The synthesis of dehydroarachidonic acid derivatives **1-3** is described together with their oxidation by soybean lipoxygenase. The results of these and kinetic studies suggest the occurrence of organoiron intermediates in lipoxygenation.

The enzymatic lipoxygenation of polyunsaturated fatty acids is a process of fundamental importance in the biosynthesis of biologically active compounds such as prostaglandins and leukotrienes. In connection with studies on the mechanism of lipoxygenation, specifically with the problem of deciding between free radical or organoiron intermediates in this reaction,[1] we required a number of analogs of arachidonic acid possessing additional unsaturation in the C(16)-C(19) segment. This paper reports the synthesis of three such compounds (**1-3**) and the study of the reaction products with soybean lipoxygenase (SBLO). The combined study of the kinetics and products of the lipoxygenation of **1-3** by SBLO has recently provided evidence in support of organoiron rather than free radical intermediates.[2]

Arachidonic acid was converted to the 14,15-epoxide methyl ester in high yield as previously described.[3] Exposure of this epoxide to 1 : 2 0.5*M* perchloric acid-tetrahydrofuran (THF) at 23°C as previously reported[4] converted it to the corresponding diol which was cleaved with lead tetraacetate in CH_2Cl_2 to aldehyde **4**.[4] Aldehyde **4** was reduced by addition in THF solution to a solution of ethanolic sodium borohydride containing cyclopentene at -40°C to give after 30 min at -40°C, extractive workup, and chromatography on silica gel ester alcohol **5** (60% overall from the above glycol).[5] Treatment of **5** with 5 equiv of iodine, 5.8 equiv of imidazole and 5.5 equiv of triphenylphosphine in 4 : 1 benzene-acetonitrile at 23°C for 15 min, pouring the mixture into aqueous hydrogen peroxide, extractive isolation, and filtration of the product through silica gel afforded iodide **6** (99%). Reaction of **6** with 1.2 equiv of triphenylphosphine at 60°C in acetonitrile (14 ml/g of **6**) for 40 h produced phosphonium iodide **7** which was isolated as a colorless oil in 99% yield. For subsequent Wittig reactions this salt was rigorously dried by three cycles of dissolving in methylene chloride-toluene and removal of solvent *in vacuo*.

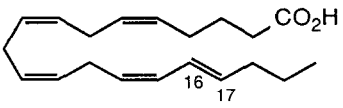
The phosphonium salt **7** was converted to the corresponding Wittig reagent by reaction under nitrogen with 1 equiv of potassium hexamethyldisilazide in THF at -20°C for 5 min, and the resulting solution was cooled to -78°C and treated with 1.2 equiv of *E*-2-hexenal. After 30 min at -78°C, gradual warming to 23°C over 2 h, extractive isolation, and column chromatography on silica gel using 10 : 1 hexane-ethyl acetate for elution the methyl ester of 16,17*(E)*-dehydroarachidonic acid (**1**) was obtained in 80% yield and >98% purity as determined by HPLC analysis (reversed phase, Dupont Zorbax ODS column, 94 : 6 methanol-water for elution). For

enzymatic studies **1** methyl ester was further purified by preparative reversed phase HPLC; UV max in ethanol 232 nm (ε, 24,600), mass spectrum (MS) (CI, *i*-butane), 317 ($M^+ + 1$), 316 (M^+). The free acid **1** was obtained in quantitative yield by saponification of the methyl ester in 2 : 1 dimethoxyethane-1N aqueous lithium hydroxide at 23°C for 3 h, acidification to pH 3.5, and extractive isolation.

In the same way the acid **2** was obtained via a Wittig reaction of the ylide from **7** with *E,E*-2,4-hexadienal (78% yield). Similarly, the acid **3** was synthesized starting with the Wittig reagent from **7** and the racemic aldehyde **8** (58% yield),[6] HPLC purification, and saponification.

16,17*(E)*-Dehydroarachidonic acid **1** proved to be a substrate for soybean lipoxygenase[7] in 0.2*M* pH 9.2 sodium borate buffer at 23°C in air, although for a given % conversion to product more SBLO was required than for arachidonate as substrate. For **1** K_m 5.7 μM and V_{max} 2,700 min^{-1} were determined as compared to K_m 13.3 μM and V_{max} 11,000 min^{-1} for arachidonate.[2] The products of oxidation of **1** by SBLO under the stated conditions were determined to be the 15-LO product **9** (6%), the 17-LO products **10** and **11** (88% and 4% resp.) the 11-LO product **12** (2%). The relative amounts of product were ascertained by reduction of hydroperoxide to hydroxyl (ethereal trimethylphosphite), esterification with diazomethane and quantitative HPLC analysis (order of elution from a silica gel column using 15 : 1 hexane *t*-butyl methyl ether for elution: **9**, **12**, **10** and **11**). The structures of these products were confirmed by ^1H NMR, UV, and MS data on the corresponding hydroxy methyl esters. For example, the ester from the 15-LO product **9** had UV_{max} 236 nm and the ^1H NMR spectrum allowed unambiguous assignment of key peaks using spin decoupling.[8] The stereochemistry of the conjugated diene system is clear from coupling constants: $J_{11,12}$ 10.8 Hz; $J_{13,14}$ 14.6 Hz. Hydrogenation of the hydroxy methyl ester corresponding to **9** afforded a product identical to the reduction product of 15-HETE methyl ester; MS: m/e 342 (M^+), 271 (C(15)-C(16) cleavage) and 242 (C(14)-C(15) cleavage). The hydroxy ester corresponding to **10** had UV_{max} 262, 272 (max) and 282 nm and after hydrogenation showed major MS peaks at m/e 342 (M^+), 299 (MeOCO $C_{15}H_{30}CHOH$) and 270 (MeOCO $C_{15}H_{31}$). The absolute configuration at C(17) of **10** was determined as *S* by conversion of the corresponding hydroxy methyl ester to the l-menthyloxy carbonyl derivative, ozonolysis with oxidative workup using peroxyacetic acid, and esterification with diazomethane to give the l-methyloxycarbonate of methyl *(S)*-2-hydroxypentanoate, identified by gas chromatography and comparison with an authentic sample.[9] It is interesting that the major product from the reaction of **1** with SBLO is the 17*(S)*-hydroperoxide **10** with the 15-hydroperoxide **9** being formed to a much smaller extent, whereas arachidonate is converted only to the 15-hydroperoxide. In the reaction of **1** with SBLO the ratio of 17-LO products to 15-LO product decreases strongly with increasing pressure of O_2, indicative of a reaction intermediate which can be trapped by O_2 to form 15-LO product or rearrange to a predecessor of the 17-LO product.[2]

16,17*(E)*,18,19*(E)*-Bisdehydroarachidonic acid **2** was not a substrate for SBLO under standard conditions (air, 23°C, pH 9.2),[10] but at 50 atm of O_2 and 23°C became a substrate.[2] The major product (94.7% at 50 atm O_2) was the 19-hydroperoxide **13**, UV_{max} 279, 298, 302 (max), 317 nm, whose structure was demonstrated by ^1H NMR, MS measurements of the corresponding hydroxy methyl ester and hydrogenation to methyl 19-hydroxyeicosanoate, which gave in the MS the expected peaks for M^+ and for products of cleavage at the C(18)-C(19) bond and the C(19)-C(20) bond. The absolute configuration at C(19) in **13** was determined as *S* by conversion to the l-menthyloxycarbonyl derivative and oxidative transformation as described above to the l-menthyloxycarbonyl ester of methyl *S*-lactate (identified by capillary gas chromatographic comparison with an authentic sample). HPLC analysis and separation were used to obtain the minor products from **2** and SBLO at 50



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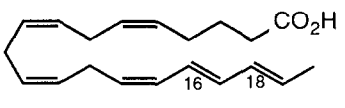

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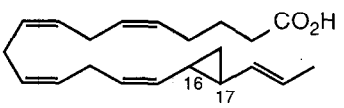

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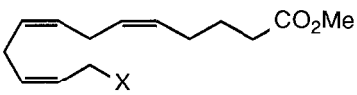
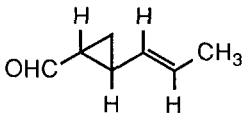


4 X = CHO
5 X = CH_2OH
6 X = CH_2I
7 X = $CH_2PPh_3^+ I^-$



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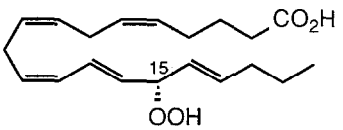

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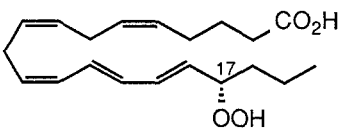

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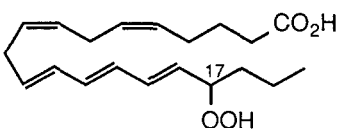

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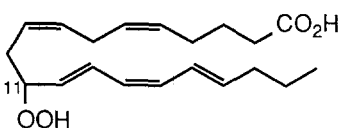

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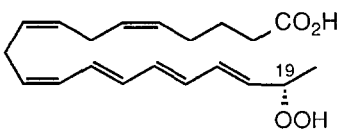

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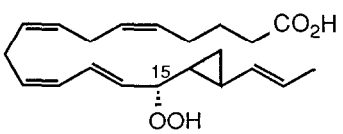

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atm of O_2, identified by spectroscopic studies paralleling those described above, 11-LO product (2.9%), 15-LO product (0.7%) and 17-LO product (1.5%).

The "radical clock" substrate **3** when incubated with SBLO at 23°C in air was also not a substrate but a time dependent inactivator.[2,11] At 50 atm O_2 and 23°C, however, lipoxygenation of **3** occurred, predominantly at C(15) to form **14** (stereochemistry assumed); 11-, 8-, and 15-LO products (without cyclopropane cleavage), amounted to another 28%.[12] Two products of cyclopropane cleavage, methyl 18-hydroxy-5,8*(Z)*,11,13,15,19*(E)*-heneicosahexaenoate (4.7%) and methyl 20-hydroxy-5,8*(Z)*,11,13,15,18*(E)*-heneicosahexaenoate (4.3%) were also isolated (after reduction and esterification) and characterized.[12] The major products containing the intact cyclopropyl unit would seem to be formed from an organoiron rather than a radical intermediate.

The results described above with SBLO substrates **1-3** demonstrate a new facet of the lipoxygenation reaction which will be useful in the eventual determination of reaction mechanism.[13]

REFERENCES AND NOTES

1. For a recent reveiw see E. J. Corey in "Stereochemistry of Organic and Bioorganic Transformations," W. Bartmann and K. B. Sharpless eds., VCH Publishers, 1986, pp 1-12.
2. E. J. Corey and R. Nagata, *J. Am. Chem. Soc.*, Submitted. For other proposals involving organoiron intermediates see (a) D. H. R. Barton *et. al.*, *Tetrahedron Letters*, **26**, 447 (1985); and (b) J. E. Baldwin *et. al.*, *Chem. Commun.*, 1305 (1986).
3. E. J. Corey, H. Niwa, and J. R. Falck, *J. Am. Chem. Soc.*, **101**, 1586 (1979).
4. E. J. Corey, S. Iguchi, J. O. Albright, and B. De, *Tetrahedron Letters*, **24**, 37 (1983).
5. All new compounds were characterized spectroscopically using chromatographically purified and homogeneous samples.
6. The aldehyde **8** was synthesized from *E,E*-2,4-hexadienol by the sequence: (1) Simmons-Smith reaction with excess zinc and 1.2 equiv of methylene iodide in ether at 35°C for 15 h to give *trans*-2,3-methano-4*(Z)*-hexenol (51%); (2) oxidation with 2 equiv of pyridinium chlorochromate and 4A molecular sieves in methylene chloride at 23°C for 2 h, filtration through silica gel and distillation (68% yield).
7. SBLO enzyme used in all experiments described herein was Sigma Co. type I further purified by DEAE-Sephadex column chromatography as described by B. Axelrod, T. M. Cheesbrough, and T. M. Laakso, *Methods Enzymol.*, **71**, 441 (1981).
8. ^{1}H NMR spectrum of **9** in $CDCl_3$ (δ): 6.55 (dd, 1H, J=14.6, 10.8Hz) H-13; 6.02 (t, 1H, J=10.8Hz) H-12; 5.73 (dd, 1H, J=7.5, 14.6Hz) H-14; 5.71 (dt, 1H, J=7.5, 14.6Hz) H-17; 5.52 (dd, 1H, J=7.5, 14.6Hz) H-16; 5.30 - 5.46 (m, 5H) H-5, 6, 8, 9, 11; 4.67 (m, 1H) H-15, 3.67 (s, 3H) OMe; 2.96 (t, 2H, J=5.7Hz) H-10; 2.81 (t, 2H, J=5.7Hz) H-7; 2.33 (t, 2H, J=7.6Hz) H-2; 2.11 (q, 2H, J=7.6Hz) H-4; 2.03 (q, 2H J=7.6Hz) H-18; 1.71 (5et, 2H, J=7.6Hz) H-4; 1.40 (6et, 2H, j=7.6Hz) H-19; 0.90 (t, 3H, J=7.6Hz) H-20.
9. (a) J. W. Westley and B. Halpern, *J. Org. Chem.*, **33**, 3978 (1968); (b) H. C. Brown and G. G. Pai, *J. Org. Chem.*, **50**, 1384 (1985).
10. Acid **2** was actually a time dependent irreversible inhibitor of the reaction of arachidonate with SBLO, K_i 1.0μM; K_{inact} 0.14 min^{-1}.[2]
11. Found for inactivation of SBLO by **3** (23°C in air) K_i 21μM, K_{inact} 0.28 min^{-1}.[2]
12. These products were separated by HPLC and characterized (after conversion to the corresponding hydroxy methyl esters) by UV, MS and 500 MHz ^{1}H NMR spectrospcopy. They were also hydrogenated to the corresponding saturated hydroxy methyl esters and characterized by MS.
13. This research was assisted financially by grants from the National Institutes of Health and the National Science Foundation.

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