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CHEMICAL CONVERSION OF ARACHIDONIC ACID TO SLOW REACTING SUBSTANCES

E. J. Corey and Alan E. Barton
Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

Summary: The synthesis of slow reacting substances, leukotrienes C, D, and E, can be accomplished conveniently by a stereoselective biomimetic route. Details are provided for the conversion of 5-HPETE methyl ester 4 to leukotriene methyl ester (2) and thence to leukotrienes C and D.

Previous publications from this laboratory have outlined both chemical and enzymic methods for the conversion of arachidonic acid to 5-(S)-hydroxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HETE)[1,2] and the corresponding hydroperoxide (5-HPETE, 1).[1] In addition, a biomimetic conversion of 5-HPETE (1) via leukotriene A (LTA) methyl ester (2) to slow reacting substances such as LTC (3) has been reported.[3] There has been much interest in these methods since they provide short pathways of synthesis for the small quantities of slow reacting substances (SRSs) that are generally adequate for biological studies. For this reason we have been continuing our studies in this area. This note reports an improved procedure for the synthesis of LTC and further details with regard to the stereoselectivity of the process which has recently been discussed by Atrache et al.[4]

In our original procedure the methyl ester of (S)-5-HPETE (4) was converted to the peroxy triflate at -110°C in 1:1 methylene chloride-ether in the presence of excess pentamethylpiperidine (PMP) which underwent transformation to LTA methyl ester 2 (ca. 25%). The major by-product in these experiments, formed in approximately equal amount, was the keto ester 5. Formation of 5 can be reduced by the use of 1:1 CH_2Cl_2-tetrahydrofuran (THF) rather than methylene chloride-ether 1:1 as solvent at -78°C which provides 2 in higher yield (33%) as follows:

Leukotriene C. Racemic 5-HPETE methyl ester (4)[1,2] (6.0 mg, 0.017 mmol) was azeotropically dried with benzene and then dissolved in dry CH_2Cl_2 (60 μl) and THF (60 μl) under argon. PMP (Fluka) (8.3 μl, 0.051 mmol) was added and the solution was cooled to -78°C. After the addition of triflic anhydride (4.3 μl, 0.025 mmol) the reaction mixture was stirred rapidly for 2 h at -78°C. Triethylamine (15 μl) was added and the reaction mixture was allowed to warm to ca. -10°C. Hexane-ether-triethylamine (50:50:1) was added, followed by saturated brine. Separation followed by drying (Na_2SO_4) afforded after evaporation crude racemic LTA methyl ester which contained 2.44 mg (43% yield) of epoxide products by ultraviolet analysis at 279 nm. Analysis by HPLC using a Waters Associates μ-Porasil column with hexane-ethyl acetate-triethylamine (100:0.4:0.4) for elution revealed three significant components (fig. 1): (1) major peak (center) LTA methyl ester, (2) less polar peak (left) keto ester 5, and (3) most polar peak (right) 9-cis LTA methyl ester (6). The yield of LTA methyl ester calculated from these data is approximately 33%. The by-products 5 and 6 were identified by HPLC separation and spectroscopic comparison with known compounds.[3,5] Pure 2 is readily obtained by HPLC separation.

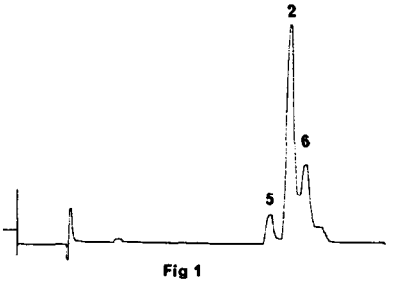


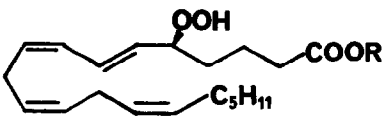
Fig 1

For the next step, coupling with glutathione to form LTC, the crude mixture containing 2 was heated in benzene under argon at 50-60°C for 4 h which selectively effects conversion of 6 to a less reactive $\Delta^{8,10,12,14}$ isomer[5] which like 5 is unreactive toward glutathione. After evaporation of benzene the resulting mixture was treated with N-trifluoroacetyl glutathione dimethyl ester and triethylamine in methanol at 23°C for 4 h as previously described.[6] HPLC analysis of the crude product showed only two peaks in the general vicinity of LTC corresponding to N-trifluoroacetyl LTC trimethyl ester and the 5R,6S-isomer (1:1), the latter arising from the use of racemic 4 in the experiment. Isolation of pure N-trifluoroacetyl LTC trimethyl ester was accomplished by HPLC using a Waters μ-Cyanpropyl column with hexane-CH_2Cl_2-CH_3OH (80:20:1) for elution which separated the LTC derivative (30% yield) (retention vol., R_V 4.5) from its 5R,6S-diastereomer (R_V 5.25) (30% yield). Deprotection of N-trifluoroacetyl LTC trimethyl ester using 15 equiv of lithium hydroxide in 4:1 dimethoxy ethane-water at 0°C for 1 h and 23°C for 3 h under argon followed by neutralization with acetic acid to pH 7 proceeded cleanly to give LTC (3) which was shown to be homogeneous by reversed phase HPLC analysis[5] and identical with an authentic sample.

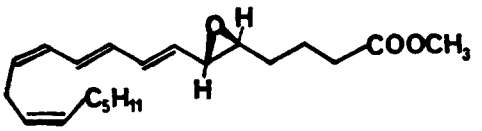
Although the use of 5S-HPETE in the above process has the advantage of affording LTC (20% overall) free of the 5R,6S-diastereomer, the availability of racemic 5-HPETE in unlimited quantities and the ease of purification of LTC makes the above procedure quite serviceable. Pure LTD (7) has been synthesized by an analogous procedure.

In summary, pure LTC and LTD are conveniently prepared from arachidonic acid via 5-HPETE as outlined above. The synthesis of 2 from 5-HPETE is stereospecific with regard to the oxirane and reasonably stereoselective for the olefinic units. Although minor amounts of two by-products (5 and 6) can be detected, they present no problem for the synthesis of the SRSs LTC-E. Atrache et al.[4] report less success in the biomimetic route to LTA methyl ester. This is not surprising since in our experience reaction conditions (solvent, temp., time of reaction, quench conditions, isolation, etc.) are very critical. As pointed out by Atrache et al. under non-optimal conditions the amounts of by-products 5 and 6 are increased with a corresponding decrease in the yield of 2. Further, we have found that LTA methyl ester (2) tends to decompose to an appreciable extent on HPLC columns so that prolonged chromatographic exposure such as reported by Atrache et al. (up to 1 h) can lead to erroneously low analytical values for 2.

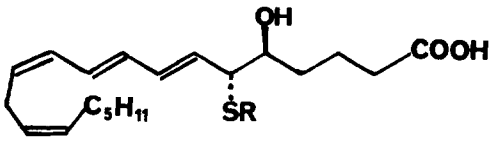
The conversion of 15S-HPETE methyl ester (8) via the 14,15-analog of leukotriene A methyl



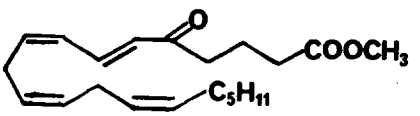
1, R = H
4, R = CH_3



2



3, RS = glutathionyl
7, RS = cysgly



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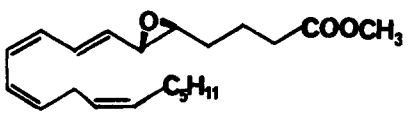

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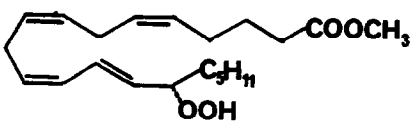

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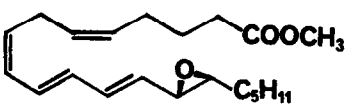

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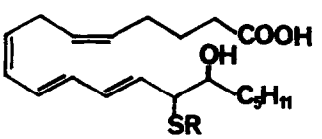

10, RS = glutathionyl
11, RS = cysgly

ester (14,15-EPETE methyl ester) (9) to the 14-thio peptide conjugates 10 and 11 which are analogs of LTC and LTD has previously been reported from this laboratory.[7] We provide below experimental data for this transformation and the further reaction to form 11 which has been effected in a manner closely analogous to that described above for 4 → 2 → 3.

Conversion of 15S-HPETE methyl ester to 11. Dry 15S-HPETE methyl ester (8) (3.6 mg, 0.01 mmol) in dry CH_2Cl_2 (40 μl) and THF (40 μl) under argon was treated with PMP (6 μl, 0.035 mmol) at -78°C and triflic anhydride (3.0 μl, 0.018 mmol) was added with rapid stirring. After 2 h at -78°C 10 μl of triethylamine was added, the reaction vessel was removed from the cooling bath and the reaction mixture was diluted with hexane-ether-triethylamine. The organic layer was washed with brine, and dried to give 1.8 mg (52%) crude 14,15-EPETE methyl ester. Analysis of this mixture using the same HPLC parameters employed for 2 indicated an epoxide ratio of ca. 2.5 (9) : 1 (10-cis 9), thus the yield of (9) is 37%. This crude reaction product was conjugated with N-trifluoroacetyl cysteinyl glycine methyl ester under the conditions described earlier to give a mixture of adducts (separable on a Waters Associates μ-Porasil Radial Pak column using hexane-methylene chloride-methanol (80:20:1) for elution). The mixture was hydrolyzed using lithium hydroxide in 4:1 dimethoxyethane-water at 23°C for 4 h to give crude (11) which showed a single major component by reversed phase HPLC using a Waters C18 column (eluant 65% methanol, 35% water, 0.1% acetic acid buffered to pH 5.6 with ammonium hydroxide, retention volume of (11) = 7.4). The 10-cis isomer gives products on conjugation followed by hydrolysis which are readily separable (retention volumes 5.6 and 6.3) from (11).

The conjugates 10 and 11 have been found to be much less active than LTC or LTD in causing contraction of guinea pig parenchymal strips.[8] Although 10 and 11 have recently been detected[9] as products of incubation of 15-HPETE with rat basophilic leukemia cells, their potential physiological role is unclear.[10]

References and Notes

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