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TOTAL SYNTHESIS OF SLOW REACTING SUBSTANCES (SRS). "LEUKOTRIENE C-2" (11-trans-leukotriene C) (3) and Leukotriene D (4)

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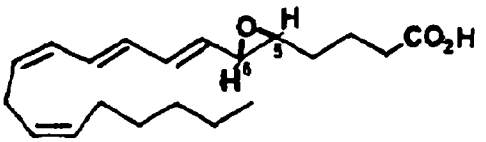
Summary: Syntheses are described for the "slow reacting substances" 11-trans-leukotriene C (3) (previously known as leukotriene C-2) and leukotriene D (4), the cys-gly analog of leukotriene C (The synthesized leukotrienes 3 and 4 were instrumental in the assignment of structure to these members of the family of naturally occuring slow reacting substances which includes also 2.

"Slow reacting substance" (SRS), is the name applied to a biologically active material produ by the action of an antigen on, for example, guinea pig lung.[1] SRS is considered to be an important agonist in various forms of immediate hypersensitivity including asthma and allergic rhinitis.[2] Samuelsson and coworkers have purified two leukotrienes (LT's) designated originally C-1 and C-2, from murine mastocytoma cells and have proposed that LTC-1 is a C(6)-S conjugate of leukotriene A (LTA) with cysteine or a derivative thereof.[3] Recent work has confirmed the struct of LTA to be 1 (as first suggested by one of us in March 1977) by isolation and comparison[4] with totally synthetic 1.[5-8] Further the structure of LTC-1 has been shown to be 2 by total synthesis and rigorous comparison[9-11] with naturally derived material. By comparison of synthetic and naturally derived compounds LTC-2 emerges as the 11-trans isomer of LTC-1 (3). In addition to 2 and 3 another biologically active SRS has been detected and isolated from rat basophilic leukemia cells[12] and from human lung[11] and challenged rat peritoneal leukocytes.[11] The structure of this compound, for which Samuelsson has proposed the trivial name LTD,[12] is assigned as 4, i.e. the cys gly conjugate of leukotriene A, on the basis of correspondence of naturally derived and synthetic material.[11,12] In this paper we present data relating to the synthesis and identification of 11-trans-LTC (3) and LTD (4). The latter substance appears to be of exceptiona interest since it accounts for most (>90%) of the biological activity in hypersensitized human lung.[1]

Synthesis of LTC-2 (11-trans-LTC): The contrast between the ultraviolet absorption of LTC-2 (λ_{max} 278 nm) and LTC-1 (λ_{max} at 280 nm) and reactivity toward soybean lipoxygenase[3,13] (LTC-1 reactive, LTC-2 unreactive) suggested that LTC-2 might differ from LTC-1 in the geometry of the

Δ^{11}-bond. On the other hand it was observed that hydrolysis of pure N-trifluoroacetyl LTC-1 trimethyl ester[6] [purity established by both high performance liquid chromatographic (HPLC) analysis and hydrolysis to pure LTC-1 with 0.1 M aqueous potassium carbonate under argon at 23° for 4 hrs. by means of 15 equiv. of lithium hydroxide in 4:1 dimethoxyethane-water under argon at 23° for 4 afforded a mixture of LTC-2 and LTC-1 containing as much as 80% of the former.[14] Purified LTC-1 was not converted to LTC-2 under the same conditions. To test the possibility that base-catalyze epimerization was occurring in the peptide moiety, pure S-α-napthylmethyl-N-trifluoroacetyl-glutathione dimethyl ester was prepared and subjected to the action of excess lithium hydroxide (5-19 equiv.) in 4:1 dimethoxyethane-water at 23° for 4 hr. Pure S-α-napthylmethyl-glutathione, mp 153-154°C, homogeneous by HPLC analysis, was obtained quantitatively, arguing against stereomutation in the peptide moiety. Epimerization at C-6 of the eicosanoid moiety, another formal possibility could be excluded by comparision with 6-epi-LTC obtained by unambiguous synthesis.[15]

The synthesis of 11-trans-LTC for comparison with naturally derived LTC-2 was achieved in an expeditious manner using the same intermediates employed for the synthesis of LTC (i.e. LTC-1). As previously reported[6] reaction of the trans,trans-dienal-ester 5 with the Wittig reagent 6 at -78°C in tetrahydrofuran (THF)-hexamethylphosphoramide proceeds stereospecifically as a cis olefination to give the methyl ester of leukotriene A (1). Modification of these conditions aimed at promoting trans olefination was therefore studied. Reaction of a mixture of the ylide 6 and 8.6 equiv of dry lithium iodide at 0° in 4:1 ether-THF with the aldehyde 6 (all under argon, addition of 5 to 6) for 35 min. produced in addition to LTA (1) its isomer, 11-trans-LTA. The mixture was not analyzed directly, since the components were not easily separated chromatographically nor distinguished spectroscopically (UV, IR, PMR). However, reaction of the mixture with 3 equiv. of glutathione and 12 equiv. of triethylamine in concentrated methanol solution at 23° for 4 hr.[6] afforded a mixture of monomethyl esters of 2 and 3 which was readily separable by using reverse-phase HPLC analysis (Waters Associates μ-Porasil-C_{18} column with 65:35 methanol-water containing 0.1% acetic acid buffered to pH 5.6 with ammonium hydroxide) with peaks at 18 min. (LTC monoester and 20.5 min. (11-trans-LTC mono ester) in a ratio 3:1. Chromatography gave the pure components (to yield 65%). The monomethyl ester of 11-trans-LTC (UV_{max} at 278 nm as compared to 280 nm for 2 methyl ester) upon treatment with 0.1 M aqueous potassium carbonate at 23° for 4 hr. produced pur 11-trans-LTC (3) UV_{max} 278 nm, ε 40,000, homogeneous by reversed-phase HPLC analysis (95%). Synthe 3 and LTC-2 isolated from mastocytoma cells were indistinguishable.[16]



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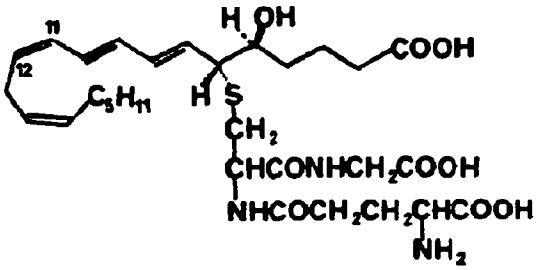

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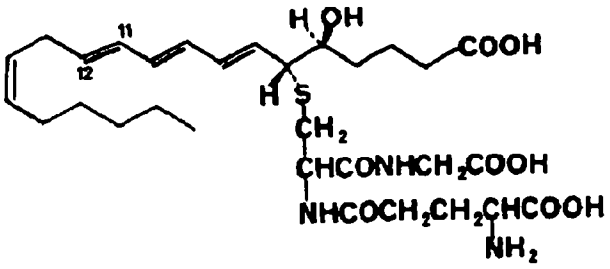

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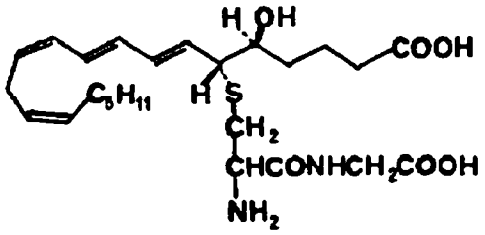

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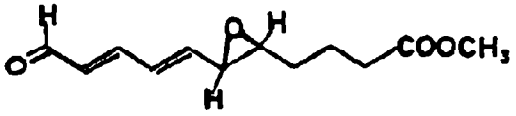

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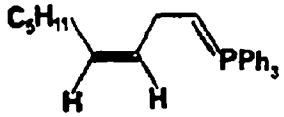

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Synthesis of LTD (4): The cys-gly analog of LTC was synthesized from the methyl ester of LTA (1) and N-trifluoroacetylcysteinylglycine methyl ester. N-trifluoroacetyl-L-cystine, mp 166-167°C was converted to the acid chloride (phosphorous pentachloride in ether at 0°C for 0.5 hr.), and allowed to react with glycine methyl ester in tetrahydrofuran at 0-23°C for 2 hr. Reduction of th crystalline cystine derivative so obtained (87%) mp 189.5°C, with triphenylphosphine (1.1 equiv) : 2:1 dimethoxyethane-water at 23°C for 3 hr gave after extractive isolation and recrystallization from ether N-trifluoroacetylcysteinylglycine methyl ester, mp 99-100°C (84%). Reaction of the methy ester of 1 with 2 equiv of N-trifluoroacetylcysteinylglycine methyl ester and 4 equiv of triethyl-amine in a minimum of methanol under argon at 23°C for 4 hr afforded after extractive isolation a single major product which was purified by thin layer chromatography to yield the dimethyl ester of N-trifluoroacetyl leukotriene D (58%) as homogeneous material (by HPLC analysis on μ-Porasil)

with characteristic ultraviolet absorption at 270, 280, and 290 nm (ε 31,000, 40,000, 31,000). Reaction of this LTD derivative under argon with 0.13 M potassium carbonate in water-methanol (3:1 at 23° for 18 hr followed by isolation and purification by reversed phase HPLC as described earlie for LTC-1 afforded pure LTD (4) in ca. 90% yield. This purification of 4 served to separate 4 (retention volume 9.3) from a minor impurity (<5%, ultraviolet max at 278 nm). Use of the milder conditions which were used for the synthesis of leukotriene C resulted in incomplete deprotection to give a mixture of 4 and its N-trifluoroacetyl derivative. The latter was synthesized unequivocally from the methyl ester of 1 by coupling with N-trifluoroacetylcysteinylglycine followed by selective cleavage of the resulting monomethyl ester using 0.05 M potassium carbonate in aqueous dimethyoxyethane at 23°C for 4 hr.[11,12]

The syntheses of 11-trans-LTC (3) and LTD (4) reported herein allowed rigorous assignment of structure. The question of the mechanism of the conversion of 2 methyl ester to 3 under basic conditions is under study. It is possible that the 11-cis → 11-trans isomerization is catalyzed a trace contaminant, e.g. thiyl radical (RS•), generated by base-catalyzed β-elimination of RS^- ir the cysteine moiety followed by oxidation by traces of air.

The biologically very potent LTD is now readily available by synthesis as described herein.[17]

References and Notes

1. W. Feldberg and C. H. Kellaway, J. Physiol. (Lond.), 94, 187 (1938).
2. See (a) "Immediate Hypersensitivity", M. K. Bach, ed., Marcel Dekker, New York, 1978; (b) K. F. Austen, Harvey Lecture Series, 73, 93 (1978); (c) K. F. Austen, J. Immunol., 121, 793 (197
3. R. C. Murphy, S. Hammarström and B. Samuelsson, Proc. Nat. Acad. Sci. U.S., 76, 4275 (1979).
4. (a) O. Rådmark, C. Malsten, B. Samuelsson, D. A. Clark, G. Goto, A. Marfat and E. J. Corey, Biochem. Biophys. Res. Commun., 92, 954 (1980); (b) O. Rådmark, C. Malsten, B. Samuelsson, G. Goto, A. Marfat, and E. J. Corey, J. Biol. Chem., in press (1980).
5. E. J. Corey, Y. Arai and C. Mioskowski, J. Am. Chem. Soc., 101, 6748 (1979).
6. E. J. Corey, D. A. Clark, G. Goto, A. Marfat, C. Mioskowski, B. Samuelsson, and S. Hammarstr ibid., 102, 1436 , 3663 (1980).
7. E. J. Corey, H. Niwa, J. R. Falck, C. Mioskowski, Y. Arai, and A. Marfat, Adv. Prostaglandin Thromboxane Res., 6, 19, 1980.
8. E. J. Corey, A. E. Barton and D. A. Clark, J. Am. Chem. Soc., 102, in press (1980).
9. S. Hammarström, B. Samuelsson, D. A. Clark, G. Goto, A. Marfat, C. Mioskowski and E. J. Corey Biochem. Biophys. Res. Commun., 92, 946 (1980).
10. S. Hammarström, R. C. Murphy, B. Samuelsson, D. A. Clark, C. Mioskowski and E. J. Corey, Biochem. Biophys. Res. Commun., 91, 1266 (1979).
11. R. A. Lewis, K. F. Austen, J. M. Drazen, D. A. Clark and E. J. Corey, Proc. Nat. Acad. Sci. U.S., 77, in press (1980).
12. L. Örning, D. Widegran, S. Hammarström and B. Samuelsson, Proc. Nat. Acad. Sci. U.S., 77, in press (1980).
13. P. Sirois, Prostaglandins, 17, 395 (1979).
14. The ratio of LTC-2 to LTC-1 and total yield varied considerably.
15. Work to be described separately in a forthcoming publication.
16. Details of this comparison are described separately; D. A. Clark, G. Goto, A. Marfat, E. J. Corey, S. Hammarström and B. Samuelsson, Biochem. Biophys. Res. Commun., 92, in press (1980)

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