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SMOOTH MUSCLE CONTRACTILE ACTIVITIES OF LEUKOTRIENE ANALOGUES

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Chemical modification in the hydrophilic and hydrophobic regions of leukotrienes was investigated in relation to their smooth muscle contractile activities. The conjugated diene or triene moiety in the hydrophobic region is crucial for potent contractile activity. The hydrophilic region at the C-6 position is not dependent only on amino acid or peptide for potent contractile activity; some heterocyclic compounds can be used instead.

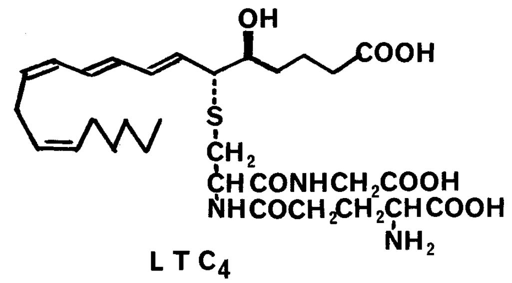
KEYWORDS——slow reacting substance; leukotriene C_4; leukotriene D_4; leukotriene analogue; smooth muscle; contractile activity; guinea pig ileum; guinea pig trachea

The "Slow Reacting Substance of Anaphylaxis" (SRS-A), first described in 1938 by Feldberg and Kellaway,[1] is an important mediator in asthma and other immediate hypersensitivity reactions (allergic rhinitis). Samuelsson et al.,[2] in 1979, suggested that SRS may be a derivative of 5-hydroxy-7,9,11,14-eicosatetraenoic acid with a cysteine residue at C-6. However, the correct structure of SRS from mouse mast cell tumor was shown by Corey's synthetic study[3] in 1980 to be 5(*S*)-hydroxy-6(*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (leukotriene C_4). Subsequently, the structure of LTD_4 was determined by Samuelsson et al.[4]

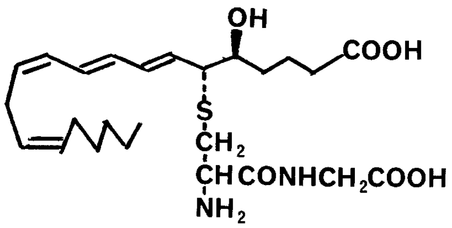
Since then, a large number of leukotriene analogues have been synthesized in order to find the biological active sites and antagonists.[4][5] We now wish to report briefly a part of our study toward finding the biological active sites and antagonists of leukotriene.

The optically active epoxide (I) was synthesized by the Wittig method of treating the esters,[4][5] methyl 6-formyl-5(*S*),6(*S*)-oxidohexanoate, methyl 8-formyl-5(*S*),6(*S*)-oxido-7(*E*)-octenoate, and methyl 10-formyl-5(*S*),6(*S*)-oxido-7(*E*),9(*E*)-decadienoate with the ylide (A), $(C_6H_5)_3P{=}CHCH_2CH{=}CHC_5H_{11}$. Similarly, the epoxide(II) was prepared from the esters and the ylide (B), $(C_6H_5)_3P{=}CHCH_2C{\equiv}CC_5H_{11}$.[6]

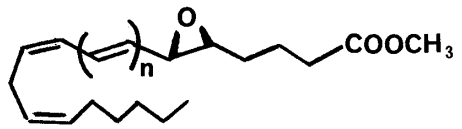
Next, treating (I) or (II) in MeOH with 3 eq of the peptide or heterocyclic compounds (*N*-trifluoroacetyl-cysteinylglycine methyl ester, methyl β-mercaptopropionate, 2-benzoxazolethiol, 6-mercaptopurine, α-mercapto-*N*-2-naphthylacetamide, or 2,5-dimercapto-1,3,4-thiadiazole) in the presence of Et_3N at room temperature for 3 h gave the corresponding products. Hydrolysis of the products with 0.3 M



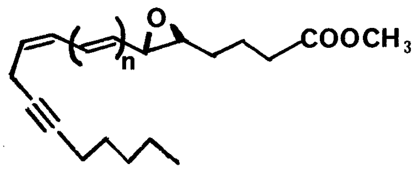
L T C_4



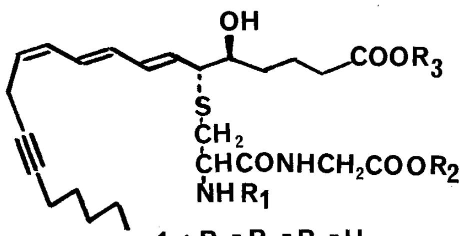
L T D_4



I : n = 0, 1, 2

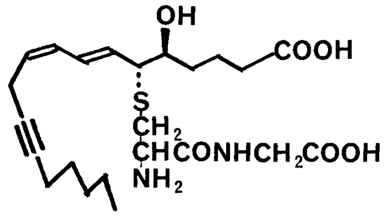


II : n = 0, 1, 2



1 : $R_1 = R_2 = R_3 = H$

2 : $R_1 = COCF_3$ $R_2 = R_3 = CH_3$



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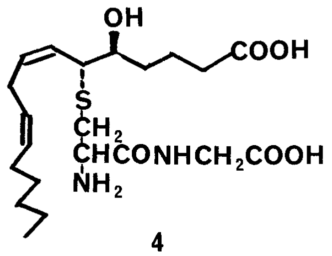

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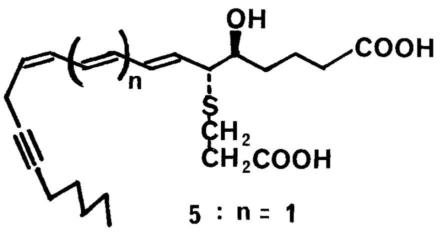
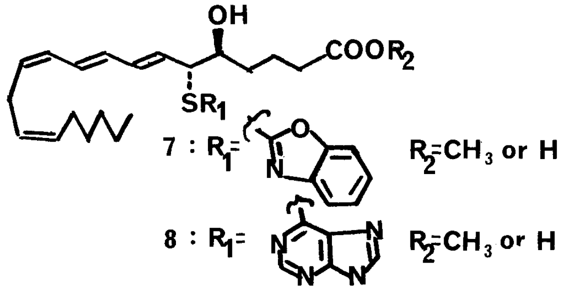


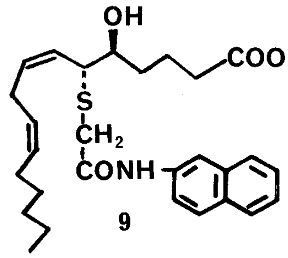
5 : n = 1

6 : n = 0



7 : R_1 = ; $R_2 = CH_3$ or H

8 : R_1 = ; $R_2 = CH_3$ or H



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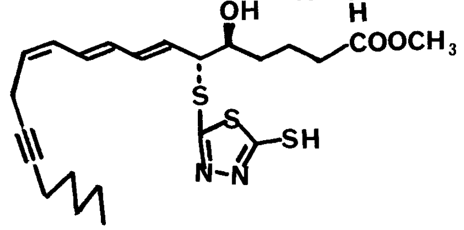

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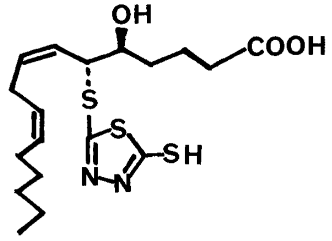

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Table. Effect of LTD_4 and Its Analogues on the Contraction of Guinea Pig Ileum and Trachea

Compound	Effective concentration* to elicit contraction of: ileum	trachea
LTD_4	0.28 ($\times 10^{-8}$M)	0.43 ($\times 10^{-8}$M)
1	0.30	2.1
3	6.8	125
5	1.9	12.5
6	1.8	6.2
10	2.5	46

* A concentration eliciting 50% as much contraction as that induced by 5×10^{-6}M histamine.

K_2CO_3 aq. in MeOH at room temperature afforded the corresponding carboxylic acids.[7)]

The biological activities of these leukotriene analogues were tested in smooth muscle contraction according to the usual Magnus method in which isolated guinea pig ileum or trachea was suspended in oxygenated Tyrod's solution.[8)9)]

The results are summarized in the table. The compounds not listed in the table showed no agonistic activity, and none of the compounds showed any appreciable antagonistic activity against LTD_4 up to a concentration of 1×10^{-5}M.

Although LTD_4 showed the same order of contractile activity on both ileum and trachea, the newly synthesized analogues tended to react more weakly on the trachea than on the ileum. However, it is obvious that these compounds act on the leukotriene receptor sites of these smooth muscles, since the contractile activity was antagonized by FPL-55712,[10)] a specific leukotriene antagonist.

As far as the ileum is concerned, conversion of the C-14,15 double bond of LTD_4 into the triple bond (1) did not affect the contractile response. Shortening of the chain length by deleting C-11, 12 double bond (3) weakened the activity to some extent but not completely. However, further deletion of C-9 to 12 (4) resulted in complete loss of activity. This suggests that the conjugated triene or diene structure in the chain is essential for the LTD-like activity.

The amino acid moiety attached to the C-6 position could be replaced by β-mercaptopropionic acid residue (5), (6) with one seventh as much activity as LTD_4, but replacement by 2-benzoxazolethiol (7) or 6-mercaptopurine (8) led to the loss of activity. It is interesting to note that the replacement of cysteinylglycine residue by 2,5-dimercapto-1,3,4-thiadiazole residue (10) resulted in approximately one tenth as much activity in the ileum. This suggests that the hydrophilic region at the C-6 position is not dependent on amino acid or peptide for potent contractile activity; some heterocyclic compounds can be used instead.

The chemical modification and biological results of our investigation may provide some information about the relation of structure to biological activity.

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6) The ylide (A) was prepared from the known phosphonium salts $(C_6H_5)_3\overset{I^-}{P^+}-CH_2CH_2\overset{H}{C}=\overset{H}{C}-C_5H_{11}$ and n-BuLi as the literature.$^{3)}$ The ylide (B) was generated from the new phosphonium salts $C_5H_{11}-C\equiv C-CH_2CH_2I^-P^+(C_6H_5)_3$, mp 39-40° C, and 1.0 eq n-BuLi in THF-12 eq HMPA at -78° C, which were obtained from the successive reactions of 3-nonyl-1-ol with TsCl, NaI, and $(C_6H_5)_3P$. To the resulting ylide solution was added a solution of 0.8 eq the formyl-ester in THF at -78° C. After 10 min, the reaction was quenched with pH 7 buffer phosphate aq. solution at -78° C.
7) Satisfactory IR, UV, NMR spectra, and, in the case of thermally stable compounds , MS spectra were obtained for all new compounds.
As for the stability of the molecule, the ene compounds (4), (9), and (11) are stable as expected but the others, as well as leukotriene C_4 and D_4, are thermally unstable.
NMR data: the compound (10) as the salts of Et_3N: $(CDCl_3)$ δ 0.68-1.24 (3H, m, CH_3), 3.67 (3H, s, $COO\underline{CH_3}$), 5.20-6.68 (6H, m, olefinic protons).
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