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# THE SYNTHESIS OF A LEUKOTRIENE WITH SRS-LIKE ACTIVITY

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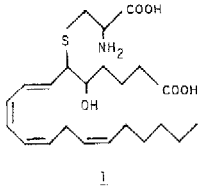
Summary: The synthesis and biological characteristics of an SRS-like leukotriene are described.

Since its discovery in 1938[1] SRS-A (slow reacting substance of anaphylaxis) has been implicated as one of the mediators released during anaphylactic reactions, and it may be the major mediator in allergic asthma. Although the biological properties of this substance including its release from various tissues by ionophores have been investigated extensively, its structure has remained elusive.

The recent report by Samuelsson[2] that the basic structure of SRS might be 1 stimulated our interest in a total synthesis of SRS. The suggestion by others[3] that the amino acid portion of SRS might be glutathione rather than cysteine and the known effect of various thiols[4] on yield enhancement for a family of isolates with SRS-like activity from rat peritoneal cells influenced the synthetic strategy which we developed and describe here.

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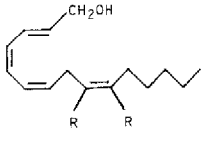



2 R = bond (acetylene)
3 R = O
4 R = H

Thus, our synthetic plan had to take into consideration three uncertainties: 1) the geometry of the 7,8- and 9,10-double bonds; 2) the stereochemistry of the 5,6-substituents; and 3) the exact nature of the sulfur-containing side chain.

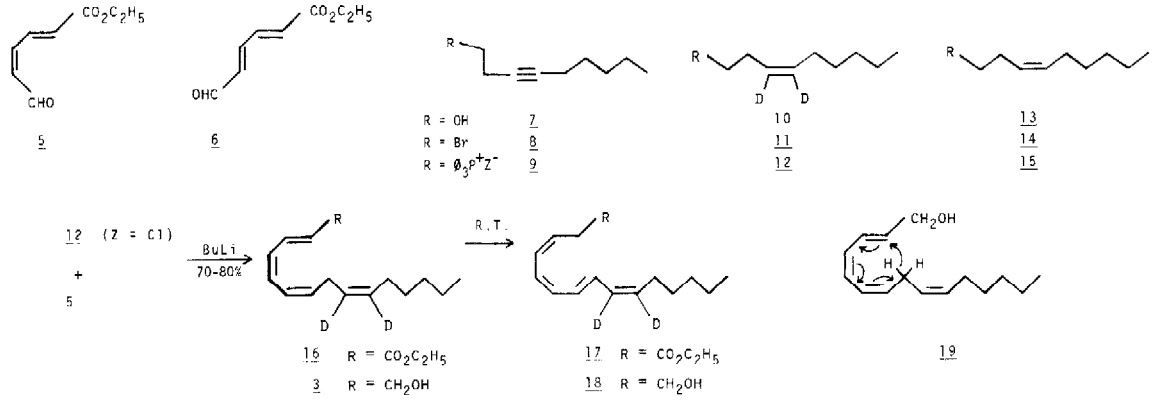
In the present approach our initial target was the synthesis of alcohols 2, 3, and 4 from which we envisioned a number of alternatives to add the remaining 5-carbon piece.

The readily prepared diene ester aldehyde[5] 5 and the commercially available alcohol 7 appeared to be excellent synthons for the assembly of these alcohols. Synthon 5 was selected because of its two readily distinguishable carbonyl functionalitites and the presence of two double bonds with the stereochemistry required in 1. In addition, the known transformation of 5 to 6 would be available should that alternative prove necessary (Scheme I).

The functionalization of 7 to yield 8-15 was straightforward[6], as was the Wittig reaction of 12 (Z=Cl) with 5 to yield ester 16. However, on standing at room temperature for 1-2 days, 16

was transformed to 17. The same isomerization was observed with the product of the Wittig reaction between the acetylene 9 (Z=Br) and 5. Disconcertingly, 3, obtained (80-90%) by $AlH_3$ reduction of pure 16 also showed the same behaviour and, after 18 hours at room temperature it had isomerized to 18 to the extent of approximately 50%. These isomerizations presumably proceed by a particularly facile [1,7]-hydrogen migration[7a] as shown in 19[7b].

Scheme I



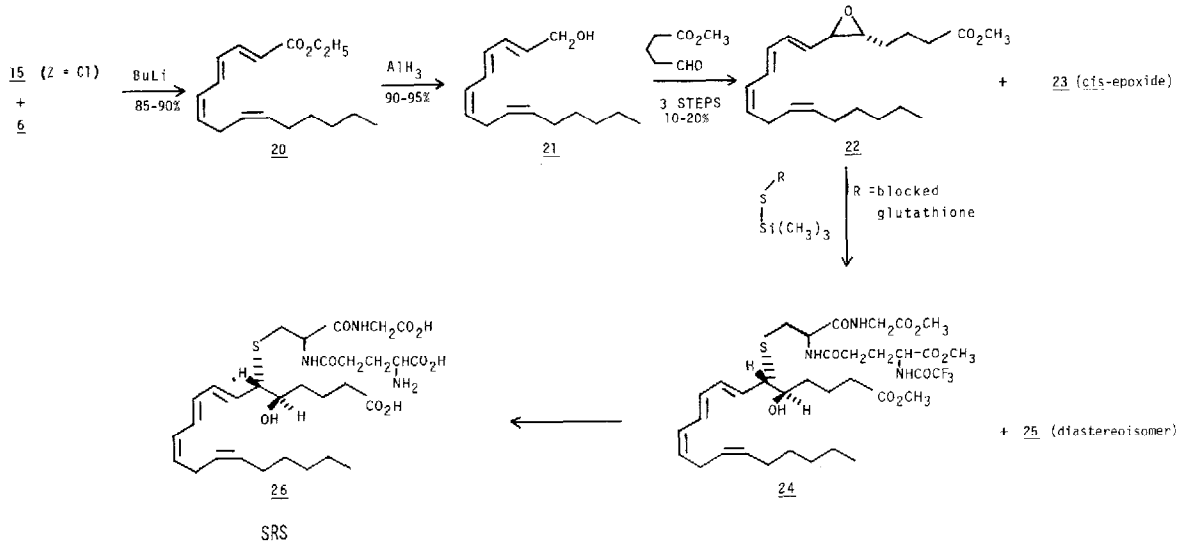

At this point we felt that the observed propensity of the trans, cis, cis-triene unit to spontaneous isomerization cast serious doubts on the possibility that the 9,10-double bond in SRS was cis, since there is no obvious reason why a similar [1,7]-hydrogen migration would not occur in that case as well. We therefore redirected our efforts to prepare the trans, trans, cis, cis-tetraene alcohol 21 by the same series of reactions using dienal ester 6 as shown in Scheme II[8]. The resulting ester 20 and derived alcohol 21 proved to be stable with respect to the [1,7]-hydrogen migration, although they were prone to polymerization if kept neat.

Alcohol 21 was converted to the mesylate, dimethyl sulfonium salt and thence to the sulfonium ylid which condensed with methyl 5-oxopentanoate to provide a mixture of the epoxides 22 and 23[9] in low yield (10-20%). The cis and trans epoxides were readily separable by HPLC[10].

For the introduction of thio-substituents into position 6 of these epoxides, a novel, mild and general method for the stereospecific opening of epoxides using alkylthiotrimethylsilanes was devised[11]. Model experiments indicated that the reaction was stereo- and regioselective as proved to be the case when applied to 22 and 23, leading to the desired C-6 thio adducts.

Thus 22 and 23 reacted separately at $0^{\circ}C$ in dichloroethane with the S-trimethylsilyl derivatives of cysteine methyl ester N-trifluoroacetamide[12, 13], glutathione dimethyl ester N-trifluoroacetamide, and other substituted thiotrimethylsilyl derivatives[11]. After aqueous or methanolic work-up, two diastereoisomers were obtained from both the cysteine and glutathione additions to each epoxide. These were separated by HPLC. After basic hydrolysis (24 hours, $K_2CO_3$, $CH_3OH$), the resultant products (essentially pure by reverse phase HPLC) were tested for biological activity. Only one of the compounds (26)[14], derived from the trans epoxide 22, and glutathione via the protected adduct 24[15], showed the potent activity characteristic of natural SRS[16].

Scheme II




Compound 26 caused dose dependent contractions of the guinea pig ileum and trachea with $PD_2$ values[17] of approximately 8. The effects of 26 on the ileum and trachea were specifically antagonized by FPL 55712[18] with $pA_2$ values[17] of about 7. After incubation with lipoxidase (1 hour, 37°C), the biological activity of 26 was destroyed[19], and the ultraviolet spectrum underwent a concurrent change, complete in 10 minutes at 25°C ($\lambda_{max}^{MeOH}$ 280 → 310 nm), identical with that published for natural SRS[2b].

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## References and Notes

1. W. Feldberg and C.H. Kellaway, J. Physiol. (Lond.), 94, 187 (1938).
2. a) First disclosed at the Fourth International Prostaglandin Conference, Washington, D.C., May 27-31, 1979.
   b) R.C. Murphy, S. Hammarstrom and B. Samuelsson, Proc. Natl. Acad. Sci. U.S.A., 76, 4275 (1979).
3. C. Parker, International Congress on Prostaglandin Synthetase Inhibitors in Clinical Medicine, Sept. 27-28, 1979.
4. M.K. Bach and J.R. Brashler, Life Sciences, 23, 2119 (1978).
5. G.O. Schenck and R. Steinmetz, Ann., 668, 19 (1963).

6. a) Bromides 8, 11, and 14 were best prepared by the method of P.J. Kocienski, G. Cernigliaro and G. Feldstein, J. Org. Chem., 42, 353 (1977).

   b) Alcohols 10 and 13 were made from 7 using the P-2 nickel catalyst of C.A. Brown and V.K. Ahuja, Chem. Commun., 553 (1973).

   c) The phosphonium salts 9, 12, and 15 (Z=Br) were prepared by quaternization of the corresponding bromides, the iodide salts by reaction of the alkyl bromides with NaI followed by quaternization, and the chloride salts by ion exchange with the bromides.

7. a) C.W. Spangler, Chem. Rev., 76, 187 (1976).

   b) The isomerization can be monitored by a shift in the UV spectrum: $\lambda_{max}^{MeOH}$ (260 sh, 269, 279 → 287, 303, 317 nm) and by the appearance of the $-CH_2CH_2OH$ unit in the PMR spectrum.

8. It is interesting to note that while 15 (Z=Cl) gave approximately 90% cis geometry in the Wittig reaction, the bromide and iodide salts under the same conditions gave significantly more trans olefin. The undesired trans isomer can be removed by preparative HPLC.

9. The structure of the cis/trans epoxides was disclosed originally by E.J. Corey (ref. 2a) and the preparation of this mixture has subsequently been published. E.J. Corey, Y. Arai and C. Mioskowski, J. Am. Chem. Soc., 101, 6748 (1979).

10. μ-Porasil column by Waters Associates, hexane, ethyl acetate, triethylamine/100:1:1.
    22: $\lambda_{max}^{MeOH}$ 269, 278, 290 nm (ε 30,900, 37,800, 30,300), PMR (300 MHz, $C_6D_6$) confirmed structure; signals for epoxide protons at δ 2.98 (H6) and δ 2.65 (H5), $J_{5,6}$ = 2.1 Hz.
    23: $\lambda_{max}^{MeOH}$ 269, 278, 290 nm (ε 33,000, 47,500, 29,900), PMR (300 MHz, $C_6D_6$) confirmed structural assignment; epoxide protons at δ 3.21 (H6) and δ 2.77 (H5), $J_{5,6}$ = 4.2 Hz.

11. Manuscript in preparation.

12. We found in model experiments that Cbz was unsatisfactory as a blocking group for nitrogen, and that the formyl group, while acceptable, required a stronger base (1N NaOH) and longer reaction time for complete hydrolysis.

13. The R-S-Si$(CH_3)_3$ derivatives were prepared in situ by treatment of the thiols with n-BuLi (1 eq., $ClCH_2CH_2Cl$, R.T.) then $(CH_3)_3SiCl$ (1 eq., 0°C).

14. $\lambda_{max}^{MeOH}$ 270, 280, 290 nm. Our synthesis proves that C-5 and C-6 are in the erythro configuration, having been formed from the trans epoxide, but does not indicate the absolute stereochemistry since the epoxide is racemic.

15. Homogeneous by HPLC, μ-Bondapack CN, heptane, methylene chloride, methanol/85:15:1.5. $\lambda_{max}^{MeOH}$ 265 sh, 278, 288 nm.

16. After the completion of this manuscript, a publication appeared describing the positive identification of leukotriene $C_1$ (SRS) as having the glutathione side chain: S. Hammerstrom, R.C. Murphy, B. Samuelsson, D.A. Clark, C. Mioskowski and E.J. Corey, Biochem. and Biophys. Res. Commun., 91, 1266 (1979).

    We repeated the ring opening of our trans epoxide 22 with glutathione and triethylamine, as described in the cited paper. One of the two diastereoisomers obtained is identical by UV, HPLC retention time and biological activity with 26 prepared with our reagent.

17. F.G. Van den Brink and E.J. Lien, Eur. J. Pharmac., 44, 251 (1977).

18. N. Chand, Agents and Actions, 9, 133 (1979).

19. P. Sirois, Prostaglandins, 17, 395 (1979).

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