Tetrahedron Letters,Vol.27,No.13,pp 1457-1460,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain

USE OF SULFUR AS A CHEMICAL CONNECTOR

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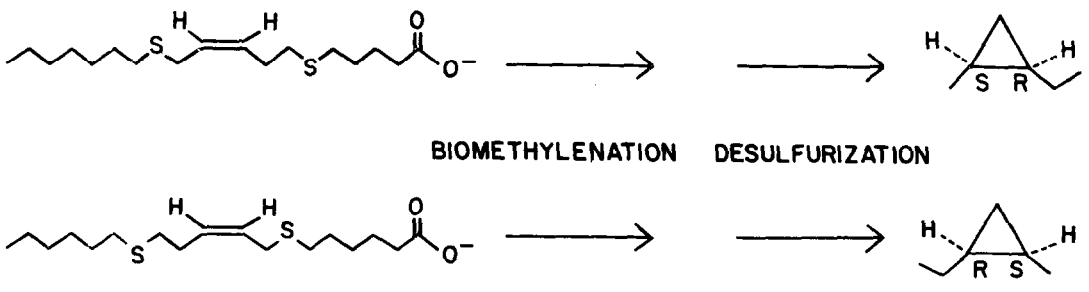
Abstract: Replacement of a methylene group by a sulfur atom at the 6-position of oleic acid does not prevent biomethylenation at the olefinic bond.

One the major problems in the use of enzymic catalysts to perform enantio- and regioselective operations is the fact that enzymic selectivity has been optimized with respect to their natural substrates. In this context, it occurred to us that many highly stereo- and regioselective enzymatic transformations of fatty acid chains such as desaturation, epoxidation, hydroxylation, hydration and methylenation[1] remain unexploited. One strategy which might be used to overcome this problem is to mask substrates as fatty acid derivatives so they conform to enzymic demands.

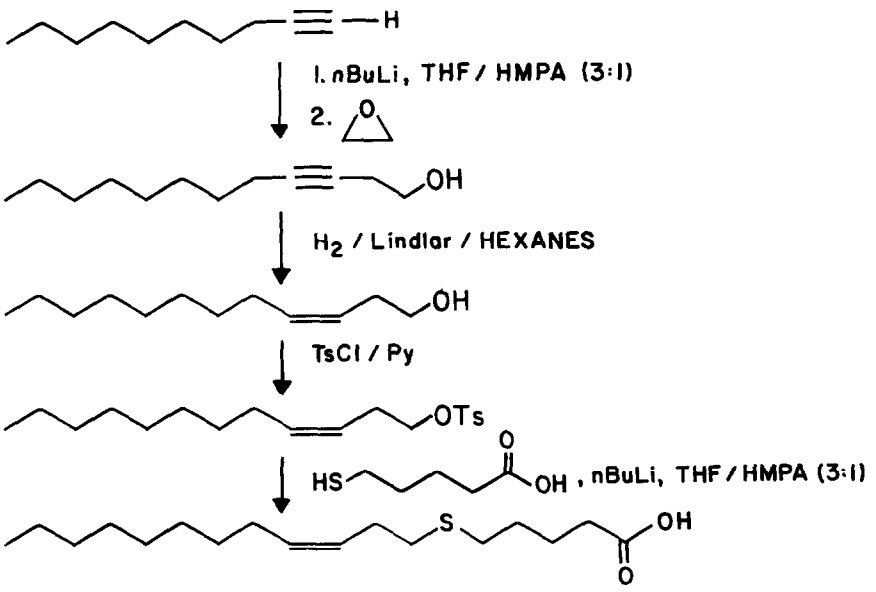
We would like to propose the use of sulfur atoms as chemical connectors which would serve to connect alkyl or alkenyl chains so as to form analogues of long-chain fatty acids. Stereo- and regioselective biotransformation of these thia-analogues followed by severance of the sulfur link(s) would then in principle yield a variety of stereochemically and regiochemically ultra-pure products. For example, biomethylenation of the two dithia-oleates shown below would in principle yield the two enantiomers of cis-1-ethyl-2-methyl-cyclopropane.



Scheme 1

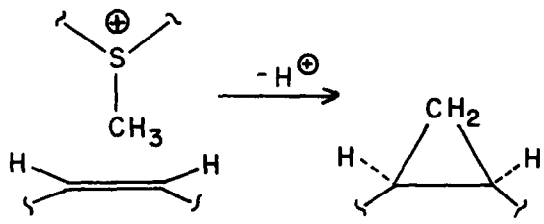
The success of this proposed scheme depends to a large extent on enzymic toleration of sulfur in substrate positions which are remote to the site of functionalization. We chose to synthesize a substrate with one sulfur atom in a position between the point of functionalization and the carboxyl group and to investigate its ability to act as a substrate for biomethylenation using a whole-cell system - Lactobacillus plantarum. We selected 6-thiaoleate as our test substrate because of its relative ease of preparation.[2]

In our synthesis, we exploited the opening of ethylene oxide by an acetylide as shown in Scheme 2. Semi-hydrogenation of the resultant homopropargyl alcohol followed by a tosylate displacement sequence gave the resultant 6-thiaoleic acid in an overall yield of 29% based on starting alkyne. All spectral data of all intermediates and the target compound supported our structural assignments.[3]



Scheme 2

Biomethylenation of 6-thiaoleate on an analytical scale was effected by a whole cell system which has been under investigation for some time in our laboratory. Growing cells of L. plantarum, when denied biotin, will incorporate exogenous fatty acids, from the medium into the cellular phospholipids. Fatty acyl phospholipids with double bonds at the 9 or 11 position of the fatty acid chain, are biomethylenated by this microorganism to form enantiomerically pure[4,5] cyclopropyl derivatives. This reaction is thought to involve methyl transfer from S-adenosyl-L-methionine (S.A.M.) to the olefin followed by proton elimination as shown in Scheme 3.[6] Biochemical precedents for this type of process are abundant.[7,8] Very recently, chemical precedent for the methyl transfer step has been also achieved.[9]



Scheme 3

Table 1: Biomethylenation of Vaccenate, Oleate and 6-Thiaoleate by L. plantarum

Exp #	Compounds administered*	% Biomethylenation of olefinic substrate	% Biomethylenation of thiaolefinic substrate
1	biotin (10μg/L)	90	
2	oleate	92	
3	6-thiaoleate		13
4**	oleate + 6-thiaoleate (1:1)	90	16

*The fatty acids were fed as their methyl esters at a concentration of 40 mg/L. Cells grew normally in all four experiments as determined by culture pH. Fatty acid distribution was determined by basic workup of the cells and capillary G.C. analysis of the fatty acid methyl esters as previously described.[10,11]

** The ratio:(oleate + cyclopropyl oleate)/(thiaoleate + thia-product) was 1.38.

As can be seen from the data in Table 1, 6-thiaoleate was methylenated successfully albeit at a reduced level. No attempt was made to optimize the conversion rate.[12] Competitive feedings of oleate and its thia-analogue did show that the activity of the microbial cyclopropane synthetase was not affected by the thia-analogue since oleate was biomethylenated to the normal extent.

Our results also demonstrate the potential for this type of system to exhibit reversed chemoselectivity because of a built-in regiochemical imperative. In our system, methylation of the less nucleophilic double bond in the presence of a sulfide has been achieved.

The anticipated 100% enantioselectivity of this reaction remains to be demonstrated experimentally and must await scale up of this process. In this connection, the possibility of synthesizing olefinic precursors in one step from the readily accessible thiastearates via enzymic desaturation[1] is being pursued.

It should also be pointed out that apart from their potential utility in organic synthesis of stereochemically ultra-pure products, simple thia-fatty acids may have interesting biological activities should they be further transformed into poly-unsaturated compounds. At that point, the sulfur atom would now come into range with potentially interesting consequences.[2]

Acknowledgements

We wish to thank the National Science and Engineering Research Council of Canada and Carleton University for financial support of this work. We also thank Professor E. Block for useful discussions.

References and Notes

1. A.J. Fulco. Prog. Lipid Res. 22, 133 (1983) and ref. cited therein.
2. Relatively few thia-analogues of olefinic fatty acids have been prepared. Current interest in these types of compounds is focussed on selective enzyme inhibition due to the involvement of the sulfur atom in active site chemistry: M.O. Funk Jr. and A.W. Altenader. Biochem. Biophys. Res. Commun. 114, 937 (1983), E.J. Corey, J.R. Cashman, T.M. Eckrich, and D.R. Corey. J. Am. Chem. Soc., 107, 713 (1985), E.J. Corey, M. d'Alarcao and K.S. Kyler. Tet. Lett. 26, 3919 (1985).
3. For feeding experiments, 6-thiaoleic acid was methylated with diazomethane and purified using reverse phase HPLC. Capillary G.C. analysis showed the material to be homogeneous. ^{1}H NMR ($CDCl_3$): δ 9.88 (1H, s, COOH), 5.4 (2H, m, -CH=CH-), 2.2-2.6 (8H, m, -CH=CHCH$_2$CH$_2$SCH$_2$-CH$_2$COOH), 1.5-2.2 (6H, m, -CH$_2$CH=CH-, -SCH$_2$CH$_2$CH$_2$-), 1.3 (12H, bd. s, $CH_3(CH_2)_6$-), 0.88 (3H, t, CH_3-).
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11. The G.C. retention times of the olefinic fatty acids and their methylenated derivatives were as follows: oleate (9.5 min), cyclopropyl oleate (10.8 min), thiaoleate (11.6 min), methylenated thiaoleate (12.6 min).
The mass spectra of these thia-fatty acids is characterized by the cleavage of the carbon-sulfur bond on the carboxyl side. Thus methyl 6-thiaoleate cleaves to give a fragment ion at m/e 115 due to -$(CH_2)_4CO_2Me$ and a fragment at m/e 199 due to $CH_3(CH_2)_7CH=CH(CH_2)_2S$-. The methylenated product gives a fragment ion at m/e 115 and at m/e 213-14 mass units higher as is typical of cyclopropyl derivatives. J.A. McCloskey in "Topics in Lipid Chemistry", Vol. 1, F.D. Gunstone, Ed., Wiley-Interscience, New York, N.Y., 1970, p. 409.
12. It should be noted that the biomethylenation reaction yields only one product. No by-products, isomeric or otherwise, have ever been observed in this system.

(Received in USA 15 November 1985)