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HIGHLY CHEMO-, REGIO-, and STEREOSELECTIVE INTRODUCTION OF A CIS-DOUBLE BOND INTO A THIA-ANALOGUE OF STEARIC ACID

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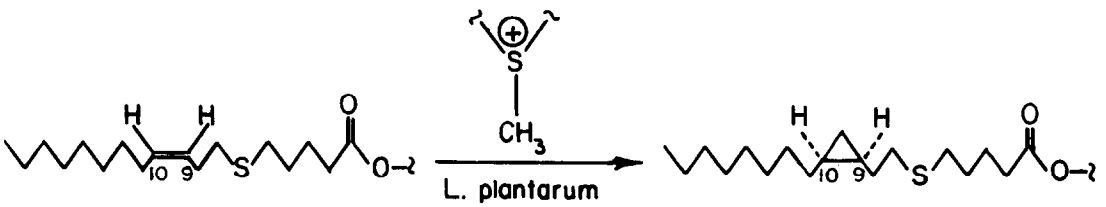
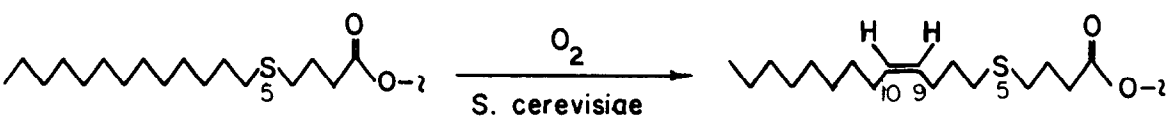
Abstract: Replacement of a methylene group by a sulfur atom at the 5-position of stearic acid does not prevent the introduction of a cis-double bond at the 9-,10-positions.

In a previous communication[1], we showed that an enzymatic methylating system was able to attack an unactivated double bond in the presence of a more nucleophilic thioether linkage only two carbons removed. Thus 6-thiaoleic acid was converted to its cyclopropyl analogue with presumably 100% enantioselectivity by L. plantarum (Scheme 1). This apparent reversal of normal chemoselectivity is probably due to a strict regiochemical imperative imposed by the enzyme in question.



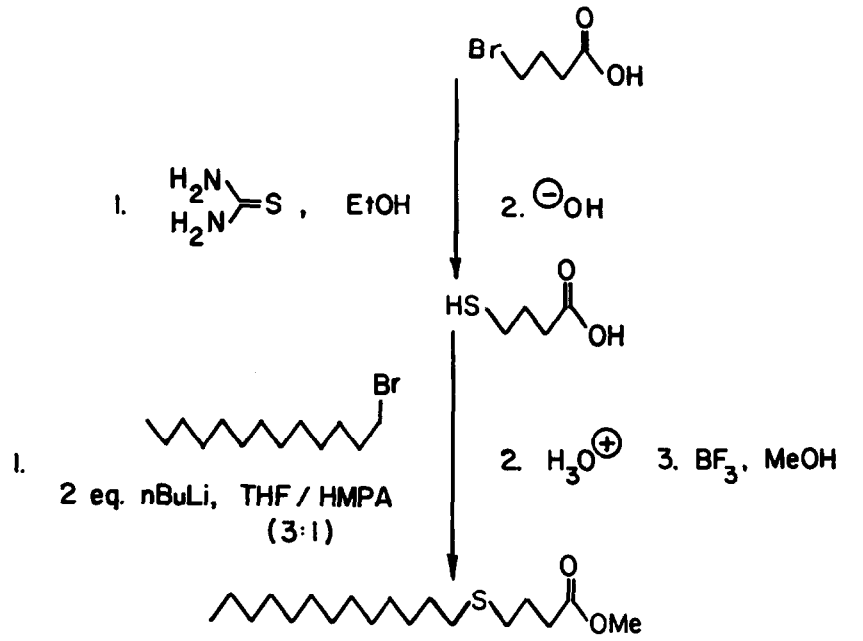
Scheme 1

We now wish to report a much more dramatic example of reversed chemoselectivity, namely the biological desaturation of 5-thiastearic acid to give 5-thiaoleic acid (Scheme 2).



Scheme 2

5-thiastearic acid was synthesized in straightforward fashion[2] as shown below.



Scheme 3

All spectral data was in accord with our structural assignments. Analysis by HPLC (Waters Novapak C-18, 5% H_2O in acetonitrile, refractive index detection), capillary GC (.25 μ DB 225, programmed from 150°C to 220°C with injector at 180°C and FID detector at 300°C) and TLC (silica gel, 10% ethyl acetate in hexane) of synthetic methyl 5-thiastearate showed that this material could be used without further purification in preliminary biological testing. We chose the yeast, Saccharomyces cerevisiae, as our biological system in the hope that this organism would incorporate methyl 5-thiastearate, activate it by forming a thioester linkage with coenzyme A and then convert this derivative to a thiaoleate using its desaturase system. We reasoned that a sulfur atom poised mid-way between the carboxyl group and the incipient double bond would present a suitable challenge to this biological system.

Thiastearic acid (50 mg) as its methyl ester was administered in 1.0 mL of 95% ethanol to 300 mL of a freshly innoculated culture of Saccharomyces cerevisiae NRC #2335 contained in a 1 L flask. The culture medium was that used by Bloomfield and Bloch[3] in their early studies on yeast desaturases. A culture without added thiastearate served as a control. After 48 hours of growth at 30°C in a rotary shaker operating at 150 r.p.m., the cells were harvested by centrifugation, washed with distilled water and filtered. The resultant cake of cells was stored under nitrogen at -20°C. The cells were then hydrolyzed in base and the fatty acids extracted and converted to their methyl esters using standard procedures.[4] The culture medium was extracted with methylene chloride and the residue after evaporation of this extract was treated in the same manner.

Table 1: Analysis of Fatty Acids of S. cerevisiae by Capillary G.C.

Culture mg of Methyl 5-thiastearate added	Fatty Acid Distribution					
	C_{16}:0	C_{16}:1	C_{18}:0	C_{18}:1	5-thia-C_{18}:0	5-thia-C_{18}:1
	as % of Total Fatty Acids					
0	8	62	3	27	-	-
50	4	33	tr.	18	14	31
50	6	38	1	16	14	25

The common names of the fatty acids are: palmitic (C_{16}:0), palmitoleic (C_{16}:1), stearic (C_{18}:0), oleic (C_{18}:1), 5-thiastearate (5-thia-C_{18}:0), 5-thiaoleate (5-thia-C_{18}:1).

The addition of methyl 5-thiastearate to the culture medium had no observable effect on the growth of the yeast. However, the fatty acid profile in the cell extracts was dramatically altered as can be seen by examination of Table 1. Substantial incorporation of the thia-analogue was achieved and more importantly an average 66% conversion to product was obtained. Product (10.2 mg) was isolated by preparative HPLC, using a Whatman Magnum-9 ODS-2 column and 10% ethyl acetate in acetonitrile as the mobile phase. The isolated material was shown to be methyl 5-thiaoleate by a variety of techniques.[5]

We believe these results are significant for the following reasons:

1). Desaturation has occurred in chemoselective fashion. The catalyst is not "poisoned" by sulfur presumably because the sulfur atom is "out of reach" as it were. Appreciable formation of sulfoxides or sulfones does not appear to have taken place - HPLC and TLC analysis of both the cell and culture medium extracts showed only traces of polar material which were not identified further.

2). Desaturation has occurred in a regioselective manner. Introduction of a sulfur atom with the concomitant change in bond lengths and bond angles along the chain does not prevent proper alignment of the 9- and 10-methylene groups. Work with other thiastearates is in progress in order to determine how tolerant of sulfur substitution the enzymic catalyst is.

3). Desaturation has occurred stereoselectively in the sense that no trans-olefin could be detected. This feature appears to be an intrinsic property of the catalyst.

4). Little is known about the detailed mechanism of the desaturation process.[7] Valuable information on the nature of the mysterious non-heme iron oxidant may be gleaned when thiastearates bearing sulfur within range of the oxidant are fed to S. cerevisiae.

5). We have demonstrated, for the second time, the remarkable versatility of biocatalysts operating on an unactivated fatty acid hydrocarbon backbone. We believe 5-thiaoleic acid represents the second of what will become a very large family of enzymatically generated thia-fatty acids, all of which can potentially be desulfurized to yield stereochemically ultra-pure cis-olefins, cis-cyclopropanes,[1] epoxides,[8] and alcohols.[8]

Acknowledgements

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References and Notes

1. P.H. Buist and G.P. Dimnik. Tetrahedron Letters, 27, 1457 (1986).
2. L. Rapoport, A. Smith and M.S. Neuman. J. Am. Chem. Soc. 69, 693 (1947).
3. D.K. Bloomfield and K. Bloch. J. Biol. Chem. 235, 337 (1960).
4. P.H. Buist and J.M. Findlay. Can. J. Chem. 63, 971 (1985).
5. The following diagnostic differences between the spectral data of the product and that of the starting material were noted:
   MS: Fragment ions due to loss of MeO- and $-(CH_2)_3CO_2Me$ were shifted to lower values by two mass units.
   ^{13}C NMR: Resonances at 130.0 and 128.4 ppm due to vinyl carbons and at 27.3 and 26.3 ppm due to allylic carbons adjacent to a cis-double bond were observed.
   ^{1}H NMR: An AB quartet of triplets at 5.35 ppm (J_{AB} = 10.9 Hz) due to 2 vinyl hydrogens and a pair of overlapping doublet of triplets at 2.0 and 2.13 ppm due to 4 allylic hydrogens were observed. In addition, a quintet at 1.63 ppm due to the methylene group at C-7 was observable. In addition, von Rudloff oxidation[6] of the product gave only one monoester product - methyl nonanoate as determined by capillary G.C. analysis.
6. E. von Rudloff, Can. J. Chem. 34, 1413 (1956).
7. K. Bloch, in "Oxygenases and Oxygen Metabolisms", ed. M. Nozaki, S. Yamamoto, Y. Ishimura, M.J. Loon, L. Ernster, and R.W. Estabrook, Academic Press, New York, p. 651 (1982).
8. A.J. Fulco. Prog. Lipid Res. 22, 133 (1983) and references cited therein.
9. Upon close examination by capillary G.C., trace levels of what appears to be methyl octanoate and methyl decanoate were detected in the von Rudloff oxidation. We are currently attempting to determine whether the substitution of sulfur at the 5-position of methyl stearate has induced "errors" in desaturation.

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