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USE OF SULFUR AS AN OXIDANT DETECTOR

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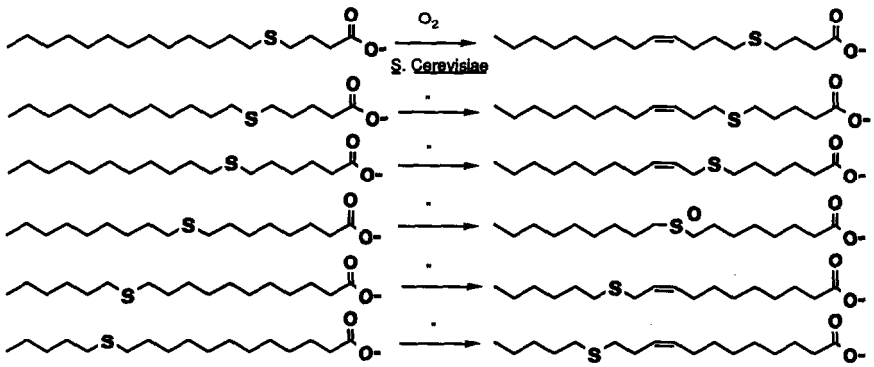
Abstract: The oxidation of a number of methyl thiastearates has been examined as a function of sulfur position using the yeast, Saccharomyces cerevisiae.

We have shown previously that the presence of a sulfur atom in methyl 5-thiastearate does not prevent its stereo-, regio- and chemo-selective dehydrogenation at the unactivated C-9 and C-10 positions by S. cerevisiae. (Scheme 1.)[1] It is of considerable interest both from a synthetic and mechanistic point of view to determine the fate of thiastearates bearing a sulfur atom which is within closer range of the dehydrogenating agent. We have therefore synthesized a number of thiastearates with sulfur atoms at positions 6 to 13 and wish to report the biosynthesis of 6-, 7-, 12-, and 13-thiaoleate and the sulfoxide of 9-thiastearate. (Scheme 1.)



Scheme 1

The thiastearates were prepared as previously reported.[1] The 9- and 10-thiastearic acids have also been prepared by others.[2,3] All methyl thiastearates were purified to homogeneity prior to each feeding experiment. The pertinant chromatographic and spectral data for the thiastearates is included in the experimental section.

Each methyl thiastearate (50 mg) was administered separately to a freshly innoculated culture (300 mL) of the yeast, S. cerevisiae NRC # 2335. The cells were harvested after growth, and the cellular fatty acids extracted and methylated as previously described.[1]

All cultures grew out normally and approximately 50 mg of total cellular fatty acid methyl esters was obtained from each culture. The fatty acid profiles were determined by HPLC and GCMS and the results are given in Table 1:

Table 1: Analysis of Feeding Experiments using S. cerevisiae

Position of sulfur atom in thiastearate fed	C_{16}:0	C_{16}:1	C_{18}:0	C_{18}:1	Thia-C_{18}:0	Thia-C_{18}:1	% Incorp. Thia FA	% Desat. ThiaSt.
Control	10.5	52.4	2.7	34.4	-	-		
5	4.5	26.9	0.2	13.1	19.9	35.4	55	64
6	5.6	26	1.4	15.3	10.7	41	52	79
7	6.9	22.6	1.4	12.9	16.6	39.6	56	70
8	10.5	32.2	3.6	14.8	38.9	-	39	-
9	10.6	23.6	3.5	25.9	36.4	-	36	-
10	11.3	23.9	2.2	14.6	48	-	48	-
11	7.6	41.7*	1.2	29.6	19.9*	-	20	-
12	7.0	26.5	1.6	15.5	29.2	20.2	49	41
13	6.3	22.8	1.3	9.8	19.9	39.9	60	67

The relative amounts of each fatty acid was determined by reverse phase HPLC (Whatman Magnum 9 ODS-2, 50 cm x 9.4 mm i.d., 10% EtOAc/CH_3CN, 4.0 ml/min, RI detection). The fatty acids are palmitic (C_{16}:0), palmitoleic (C_{16}:1), stearic (C_{18}:0), oleic (C_{18}:1), thiastearic (thia-C_{18}:0), thiaoleic (thia-C_{18}:1).

% Incorp. Thia FA refers to the ratio: [(Thia-C_{18}:0 + ThiaC_{18}:1) x 100]/ Total Fatty Acids

% Desat. ThiaSt. refers to the ratio: [Thia-C_{18}:1 x 100] / [ThiaC_{18}:0 + Thia C_{18}:1]

* These values were estimated by GCMS since C_{16}:1 and Thia-C_{18}:0 co-eluted.

Olefinic products were isolated by preparative HPLC and the structures determined by MS and ^1H NMR. (See experimental section.) All products are thiaoleates bearing the sulfur atom in the same position as in the thiastearate precursor. The *cis*-double bond is always inserted between C-9 and C-10. We have been unable to detect any *trans*-olefin or any positional isomers. It is interesting to note the apparent insensitivity of the yeast desaturase system to the position of the sulfur atom in the thiastearate precursor. Thus 5-, 6-, 7-, 12- and 13-thiastearates are incorporated and desaturated to a similar extent.

Having thus firmly established the validity of thiastearates as substrates for the yeast desaturase, it was of great mechanistic interest to determine the nature of products derived from 8-, 9-, 10- and 11-thiastearates where normal olefinic products are obviously not possible. We thus extracted the culture media of each feeding experiment to look for new oxidized derivatives. The media was acidified to a pH of 2 and extracted with several portions of methylene chloride and the extracts dried over anhyd Na_2SO_4. The residues obtained upon evaporation of solvent were treated with boiling 1 M NaOH in 50% ethanol. The fatty acids obtained from the hydrolyzates were methylated in the usual way.[4] The fatty acid methyl esters from each feeding experiment were spotted as 1 % solutions on a silica gel plate and developed in EtOAc to search for sulfoxides and in 10 % EtOAc/hexanes to search for sulfones. Synthetic standards were synthesized by chemical oxidation of the methyl thiastearates. (See experimental section.)

Using a water spray to vizualize all fatty acids, it was clear that while sulfoxidation had occurred to some extent in all feeding experiments, the sulfoxide from 9-thiastearate had been produced in by far the greatest amount. Sulfone could not be detected in any extract.

In order to confirm the production of 9-sulfoxide, a 9-thiastearate feeding experiment was conducted on a 1.4 L scale. The culture medium was worked up in the manner described above and the sulfoxide of methyl 9-thiastearate so isolated was purified by two successive flash chromatographies on silica gel using EtOAc as the first eluant and 4% MeOH/CH_2CL_2 as the second eluant. The 9-sulfoxide was obtained in 27% isolated yield as a colorless solid (mp 71-72 °C). All chromatographic and spectral data for this material was identical with that of the colorless solid (mp 64-66 °C) obtained by chemical oxidation of methyl 9-thiastearate. (See experimental section.) The enantiomeric purity of biologically produced sulfoxide is currently under investigation in our laboratory.

We next carried out two competition experiments in order to examine the formation of sulfoxide as a function of sulfur position. Thus an equimolar mixture of methyl 5-, 6-, 7-, 8-, and 9-thiastearate and an equimolar mixture of methyl 9-, 10-, 11-, 12-, and 13-thiastearate were administered separately to S. cerevisiae in the usual way. The sulfoxide fraction was isolated, purified by flash chromatography and analyzed by direct probe mass spectrometry. The fragment ion: $-CO(CH_2)_mSO-$, m = 3 -11, was found to be unique for each sulfoxide isomer and the relative abundance of each sulfoxide isomer could be estimated using this ion. In the first competition experiment the percentage of each sulfoxide was as follows: 5-sulfoxide (10%), 6-sulfoxide (5%), 7-sulfoxide (12%) 8-sulfoxide (3%) 9-sulfoxide (70%). In the second competition experiment the percentage of each sulfoxide isomer was as follows: 9-sulfoxide (44%), 10-sulfoxide (21%), 11-sulfoxide (7%), 12-sulfoxide (9%), 13-sulfoxide (19%). It is clear that in each case the 9-sulfoxide was produced in the greatest amount.

The simplest interpretation of our results is as follows: When the sulfur atom is at the 9- and 10-position of a thiastearate, dehydrogenation is not possible but oxygen transfer occurs instead. (See Scheme 3.) The fact that the 9-sulfoxide predominates over the 10-sulfoxide may indicate that the non-heme iron oxidant initiates dehydrogenation of a normal substrate by oxygen insertion or hydrogen atom abstraction at C-9 as has been suggested by others on the basis of isotope effect studies.[5] The presence of other sulfoxides may be due to autooxidation, or the operation of random P_{450}-type oxidation.[6] It should be noted that traces of sulfoxides are obtained when control experiments using autoclaved yeast are performed. Insufficient material was obtained from these extracts to allow mass spectral analysis.

These results were presented at ESOC V, Jerusalem, Israel, Aug. 30-Sept.4, 1987. Book of Abstracts, p.264.

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Physical Data: Methyl Thiastearates

All methyl thiastearates were purified by flash chromatography. (silica gel, 4% EtOAc/hexanes.) Methyl 8-, 9-, 10-, 11-, 12-, and 13-thiastearates are colorless liquids at 21 °C. Methyl 5-, 6-, and 7-thiastearates are colorless, waxy solids with mps 30-31 °C, 26-26.5 °C and 23-

25 °C respectively. The purity of the thiastearate samples was assessed according to the folowing criteria: TLC (10 % EtOAc/hexanes): R_f: 0.31 (5-thiastearate) - 0.37 (13-thiastearate); capillary GC (J. + W. DB 225, 0.25 μ film, Inj. 180 °C, FID Det. 300 °C , temp. program: 180 °C to 300 °C at 10 °C/min): t_r: 15.67 min (5-thiastearate) -16.41 min (13-thiastearate). HPLC (see Table 1): t_r: 11.60 min (5-thiastearate) -13.53 min (13-thiastearate). The presence of the sulfide group was determined by ^1H NMR spectra (300 MHz, $CDCl_3$) which showed two overlapping triplets at 2.5 ppm and by MS (EI, 70 eV) which exhibited a molecular ion at m/e 316 and characteristic fragment ions due to cleavage alpha to the sulfur atom.

Physical Data: Methyl Thiaoleates

All methyl thiaoleates were isolated as colorless liquids by preparative reverse phase HPLC (See Table 1) The retention times of the methyl thiaoleates was 3-7 minutes shorter than the corresponding thiastearates. The ^1H NMR spectrum ($CDCl_3$, 300MHz) of each isomer showed an AB quartet of triplets, J = 10-11 Hz at ca. 5.4 ppm - diagnostic for a *cis* -olefin. The MS (EI, 70eV) of each isomer exhibited a weak molecular ion at m/e 314 and fragment ions due to cleavage alpha to the sulfur atom.

Physical Data: Sulfoxides and Sulfones of Methyl Thiastearates

The sulfoxides were prepared by oxidation of the corresponding sulfides using one equivalent of MCPBA in CH_2Cl_2 at O °C for two hours. The sulfoxides were purified by flash chromatography on silica gel using EtOAc as eluant. All sulfoxides are colorless solids with mps ranging from 82-82.5 °C (5-sulfoxide) to 67-68 °C (13-sulfoxide). The R_f values on silica gel TLC (EtOAc) ranged continuously from 0.21 (5-sulfoxide) to 0.32 (13-sulfoxide). Evidence for sulfoxide formation was obtained from ^1H NMR spectra (300 MHz, $CDCl_3$) which exhibited a multiplet integrating for 4 hydrogens centered at 2.55-2.70 ppm, IR spectra which showed an SO stretch at 1025 cm^{-1} and MS (EI, 70 eV) which featured diagnostic fragment ions due to cleavage alpha to the sulfinyl group.

Sulfones were prepared by oxidation of the corresponding sulfides using two equivalents of MCPBA in CH_2Cl_2 at 30 °C for two hours. The sulfones were purified on a silica gel column using 10 % EtOAc/hexanes as eluant to give colorless solids with mps as follows: 82-84.5 °C (5-sulfone) to 64-66 °C (13-sulfone). The Rf values on silica gel TLC (30 % EtOAc/hexanes) ranged from 0.25 (5-sulfone) to 0.34 (9-sulfone) to 0.25 (13-sulfone). The MS of sulfones exhibited a weak molecular ion at m/e 348 and the IR ($CHCl_3$) possessed diagnostic bands at 1300 cm^{-1} and 1135 cm^{-1}.

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