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MICROBIAL DEHYDROGENATION OF A DITHIA-ANALOGUE OF STEARIC ACID

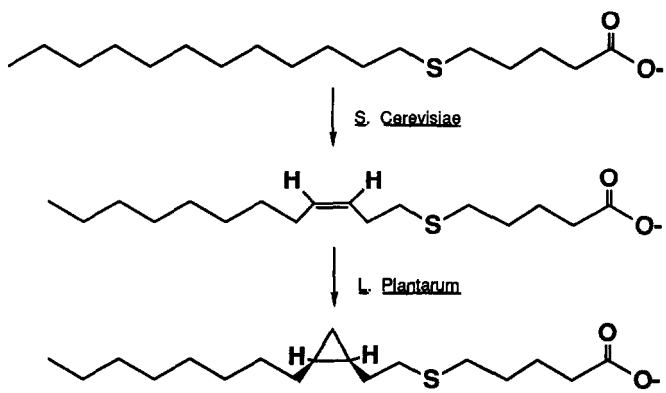
Peter H. Buist* and H. Garry Dallmann

The Ottawa-Carleton Institute for Research and Graduate Studies

Carleton University, Ottawa, Ontario, Canada K1S 5B6

Abstract: The substitution of a methylene group by a sulfur atom at C-6 and C-13 of stearic acid does not prevent the bio-introduction of a *cis* - double bond between the two sulfur atoms.

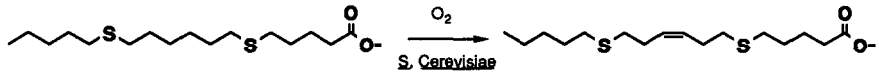
Biological transformations of thia-analogues of fatty acids are being studied extensively in this laboratory. It was previously demonstrated that the substitution of a methylene group by a sulfur atom at C-6 in oleic acid did not prevent its biomethylenation by the cyclopropane synthase of the bacterium, L. plantarum .[1] Also, the production of a number of thiaoleic acids from the corresponding thiastearic acids, by the desaturase system of the yeast, S. cerevisiae, has been shown to occur in a highly regio-, chemo- and stereoselective manner.[2] These findings point to the potential use of *in vivo* systems to produce compounds of ultra high stereo- and regiochemical purity from simple achiral starting materials (Scheme 1).



Scheme 1

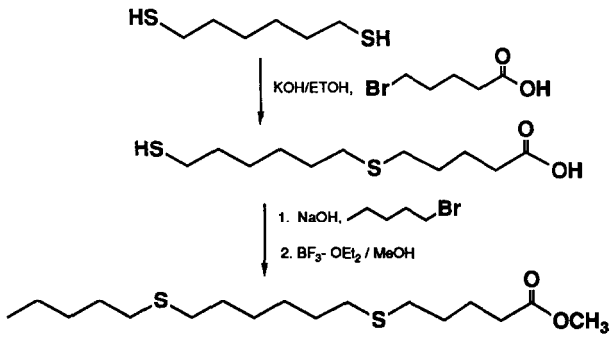
Reductive desulfurization of the final product would in principle yield an enantiomerically pure cyclopropyl compound which is free of regioisomers.

It was of interest to see if these types of transformations could be extended to include dithia-fatty acid analogues as was originally proposed.[1] We now wish to report the first example of the functionalization of a dithia-fatty acid - the desaturation of 6,13-dithiastearic acid by the yeast, S. cerevisiae # NRC 2335 (Scheme 2).



Scheme 2

Methyl 6,13-dithiastearate was synthesized in four steps using an adapted version of the method of Rapaport et al.[3] The synthesis consisted of two successive alkylations of 1,6-hexanedithiol with 5-bromovaleric acid and 1-bromopentane (See Scheme 3).



Scheme 3

In the initial coupling of 1,6-hexanedithiol with 5-bromovaleric acid, a twofold excess of the former compound was used to minimize dialkylation. Excess 1,6-hexanedithiol was removed by washing an aqueous solution of the carboxylate salt with ether. All reactions were done under N_2 to prevent oxidation of sulfur compounds. The final methyl ester product was obtained in an overall yield of 28% after purification by silica gel flash chromatography (4% EtOAc/hexanes). Analysis by capillary GC and TLC (silica gel, 4% EtOAc/hexanes) showed that the compound was homogeneous by these criteria. (All spectral and chromatographic data is given in the experimental section).

In order to test this dithiastearate as a substrate for desaturation, the compound was administered to freshly inoculated liquid cultures of the yeast, S. cerevisiae (350 mL cultures) as a solution in ethanol (50 mg/2 mL). The culture medium was that used by Bloomfield and Bloch[4]. After growth (48 h), the cells were harvested by centrifugation at 4 °C . The cell pellet was washed twice with deionized water and collected by filtration on a 0.45 μm filter. The cells were stored under N_2 at -20 °C and the supernatant culture medium was stored in the frozen state under toluene. The cells were hydrolyzed in 50% ethanolic KOH (1N) and the free fatty acids were methylated using BF3/etherate in methanol (12% w/v). The supernatant culture medium was acidified to pH 2 and extracted with methylene chloride. This extract was then hydrolyzed and the fatty acids methylated. The fatty acid methyl esters obtained from both the cells and the culture medium were analyzed by capillary GC (see experimental section) for the presence of desaturated

fatty acids and by TLC (silica gel, EtOAc) for possible polar fatty acids such as sulfoxides and sulfones.

The GC analyses revealed that unlike the monothiastearates,[2] the dithiastearate was not incorporated into the cells to any significant extent. The culture medium did however contain product derived from methyl 6,13-dithiastearate. As the data in Table 1 shows, the major product is in fact the desired desaturated compound (dithia C_{18}:1) along with small amounts of chain-shortened material (dithiaC_{16}:0, dithiaC_{16}:1) due to β-oxidation.[5] Initial identification of products was accomplished by GC-MS(See experimental section).

Table 1 - Fatty Acid Profiles of Culture Medium Extracts

Results expressed as percentages of total fatty acids

Dithiastearate added to culture (mg)	Weights of Culture Medium Extracts (mg)	C_{16}:0	C_{16}:1	C_{18}:0	C_{18}:1	dithia-C_{16}:0	dithia-C_{16}:1	dithia-C_{18}:0	dithia-C_{18}:1
0	10.0	tr.	tr.	tr.	tr.				
48.4	30.5	3	5	2	6	1	2	59	22
49.3	23.1	4	6	tr.	4	2	2	66	16
49.6	32.0	18	11	4	12	4	5	34	12

The common names of the fatty acids analysed are as follows: (the capillary GC retention times are given for each fatty acid): C_{16}:0 is palmitic acid (7.51 min); C_{16}:1 is palmitoleic (7.33 min); C_{18}:0 is stearic (9.52 min) C_{18}:1 is oleic (9.31 min); dithiaC_{18}:0 is 4,11-dithiapalmitic (11.59 min) ; dithia C_{16}:1 is 4,11-dithiapalmitoleic (11.28 min) ; dithia C_{18}:0 is 6,13-dithiastearic (13.49 min); dithia C_{18}: 1 is 6,13-dithiaoleic (13.24 min).

The olefinic dithia-fatty acid (dithia C_{18}:1), (4.6 mg) was isolated from one of the feeding experiments (line 3 of Table 1) by reversed phase HPLC (see experimental section) and shown to be methyl 6,13-dithiaoleate by ^{1}H and ^{13}C NMR (see experimental section). The *cis* - stereochemistry of the double bond was confirmed by the presence of a ^{13}C resonance at 27.1 ppm for the allylic carbons.[6] At the present time we have no indication that regio- or stereoisomers of methyl 6,13-dithiaoleate have been produced as "error" products - caused by the substitution of two methylene groups of stearic acid by sulfur atoms. We are currently developing ways to assess more precisely the regiochemical and stereochemical purity of this material.

TLC analysis of the cell- and culture medium extracts failed to show the presence of any new polar compounds. Thus oxidation of either sulfur atom of methyl 6,13-dithiastearate does not appear to have taken place to any detectable extent.

In summary, it appears that methyl 6,13-dithiastearate enters the yeast cell, is activated as the Coenzyme A derivative, is desaturated and than is expelled into the culture medium. This situation is ideal from a biotechnological point of view and experiments using immobilized cells now become feasible.

Acknowledgements

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Physical Data: Methyl 6,13-Dithiastearate

The capillary G.C. conditions used to analyze dithia-fatty acid methyl esters were as follows: Varian Vista 6000 GC equipped with a 30 m J. + W. DB-5 Capillary column, 0.25μ film, temperature programmed from 180°C to 300°C at 10°C/min with injection at 180°C and FID detection at 300°C. Under these conditions, methyl 6,13-dithiastearate eluted at 13.49 min. The title compound was further characterized as follows: MS (EI , 70 eV) : m/e 334 (M^+), 263 (M^+- C_5H_{11}), 219 (CH_3-$(CH_2)_4S(CH_2)_6$S-). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, CH_3CH_2-), 1.33 -1.37 (m, $S(CH_2)_2$ - $(CH_2)_2(CH_2)_2$S-, $CH_3(CH_2)_2$-), 1.53 -1.60 (m, -$CH_2CH_2SCH_2CH_2$-), 1.71(qi, CH_2CH_2 - CH_2COOCH_3), 2.32 (t, -$CH_2CH_2COOCH_3$), 2.45 - 2.52 (m, -$CH_2CH_2SCH_2CH_2$-), 3.65 (s, OCH_3).

Physical Data: Biosynthetic Products derived from Methyl 6,13-Dithiastearate

Product formation was monitered by capillary GC and products identified by GC-MS (VG 7070E mass spectrometer) operated in the EI mode at 70 eV coupled to a Varian 3300 GC equipped with a J. + W. DB-5 Megabore Column,1μ film .

Methyl 4,11-Dithiapalmitate (dithia C_{16}:0) MS: m/e 306 (M^+), 203 (M^+ - $C_5H_{11}S$), 119 (-$S(CH_2)_2COOCH_3$), 87 (-$(CH_2)_2COOCH_3$).

Methyl 4,11- Dithiapalmitoleate (dithiaC_{16}:1) MS: m/e 201 (-$(CH_2)_2CH$=$CH(CH_2)_2COOCH_3$), 217 ($C_5H_{11}S(CH_2)_2CH$=$CHCH_2CH_2$S-), 185 ($C_5H_{11}S(CH_2)_2CH$=$CHCH_2CH_2$-).

Methyl 6,13-dithiaoleate (dithia C_{18}:1) This material was isolated by fractionation of the fatty acid methyl esters on a Whatman Magnum-9 ODS-2 column (2.5 cm x 50 cm) using 10% EtOAc /CH3CN as the mobile phase at a flow rate of 4.0 mL/min. RI detection was used. MS: m/e 261 (-$S(CH_2)_2CH$=$CH(CH_2)_2S(CH_2)_4COOCH_3$), 229 (-$(CH_2)_2CH$=$CH(CH_2)_2S(CH_2)_4COOCH_3$), 217 ($C_5H_{11}S(CH_2)_2CH$=$CH_2CH_2$S-), 185 ($C_5H_{11}S(CH_2)_2CH$=$CHCH_2CH_2$-), 171 ($C_5H_{11}$-$S(CH_2)_2CH$=$CHCH_2$-), 161 (-$CH_2S(CH_2)_4COOCH_3$).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, CH_3CH_2-), 1.41 (m, $CH_3(CH_2)_2(CH_2)_2$S-), 1.54 - 1.60 (br.m. -$SCH_2CH_2CH_2CH_2COOCH_3$, $CH_3(CH_2)_2CH_2$S-), 1.69 (qi, -$CH_2CH_2CH_2COOCH_3$), 2.32 (m, - CH_2CH_2 CH_2COOCH_3, -CH_2CH=$CHCH_2$-), 2.48-2.55 (m, -$CH_2CH_2SCH_2CH_2$-) , 3.65 (s, -OCH_3), 5.46 (sym. m. -CH=CH-). ^{13}C NMR (50.3 MHz, $CDCl_3$). Diagnostic peaks were observed at δ 129.2 (-CH=CH-) 31.9 (-CH_2CH_2S-), 27.7 (-CH_2CH=CH-).

References

1. P. H. Buist and G.P. Dimnik. Tetrahedron Lett. **27**, 1457 (1986).
2. P. H. Buist, H.G. Dallmann, P.M. Seigel and R.T. Rymerson, Tetrahedron Lett. **28**, 857 (l987) and *unpublished results.*
3. L. Rapoport, A. Smith and M.S. Neuman. J. Am. Chem. Soc. **69**, 693 (1947).
4. D.K. Bloomfield and K. Bloch. J. Biol. Chem. **235**, 337 (1960).
5. J.D. McGarry and D.W. Foster. Ann. Rev. Biochem. **49**, 395 (1980).
6. J.N. Shoolery, Progress in NMR Spectroscopy, **11**, 79 (1977).

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