Chlorophyll-Sensitized Peroxidation of Saturated Fatty Acid Esters[1]

Y. SITA RAMA SASTRY and GOLLAMUDI LAKSHMINARAYANA,
Regional Research Laboratory, Hyderabad-9, India

ABSTRACT

Chlorophyll-sensitized peroxidation of methyl laurate and methyl stearate was carried out at 30-40 C under intermittent exposure to light from a 500 w tungsten bulb. Hydroperoxides were isolated by solvent partition, reduced to hydroxy esters and purified by silicic acid column chromatography and preparative thin layer chromatography (TLC). Gas liquid chromatography, TLC, IR spectrophotometry, NMR and mass spectroscopy of the hydroxy esters showed that the oxygen attack was exclusively on the α-methylenic carbon atom.

INTRODUCTION

Saturated fatty acids present in fats may be autoxidized very slowly and the products, once formed, though in small concentrations, may contribute significantly to problems of flavor and stability (1,2). Earlier autoxidation studies on saturated fatty acids, esters and glycerides were carried out at moderate (120 C) to high temperatures (200 C) to accelerate the rate of peroxidation (1-5). These studies led to various suggestions, namely, that α- (3,4), β- (4) and random (1,2,5) oxidations occurred, thus leaving unanswered the question of initial site of oxygen attack. A definite conclusion is difficult to draw because of the decomposition of the peroxides even at moderate temperatures and subsequent secondary reactions. We have employed chlorophyll as a photosensitizer to peroxidize saturated fatty esters under mild conditions at about room temperature.

Chlorophyll occurs naturally in some oils such as soybean, olive, peanut and linseed, and is known to accelerate autoxidation (6). Chlorophyll-sensitized peroxidations of unsaturated fatty esters have already been reported (7,8). Khan et al. (7) reported the attack of oxygen at the methylenic carbon atom adjacent to the double bond whereas Cobern et al. (8) stated that this occurred at each of the carbon atoms which originally formed part of the double bond with subsequent migration of double bond to an allylic position.

Our studies on chlorophyll-sensitized peroxidation of saturated esters show that the initial attack of oxygen is exclusively at the methylenic carbon adjacent to the carboxyl ester group.

EXPERIMENTAL PROCEDURES

Materials

Technical grade lauric and stearic acids were esterified with methanol and fractionated in an electrically heated and packed column under vacuum. The purity of the ester fractions was checked by determining peroxide, acid, iodine and saponification values by the AOCS methods (9) on a semi-micro scale and refractive indices. The purity was also checked by reversed phase paper chromatography of fatty acids liberated from the methyl esters on Whatman No. 4 paper impregnated with liquid paraffin using 95% acetic acid (10). Methyl esters were also analyzed both on silicone gum rubber (methyl-GE SE-30) and diethylene glycol succinate (DEGS) columns using an F and M Model 720 programmed temperature gas chromatograph equipped with a flame ionization detector. Only those samples free from peroxides and shown to be pure by all the methods mentioned were used in the studies.

Methyl 2-hydroxystearate was synthesized according to the method of LeSuer (11). It was found to be pure by thin layer chromatography (TLC), gas liquid chromatography (GLC), IR spectrophotometry and NMR spectroscopy as described later. Methyl 6(7)-, 9(10)-, 12- and 18-hydroxystearates were gifts from R. Subbarao and found to be pure by TLC and GLC as described later. *n*-Hexadecane was purchased (Fluka Ag.) and found to give a single peak by programmed temperature GLC on a SE-30 column.

Chlorophyll was extracted from fresh spinach leaves with acetone and transferred to petroleum ether by addition of water (12). The petroleum ether solution was washed with 80% methanol to remove carotenes, concentrated and adsorbed on a bed of calcium carbonate. Chlorophyll was eluted with 1% isopropanol in petroleum ether and finally ether. It was shown to consist of chlorophylls a and b, carrying traces of xanthophylls and carotenes as impurities, by TLC on Silica Gel G (13), reversed phase TLC (14) and visible spectrophotometry.

Methods

Light from a 500 w tungsten bulb was allowed to pass through a 3 cm layer of water kept in a flat glass trough resting on a tripod stand. The trough was placed midway (8 cm) between the bulb and a petridish placed above containing the ester sample spread in a 4 mm layer after incorporating chlorophyll. The whole assembly was enclosed in a chamber covered with black cloth. By alternate periods of exposure for 10 min to light and darkness and periodic replacement of water in the trough, the temperature of the ester sample was kept below 40 C. The sample was swirled every 10 min. Methyl stearate and methyl laurate were peroxidized to peroxide values of 20 and 26, respectively, in 10 g batches using 1% of chlorophyll until the green color disappeared. Hydroperoxides were concentrated by partition between heptane and 88% methanol. Hydroxy esters were obtained by treatment of the methanol extract with sodium bisulfite (15) and purified by silicic acid column chromatography (16). Three per cent ether in petroleum ether removed nonhydroxy esters, while 20% ether in petroleum ether eluted hydroxy esters. Further purification of the hydroxy esters was achieved by preparative TLC on Silica Gel G plates (500 μ thick) using a solvent system of petroleum ether-ether-acetic acid (75:25:1 v/v) and iodine vapor for locating the bands. Both hydroxystearate and hydroxylaurate were analyzed by TLC, GLC, IR spectrophotometry and NMR spectroscopy. Hydroxystearate was also analyzed by mass spectroscopy.

Analytical TLC was carried out on Silica Gel G plates (250 μ thick) using petroleum ether-ether-acetic acid (75:25:1 v/v) as solvent system and phosphomolybdic acid for locating the spots. GLC was carried out on a column of 2% SE-30 (2 ft x 3/16 in.) on Chromosorb W (60-80 mesh). Methyl hydroxylaurate was analyzed at 150 C using a flame ionization detector and N_2 as carrier gas. The flow rates of H_2, N_2 and air were 40, 100 and 400 ml/min, respectively.

[1]One of 28 papers presented at the Symposium, "Metal-Catalyzed Lipid Oxidation," ISF-AOCS World Congress, Chicago, September 1970.

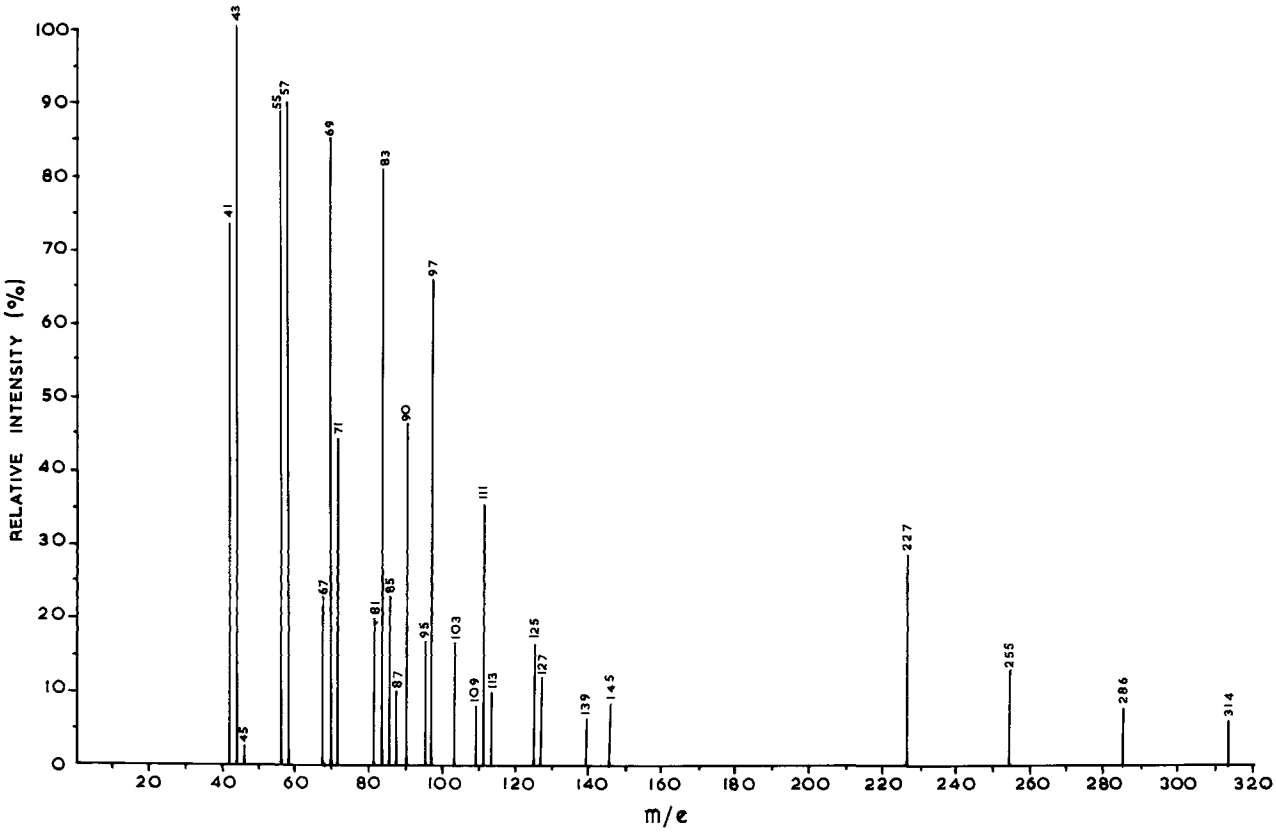


FIG. 1. Mass spectrum of methyl hydroxystearate.

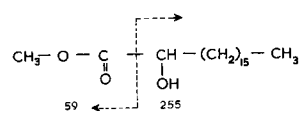
Methyl hydroxystearate was analyzed at 200 C using a thermal conductivity detector and H_2 as carrier gas (100 ml/min). Our sample of hydroxystearate was also analyzed by A.P. Tulloch before and after acetylation. IR spectra were recorded with a Perkin-Elmer IR spectrophotometer 221 using 1% chloroform solution. NMR spectra were recorded with a Varian A-60 A NMR spectrometer on a 5% solution in carbon tetrachloride using tetramethylsilane as standard.

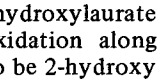
RESULTS AND DISCUSSION

TLC of peroxidized esters and the reduced hydroxy esters did not show more polar compounds than the monohydroperoxides and monohydroxy esters, respectively, indicating that the oxygen attack on the carbon chain did not occur more than once.

No unsaturation was created during chlorophyll-sensitized oxidation as evidenced by the negligible iodine values of the hydroxy and nonhydroxy esters. The pure hydroxy esters yielded were ca. 0.05% based on methyl esters taken for oxidation.

TLC of methyl hydroxystearate and hydroxylaurate obtained from chlorophyll-sensitized peroxidation along with reference compounds indicated them to be 2-hydroxy esters. Both the hydroxy esters from chlorophyll-sensitized oxidations had the same R_f value as the standard 2-hydroxystearate which had a higher value than those for other positional isomers of hydroxystearate. The 2-hydroxy ester is known to have the highest mobility (17). The ratios of the retention times on an SE-30 column of methyl hydroxystearate to methyl stearate and of methyl hydroxylaurate to methyl laurate were 2.3 and 1.6, respectively. A.P. Tulloch reported carbon numbers of 19.3 and 20.3 for our sample of methyl hydroxystearate and its acetylated sample. IR spectra of both hydroxystearate and hydroxylaurate showed a weak shoulder at 2.76 μ and a strong band at 2.81 μ, which are characteristic for 2-hydroxy ester (18). IR spectra of the hydroxystearate obtained and authentic 2-hydroxystearate were identical. NMR spectra revealed the absence of an α-CH_2 group since no signal in the region 2.0-2.5 δ was noticed. The signals at 3.69 δ attributable to OCH_3 and 4.05 δ due to α-hydroxy group were observed (19). Also the signals at 0.9 δ attributable to terminal CH_3 and 1.2 δ due to CH_2 groups were also noticed. No signals were observed to indicate the presence of other positional isomers of hydroxy esters. Tulloch reported prominent signals at 3.72 δ due to OCH_3 and at 3.98 δ due to -CHOH for our hydroxystearate sample. The mass spectrum of hydroxystearate gave characteristic peaks for 2-hydroxystearate. The parent peak m/e = 314 was present (molecule ion peak).



The peak m/e = M - 59 = 255 which is specific for 2-hydroxystearate was observed. Also m/e = 90 due to ion  which is specific for methyl 2-hydroxyesters was noticed (20). Fragments indicating other positional isomers of hydroxystearate were not observed. The relative intensity vs. m/e was presented in the graphical form (FIG. 1). It was thus confirmed that the hydroxystearate, obtained by reduction of the hydroperoxide produced in chlorophyll-sensitized peroxidation of methyl stearate under mild conditions, is 2-hydroxystearate, suggesting an exclusive attack of oxygen on the α-methylenic carbon atom adjacent to carboxyl ester. This is in agreement with the general reactivity of the α-methylene group in carboxylic acids in chemical reactions such as bromination. The effect of the carboxyl ester moiety was further evidenced by the absence of peroxide development

in chlorophyll-sensitized peroxidation of *n*-hexadecane under similar conditions. However, in thermal oxidations of saturated fatty acid esters and saturated hydrocarbons, the position of initial attack of oxygen is controversial. While some workers considered α- and β-carbons as the initial sites others noticed random attack with a preference towards the center of the carbon chain especially between the 5 and 11 positions (1-5). One explanation for these observations on thermal oxidations could be that the peroxy radical formed is originally formed at the α-carbon atom and propagated thereafter along the chain. It is also possible that at higher temperatures not only the α-methylenic carbon atoms but also other methylenic carbon atoms are activated more or less simultaneously and peroxidized. Apart from these possibilities, it may be that the mechanism of chlorophyll-sensitized peroxidation of saturated esters is different from that of thermal oxidation.

ACKNOWLEDGMENTS

R. Ryhage, Karolinska Institutet, Stockholm, did the mass spectral analysis of methyl hydroxystearate and A.P. Tulloch, National Research Council, Saskatoon, Canada, did NMR and GLC analyses of methyl hydroxystearate. The Joint Committee of the Indian Council of Agricultural Research and the Council of Scientific and Industrial Research sponsored this investigation.

REFERENCES

1. Swern, D., "Autoxidation and Antioxidants," Vol. 1, Edited by W.O. Lundberg, Interscience, New York, 1961, p. 26-28.
2. Brodnitz, M.H., J. Agr. Food Chem. 16:994 (1968).
3. Crossley, A., T.D. Heyes and B.J.F. Hudson, JAOCS 39:9 (1969).
4. Thaler, H., and H.J. Kleinau, Fette Seifen Anstrichm. 71:261 (1961).
5. Brodnitz, M.H., W.W. Nawar and I.S. Fagerson, Lipids 3:59, 65 (1968).
6. Coe, M.R., Oil Soap 15:230 (1938).
7. Khan, N.A., W.E. Tolberg, D.H. Wheeler and W.O. Lundberg, JAOCS 31:460 (1954).
8. Cobern, D., J.S. Hobbs, R.A. Lucas and D.J. Mackenzie, J. Chem. Soc. (C) 1966:1897.
9. AOCS, "Official and Tentative Methods," Second Edition, Revised to 1960, Cd 8-53, Ca 5a-40, Cd 1-25, Cd 3-25.
10. Alimova, E.K., and G.D. Volgova, Biochemistry 22:527 (1957).
11. LeSuer, H.R., J. Chem. Soc. 85:827 (1904).
12. Jacobs, E.E., A.E. Vatter and A.S. Holt, Arch. Biochem. Biophys. 53:228 (1954).
13. Anwar, M.H., J. Chem. Educ. 40:29 (1963).
14. Randerath, K., "Thin Layer Chromatography," Verlag Chemie, GMBH Weinheim/Bergstr., 1964, p. 154.
15. Perlstein, T., A. Eisner and W.C. Ault, JAOCS 43:380 (1966).
16. Fulco, A.J., and J.F. Mead, J. Biol. Chem. 236:2416 (1961).
17. Morris, L.J., and D.M. Wharry, J. Chromatogr. 20:27 (1965).
18. Morris, L.J., and S.W. Hall, Chem. Ind. (London) 1:32 (1967).
19. Tulloch, A.P., JAOCS 43:670 (1966).
20. Ryhage, R., and E. Stenhagen, Arkiv Kemi 15:545 (1960).

[Received April 5, 1971]