Hydroxy Fatty Acids Through Hydroxylation of Oleic Acid with Selenium Dioxide/*tert.*-Butylhydroperoxide[1]

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Oleic acid was hydroxylated in the allylic positions with the selenium dioxide/*tert.*-butylhydroperoxide system to give 8-hydroxy-9(*E*)-octadecenoic acid, 11-hydroxy-9(*E*)-octadecenoic acid and the novel 8,11-dihydroxy-9(*E*)-octadecenoic acid. This is a viable method for obtaining hydroxy fatty acids. The unsaturated hydroxy acids were hydrogenated with the hydrazine/air system to give the corresponding saturated products. 8,11-Dihydroxyoctadecanoic acid thus obtained is also a novel compound. The saturated and unsaturated dihydroxy products were obtained as *erythro/threo* isomers as determined by nuclear magnetic resonance.

KEY WORDS: Allylic hydroxylation, hydrazine, hydrogenation, hydroxy fatty acid, selenium dioxide, *tert.*-butylhydroperoxide.

Hydroxy fatty acids are of considerable interest in commercial applications such as lubricants and cosmetics (1,2). They occur naturally only in few vegetable oils, most notably castor oil, and some other alternative crops such as the more recently investigated lesquerella (3). There have been considerable efforts to obtain hydroxy fatty acids by microbial transformations (2,4–8). Chemically, hydroxy fatty acids have been synthesized by peracid oxidation of unsaturated fatty acids or direct oxidation of the double bond with reagents such as potassium permanganate or hydrogen peroxide/tungstic acid (9) to give vicinal diols.

One common hydroxylation reaction, allylic hydroxylation of double bonds with selenium dioxide, has been neglected almost completely in the synthesis of hydroxy fatty acids (10–12). These papers report mixtures of various reaction products. However, the products were not fully characterized by methods such as mass spectrometry (MS) and nuclear magnetic resonance (NMR). Since those earlier studies, *tert.*-butylhydroperoxide (TBHP) has been introduced as a reoxidizing agent to eliminate colloidal selenium formed during the reaction (13). TBHP remains the coreagent of choice when carrying out allylic hydroxylations with selenium dioxide (14).

Recently, bioconversions with the *Pseudomonas* strains PR3 and 42A2 yielded a novel compound, 7,10-dihydroxy-8(*E*)-octadecenoic acid from oleic acid (5) or olive oil (6). This is a unique product because it involves a double allylic hydroxylation besides a shift of the double bond. The SeO_2/TBHP system can simulate such a hydroxylation without a double bond shift. Hydrogenation of the double bond yields the corresponding saturated products.

EXPERIMENTAL PROCEDURES

Oleic acid was obtained from Nu-Chek-Prep, Inc. (Elysian, MN). Selenium dioxide and 90% TBHP solution containing 5% water and 5% *tert.*-butyl alcohol were purchased from Aldrich Chemical Co. (Milwaukee, WI). Solvents were obtained from EM Science (Gibbstown, NJ).

NMR spectra were obtained with $CDCl_3$ as solvent on a Bruker (Rheinstetten, Germany) WM-300 spectrometer operating at 300 MHz (^{1}H-NMR) and 75.5 MHz (^{13}C-NMR). To enhance solubility, the saturated dihydroxy acids were examined in cosolvents of $CDCl_3$ and CD_3OD. Gas chromatography/MS (GC/MS) analyses were performed on a Hewlett-Packard (Palo Alto, CA) 5890/5970 benchtop GC/MS system operated with electron ionization (EI). Fourier transform infrared (FTIR) spectra were recorded on a Mattson (Madison, WI) Polaris spectrometer as KBr pellets. Melting points were determined in an Electrothermal (Southend, England) 9300 apparatus and are uncorrected.

The GC/MS conditions used were described previously, as were equipment and conditions of high-performance liquid chromatography (HPLC) (15).

Hydroxylation reaction. In a typical synthesis, in a 100-mL round-bottomed flask equipped with a reflux condenser, 3.83 g (34.5 mmole) SeO_2, 15 mL TBHP solution and 40 mL dichloromethane were mixed at room temperature and stirred for 0.5 h. Then 10 g (35.4 mmole) oleic acid was added, and the reaction mixture was stirred for 24 h at room temperature. After this time thin-layer chromatography (TLC) (70:30:1, hexane/ethyl acetate/acetic acid, spots visualized with iodine vapor, spraying with H_2SO_4, and baking at 120°C for 5 min) showed that most (>80%) of the starting material had reacted. The reaction was worked up by removing the dichloromethane, adding water immediately and extracting into toluene. (CAUTION: Water must be added immediately, because the stripped mixture may form harmful fumes. Presumably, the vapors are volatile organoselenium compounds that form in the presence of air by exothermic reaction. Such vapors have been observed within 15 min following removal of dichloromethane. As an alternative procedure, add water to the reaction mixture without removing CH_2Cl_2, and then proceed with extracting as described above). The organic phase was washed three times with water, three times with brine, dried with sodium sulfate and filtered, and the solvent was then removed on a rotary evaporator. The product was dried *in vacuo* in a desiccator. The dried, often still viscous reaction mixture was subjected to several HPLC purifications. The first HPLC run was conducted with hexane/ethyl acetate/acetic acid (90:10:1), the second one with hexane/ethyl acetate/acetic acid (80:20:1) and the third and final run with CH_2CL_2/methanol (92:8). When using lesser amounts (<5 g) of starting material, the first HPLC run was left out. The first HPLC run removes keto acids and other side products from the reaction mixture as well as significant amounts of monohydroxy products. The second HPLC run removes the remaining monohydroxy products as well as other side products. The third HPLC run purifies the dihydroxy acids, which are obtained as two separate fractions (*erythro/threo* diastereomers as discussed below). The two monohydroxy products coelute in the HPLC

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runs mentioned above. However, they can be separated from a concentrate by repeating the HPLC purification on a smaller scale (≤1 g) with hexane/ethyl acetate/acetic acid (80:20:1).

Hydrogenation reactions. These reactions were carried out as described previously (15) with the hydrazine/air system in ethanolic solution. Yields of these reactions range from 80% to nearly quantitative. The products can be purified, if desired, by crystallization from CH_2Cl_2.

RESULTS AND DISCUSSION

In this paper, we report that the selenium dioxide/TBHP system is suitable for obtaining allylic mono- and dihydroxylated unsaturated fatty acids. The yields are superior to those of microbial hydroxylations, and reaction and work-up are considerably more facile. The mono- and dihydroxy fatty acids are the major products with combined yields over 50%. This contrasts with the products reported in the previous literature on oxidations with SeO_2 (10–12), where the reaction conditions differed from those used here, and there is only little mention of allylic dihydroxylated products (10). To our knowledge, other reagents for allylic hydroxylation (14) have not yet been extensively studied in such reactions of fatty acids. Figure 1 depicts the unsaturated hydroxy fatty acids arising from the present reactions.

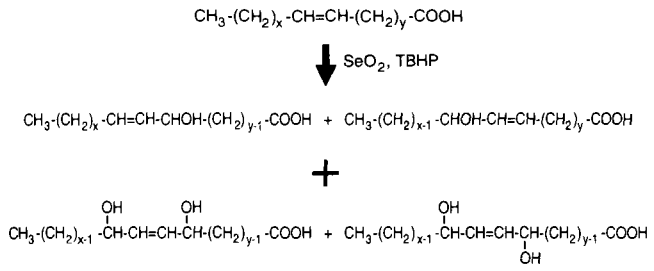

FIG. 1. Hydroxy fatty acids obtained by allylic hydroxylation of monounsaturated fatty acids with selenium dioxide/*tert.*-butylhydroperoxide. In case of oleic acid as it was investigated here, x = y = 7.

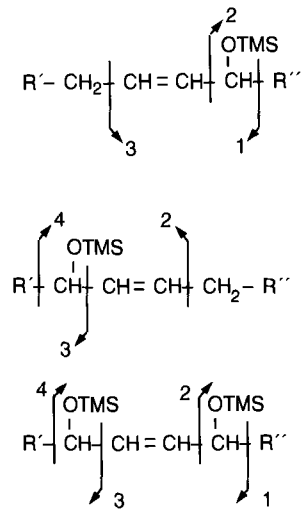

FIG. 2. Electron ionization mass spectrometry cleavage patterns for silylated allylic hydroxy fatty acids. R′ contains the methyl terminus and R″ contains the carboxylic acid functionality. These cleavage patterns, with the exception of cleavage 3 in a and cleavage 2 in b, also hold for the hydrogenated products.

The present report will focus only on oleic acid as an exemplary starting material. 8-Hydroxy-9(*E*)-octadecenoic acid, 11-hydroxy-9(*E*)-octadecenoic acid and 8,11-dihydroxy-9(*E*)-octadecenoic acid, which is a novel compound, were obtained from oleic acid. The dihydroxy product was obtained in two isomeric forms, which NMR revealed to be *erythro* (absolute configurations R,S and S,R)/*threo* (absolute configurations *R,R* and *S,S*) diastereomers. The compounds were characterized by MS, ^{1}H-NMR and ^{13}C-NMR, IR and their melting points.

Characterization of the monounsaturated hydroxy fatty acids. The pattern of cleavage in EIMS for the dihydroxy compounds has been discussed for the microbially produced 7,10-dihydroxy-8(*E*)-octadecenoic acid (5). Figure 2c shows the cleavage pattern giving rise to the most intense fragments. The dihydroxy samples were silylated to give the *bis*(trimethylsiloxy) trimethylsilyl (TMS) esters. These gave rise to peaks at *m/z* 73 (TMS) and a characteristic cleavage pattern of 431 (cleavage 4 in Fig. 2c), 341 (431-TMSOH), 329 (cleavage 1), 239 (329-TMSOH), 303 (cleavage 2) and 201 (cleavage 3). Peak intensities, as well as the base peaks, may vary from sample to sample with the peaks at *m/z* 73 and 341 being the strongest. Silylated 8-hydroxy-9(*E*)-octadecenoic acid gives a base peak at *m/z* 73 and a strong peak at *m/z* 241, resulting from cleavage 1 in Figure 2a. Correspondingly, silylated 11-hydroxy-9(*E*)-octadecenoic acid shows a base peak at *m/z* 73 and a strong peak at *m/z* 343 caused by cleavage 4 in Figure 2b. The mass spectra are depicted in Figure 3.

The IR spectra showed typical absorptions at 3307, 3222 [both ν(OH),br], 2921, 2852 and 1713 [ν(C=O)] for 8-hydroxy-9(*E*)-octadecenoic acid, 3303, 3214 [both ν(OH), br], 2925, 2852 and 1713 [ν(C=O)] for 11-hydroxy-9(*E*)-octadecenoic acid, 3219 [ν(OH),br], 2929, 2856 and 1702 [ν(C=O)] for *erythro*-8,11-dihydroxy-9(*E*)-octadecenoic acid, and 3315 [ν(OH,br], 2929, 2856 and 1698 [ν(C=O)] cm^{-1} for *threo*-8,11-dihydroxy-9(*E*)-octadecenoic acid.

The ^{1}H-NMR spectra were as follows: 8-hydroxy-9(*E*)-octadecenoic acid 5.61 ppm (*dt*, 1H at C10), 5.42 (*dd*, 1H at C9), 4.02 (*q*, 1H at C8), 2.33 (*t*, 2H at C2), 1.64–1.25 (*m*, 24H, CH_2), 0.86 (*t*, 3H at C18); 11-hydroxy-9(*E*)-octadecenoic acid 5.61 ppm (*dt*, 1H at C9), 5.42 (*dd*, 1H at C10), 4.02 (1H at C11), 2.33 (*t*, 2H at C2), 1.65–1.22 (*m*, 24H, CH_2), 0.87 (*t*, 3H at C18); *erythro*-8,11-dihydroxy-9(*E*)-octadecenoic acid 5.68 ppm (*dd*, 2H at C=C), 4.11 (*d*, 2H at C8 and C11), 2.33 (*t*, 2H at C2), 1.65–1.27 (*m*, 22H, CH2), 0.87 (*t*, 3H at C18); *threo*-8,11-dihydroxy-9(*E*)-octadecenoic acid 5.65 ppm (*dd*, 2H at C=C), 4.10 (d, 2H at C8 and C11), 2.34 (*t*, 2H at C2), 1.63–1.25 (*m*, 22H, CH_2), 0.87 (*t*, 3H at C18).

The ^{13}C-NMR spectra exhibited the following peaks: 8-hydroxy-9(*E*)-octadecenoic acid 132.82 (C9), 132.36 (C10) 73.26 (C8), 37.18 (C7), 33.94 (C2), 14.10 (C18) and the other carbons at 32.18, 31.88, 29.44, 29.28, 29.18, 29.14, 28.99,

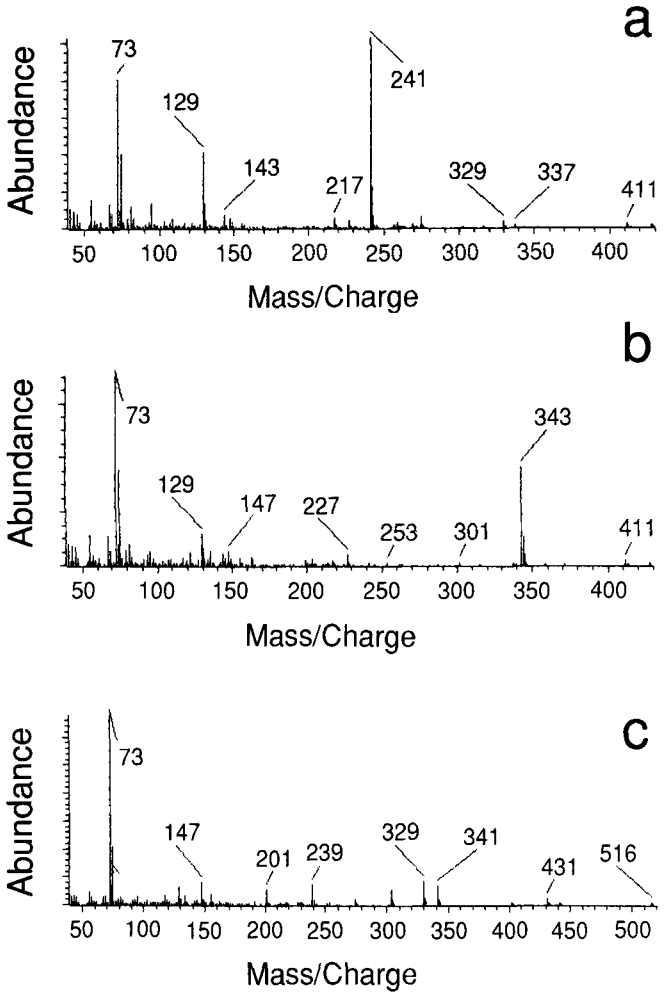

FIG. 3. Electron ionization mass spectra of a, silylated 8-hydroxy-9(*E*)-octadecenoic acid; b, silylated 11-hydroxy-9(*E*)-octadecenoic acid; c, silylated 8,11-dihydroxy-9(*E*)-octadecenoic acid. The two isomers of the dihydroxy compound are indistinguishable by mass spectrometry.

25.26, 24.62 and 22.67 ppm; 11-hydroxy-9(*E*)-octadecenoic acid 133.08 (C10) and 132.07 (C9), 73.27 (C11), 37.35 (C12), 33.86 (C2), 14.11 (C18) and the other carbons at 32.10, 31.83, 29.53, 29.28, 29.06, 28.98, 25.51, 24.65 and 22.67 ppm. The essential peaks of C7-C12 of the unsaturated dihydroxy acids are given in Table 1. The other peaks are observed at 33.59, 31.81, 29.50, 29.25, 28.97, 28.89, 25.40, 25.05, 24.51, 22.65 and 14.10 ppm for *erythro*-8,11-dihydroxy-9(*E*)-octadecenoic acid and 33.52, 31.67, 29.35, 29.12, 28.90, 28.77, 25.30, 24.97, 24.42, 22.52 and 13.96 ppm for *threo*-8,11-dihydroxy-9(*E*)-octadecenoic acid.

The melting points of 8-hydroxy-9(*E*)-octadecenoic acid and 11-hydroxy-9(*E*)-octadecenoic acid are 54-55° and 43-44°C, respectively. The melting points of the unsaturated dihydroxy acids are discussed below.

Characterization of the saturated hydroxy fatty acids. The EIMS of the silylated saturated acids also give rise to characteristic cleavage patterns. The cleavage pattern depicted in Figure 2 is also valid for these compounds. For 8-hydroxyoctadecanoic acid, the strongest fragments are at *m/z* 73 (100%), 303 (cleavage 2 in Fig. 2a) and 243 (cleavage 1). 11-Hydroxyoctadecanoic acid is characterized by *m/z* 73 (100%), 201 (cleavage 3 in Fig. 2b) and 345 (cleavage 4). The two dihydroxy isomers have their base peak at *m/z* 73 and show typical fragments at *m/z* 201 (cleavage 3 in Fig. 2c), 241 (cleavage 1-TMSOH), 303 (cleavage 2) and 343 (cleavage 4-TMSOH).

TABLE 1

^{13}C-Nuclear Magnetic Resonance Assignments of C7-C12 in the *erythro* and *threo* Isomers of 8,11-Dihydroxy-9(*E*)-Octadecenoic Acid and 8,11-Dihydroxyoctadecanoic Acid

8,11-Dihydroxy-9(*E*)-octadecenoic acid			8,11-Dihydroxyoctadecanoic acid		
	erythro	*threo*		*erythro*	*threo*
C9/C10[a]	133.72	133.67			
C9/C10	133.52	133.49			
C8	72.34	72.31	C11	71.41	71.90
C11	72.26	72.23	C8	71.29	71.77
C7	37.30	37.15	C12	36.98	37.38
C12	37.11	36.99	C7	36.83	37.33
			C9,10	32.68	33.65

[a]The ^{13}C signals of the olefinic carbons of the dihydroxy compounds could not be assigned.

Characteristic IR absorptions of 8-hydroxyoctadecanoic acid are observed at 3403 [ν(OH),*br*], 2921, 2852 and 1717 [ν(C=O)] cm^{-1}; 11-hydroxyoctadecanoic acid 3373 [ν(OH), *br*], 2925, 2852 and 1706 [ν(C=O)] cm^{-1}; *erythro*-8,11-dihydroxyoctadecanoic acid 3295, 3214 [both ν(OH),*br*], 2921, 2852 and 1702 [ν(C=O)] cm^{-1}; *threo*-8,11-dihydroxyoctadecanoic acid 3373, 3303 [both ν(OH),*br*], 2925, 2852 and 1706 [ν(C=O)] cm^{-1}.

The ^{1}H-NMR of 8-hydroxyoctadecanoic acid shows peaks at 3.57 (*br*, 1H at C8), 2.34 (*t*, 2H at C2), 1.63 (*t*, 2H at C3), 1.42-1.25 (26H, CH_2) and 0.87 (3H at C18) ppm; 11-hydroxyoctadecanoic acid 3.58 (*br*, 1H at C11), 2.33 (*t*, 2H at C2), 1.62 (*t*, 2H at C3), 1.42-1.27 (26H, CH_2) and 0.87 (3H at C18) ppm; *erythro*-8,11-dihydroxyoctadecanoic acid 3.48 (*br*, 2H at C8 and C11), 2.19 (*t*, 2H at C2), 1.52-1.17 (26H, CH_2) and 0.77 (*t*, 3H at C18) ppm; *threo*-8,11-dihydroxyoctadecanoic acid 3.45 (*br*, 2H at C8 and C11), 2.19 (*t*, 2H at C2), 1.52-1.18 (26H, CH_2), 0.78 (*t*, 3H at C18) ppm.

The ^{13}C-NMR of 8-hydroxyoctadecanoic acid exhibits peaks at 72.00 (C8), 37.52 (C9), 37.35 (C7), 33.74, 31.92, 29.71, 29.63, 29.34, 29.28, 29.02, 25.66, 25.43, 24.63, 22.70 and 14.12 ppm; 11-hydroxyoctadecanoic acid 72.10 (C11), 37.44 (C12), 37.40 (C10), 33.96, 31.84, 29.70, 29.67, 29.61, 29.48, 29.30, 29.16, 29.02, 25.65, 25.59, 24.68, 22.66 and 14.10 ppm. The ^{13}C-NMR peaks for C7-C12 of the two saturated dihydroxy acids are listed in Table 1. Other peaks of the saturated *erythro* isomer are 33.86, 31.66, 29.50, 29.11, 29.05, 28.83, 25.57, 25.27, 24.61, 22.46, and 13.83 ppm and for the *threo* isomer 33.87, 31.66, 29.51, 29.11, 29.05, 28.83, 25.54, 25.25, 24.61, 22.46, 13.83 ppm.

The ^{13}C-NMR spectra of 8-hydroxyoctadecanoic acid and 11-hydroxyoctadecanoic acid coincide with those reported in the literature for the corresponding methyl esters (17).

8,11-Dihydroxyoctadecanoic acid is also a novel compound. To our knowledge, only its ethyl ester derived from methyl oleate by chemical models of cytochrome P-450 has been reported (18).

The melting points of 8-hydroxyoctadecanoic acid and 11-hydroxyoctadecanoic acid are 81 and 71–72°C, respectively. The melting points of the isomers of the saturated dihydroxy acids are also discussed below.

Stereochemistry of the dihydroxy fatty acids. The *erythro/threo* assignments were made by comparing the ^{13}C-NMR spectra of the two hydrogenated dihydroxy isomers obtained from oleic acid with the spectra of methyl 9,10-dihydroxyoctadecanoate (19). These authors distinguished the *erythro* and *threo* forms by differences in the chemical shifts of the carbons alpha to the hydroxy-bearing carbons. Other cases of distinguishing *erythro/threo* diastereomers by ^{13}C-NMR, such as 1,3-diol derivatives (20), have been reported.

It is well known that *erythro/threo* isomers possess different physical properties, such as melting point and solubility, the latter difference facilitating their separation in HPLC as neighboring fractions. *Erythro* isomers have the higher melting points, as is shown by the melting points of *erythro*- and *threo*-8,11-dihydroxy-9(*E*)-octadecenoic acid, which are 85 and 62°C, respectively. Correspondingly, the melting point of *erythro*-8,11-dihydroxyoctadecanoic acid is 99°C and that of *threo*-8,11-dihydroxyoctadecanoic acid is 84°C. For comparison, the *erythro* and *threo* forms of 9,10-dihydroxyoctadecanoic acid have melting points of 132 and 95°C, respectively (21). Similar cases of isomerism have been reported in the halogenation of fatty acid derivatives (22) and the epoxidation of methyl linoleate (23).

The lower melting points of the *threo* compounds compared to the *erythro* compounds confirm the absolute configuration of *R,R* assigned to the microbially produced 7,10-dihydroxy-8(*E*)-octadecenoic acid (15), which has a melting point of 64°C (5), close to that of *threo*-8,11-dihydroxy-9(*E*)-octadecenoic acid.

Reactions with selenium dioxide are known to give a variety of other products (14). Side products such as keto acids were also observed here and will be discussed in a later paper. In any case, allylic hydroxy fatty acids are the predominant reaction products.

The yields of the two dihydroxy isomers obtained from oleic acid are in the range of 10–20% combined in replicated reactions. The combined yields of the two monohydroxy compounds often exceed 40%. The products are obtained by a simple, single-step chemical synthesis.

In conclusion, this work shows the potential usefulness of the SEO_2/TBHP system not only in monohydroxylation, but also in allylic dihydroxylation of monounsaturated fatty materials. The allylic dihydroxy products are obtained as *erythro/threo* diastereomers. The unsaturated products can be hydrogenated to give the corresponding saturated derivatives. Work on applying this reaction to other starting materials is in progress and will be reported in due course.

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REFERENCES

1. Villet, R.H., and M.O. Bagby, paper presented at the 80th Annual Meeting of the American Oil Chemists' Society, Cincinnati, Ohio, 1989, Abstract published in *J. Am. Oil Chem. Soc. 66*:437 (1989).
2. Bagby, M.O., and K.D. Carlson, in *Fats for the Future*, edited by R.C. Cambie, Ellis Horwood Limited, Chichester, 1989, pp. 301-316.
3. Carlson, K.D., and R. Kleiman, *Lesquerella as a Source of Hydroxy Fatty Acids for Industrial Products*, Growing Industrial Material Series, U.S. Dept. of Agriculture, 1991.
4. Ratledge, C., in *Microbial Lipids*, Vol. 2, edited by C. Ratledge and S.G. Wilkinson, Academic Press, London and San Diego, 1989, pp. 646–648, see also references cited therein.
5. Hou, C.T., M.O. Bagby, R.D. Plattner and S. Koritala, *J. Am. Oil Chem. Soc. 68*:99 (1991).
6. Parra, J.L., J. Pastor, F. Comelles, M.A. Manresa and M.P. Bosch, *Tens. Surf., Deterg. 27*:302 (1990).
7. Lanser, A.C., R.D. Plattner and M.O. Bagby, *J. Am. Oil Chem. Soc. 69*:363 (1992).
8. Koritala, S., L. Hosie, C.T. Hou, C.W. Hesseltine and M.O. Bagby, *Appl. Microbiol. Biotechnol. 32*:299 (1989).
9. Sonntag, N.O.V., in *Bailey's Industrial Oil and Fat Products*, Vol. 1, 4th edn., edited by D. Swern, J. Wiley & Sons, New York, 1979, pp. 131–132.
10. Tubul-Peretz, A., E. Ucciani and M. Naudet, *Bull. Soc. Chim. France 1966*, 2331.
11. Watanabe, Y., Y. Ito and T. Matsuura, *J. Sci. Hiroshima Univ., Ser. A20*:203 (1957); *Chem. Abstr. 52*:6816i (1958).
12. Swern. D., in *Fatty Acids, Their Chemistry, Properties, Production, and Uses*, 2nd edn., Part 2, edited by K.S. Markely, Interscience Publishers, New York, 1961, pp. 1361–1362; See also references therein.
13. Umbreit, M.A., and K.B. Sharpless, *J. Am. Chem. Soc. 99*:5526 (1977).
14. Page, P.C.B., and T.J. McCarthy, in *Comprehensive Organic Synthesis, Vol. 7 (Oxidation)*, edited by B.M. Trost and I. Fleming, Pergamon Press, Oxford, 1991, pp. 83–91.
15. Knothe, G., M.O. Bagby, R.E. Peterson and C.T. Hou, *J. Am. Oil Chem. Soc. 69*:367 (1992).
16. Sharpless, K.B., and R.F. Lauer, *J. Am. Chem. Soc. 94*:7154 (1972).
17. Tulloch, A.P., *Org. Magn. Reson. 11*:109 (1978).
18. Sakurai, H., M. Mizu, M. Nishiki, N. Mitsuo and T. Satoh, *J. Mol. Catal. 48*:175 (1988).
19. Rakoff, H., D. Weisleder and E.A. Emken, *Lipids 14*:81 (1979).
20. Hoffmann, R.W., and U. Weidmann, *Chem. Ber. 118*:3980 (1985).
21. Markley, K.S., in *Fatty Acids, Their Chemistry, Properties, Production, and Uses*, 2nd edn., Part 5, edited by K.S. Markely, Interscience Publishers, John Wiley & Sons, New York, 1968, pp. 3296–3298.
22. Sonntag, N.O.V., in *Ibid.*, 1073–1090.
23. Maerker, G., E.T. Haeberer and S.F. Herb, *J. Am. Oil Chem. Soc. 53*:505 (1966).

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