Biotransformation of Saturated Monohydroxyl Fatty Acids to 2-Tetrahydrofuranyl Acetic Acid Derivatives: Mechanism of Formations and the Biological Activity of 5-*n*-Hexyl-Tetrahydrofuran-2-Acetic Acid[1]

J.-K. Huang[a], P.F. Dowd[c], K.C. Keudell[b], W.E. Klopfenstein[a], L. Wen[a], M.O. Bagby[d,*], A.C. Lanser[d], and R.A. Norton[b]

Departments of [a]Chemistry and [b]Biological Science, Western Illinois University, Macomb, Illinois 61455, and [c]Mycotoxin Research and [d]Oil Chemical Research, NCAUR, ARS, USDA, Peoria, Illinois 61604

ABSTRACT: Transformation of 12-hydroxyoctadecanoic acid (12-HOA) to 5-*n*-hexyl-tetrahydrofuran-2-acetic acid (5-HTFA) by *Bacillus lentus* NRRL B-14864 (B-14864) was carried out in the presence or absence of oligomycin, 2-bromooctanoic acid (2-BA), or sodium azide. In addition, several saturated and monounsaturated monohydroxyfatty acids, saturated monooxofatty acids, and monounsaturated fatty acid were used as substrates for transformation reactions by B-14864 or corynebacterium FUI-2. Methyl esters of the transformation products were analyzed by gas chromatography and gas chromatography/mass spectroscopy. Various γ-lactones and tetrahydrofuran fatty acid derivatives were conversion products when saturated monohydroxyfatty acids were used as substrates; the production of 5-HTFA from 12-HOA by B-14864 cells was completely inhibited in the presence of high concentration of oligomycin, 2-BA, or sodium azide; and fatty acid β-oxidation metabolic intermediates, 6-hydroxydodecanoic, 4-oxododecanoic, and 4-oxodecanoic acids were products when 12-HOA, 10-oxo-, and 12-oxooctadecanoic acids were used as substrates. Our results suggest that the production of 5-HTFA from 12-HOA by B-14864 was through the fatty acid β-oxidation pathway. Three-day-old driedfruit beetle pupae were topically treated with 5-HTFA to test for juvenile hormone activity, and 5-HTFA was found to possess juvenile hormone-like activity in pure form but not when it was diluted to 10%.

JAOCS 73, 1465–1469 (1996).

KEY WORDS: 12-hydroxyoctadecanoic acid, 5-*n*-hexyl-tetrahydrofuran-2-acetic acid, *Bacillus lentus.*

A microorganism capable of producing small amounts of 2-hydroxymethylfuran-5-carboxylic acid was first reported by Sumiki in 1929 (1). Since then, naturally occurring furan or tetrahydrofuran fatty acids have been reported in *Exocarpus cupressiformis* seed oil, *Hevea brasiliensis* Latex, grasses, algae, a variety of plant cultivars, human urine, different organs of marine and freshwater fish and in a variety of *Streptomyces* cultures. It has been claimed that chemically synthesized tetrahydrofuran fatty acid derivatives possess some of the biological activities of both prostaglandin E and insect juvenile hormone activity, as well as serving as a constituent of the antibiotic Nonactin (2).

We have previously reported microorganisms *Bacillus lentus* NRRL B-14864 (B-14864) and FUI-2 transformed 12-hydroxyoctadecanoic acid (12-HOA) to 5-*n*-hexyl-tetrahydrofuran-2-acetic acid (5-HTFA) in good yield, and proposed that the formation of 5-HTFA from 12-HOA was through the fatty acid β-oxidation pathway (2). In order to support that hypothesis, the biotransformation of 12-HOA to 5-HTFA by B-14864 has been studied in the presence of the enzyme inhibitors 2-bromooctanoic acid (2-BA), sodium azide, and oligomycin. In addition, the substrates for biotransformation studies have been extended to include 8-hydroxy-, 10-hydroxy-, 12-hydroxy-, and 13-hydroxyoctadecanoic acids, and 14-hydroxyeicosanoic acid to see if their metabolism follows the same pathway. The biological activity of 5-HTFA was also investigated.

EXPERIMENTAL PROCEDURES

Materials. γ-Decanolactone and γ-dodecanolactone were from Aldrich (Milwaukee, WI). (R)-12-hydroxyoctadecanoic acid (12-HOA), hexadecanoic acid (palmitic acid), oligomycin, sodium azide, (R)-12-hydroxy-9(*E*)-octadecenoic acid (ricinelaidic acid), (R)-12-hydroxy-9(*Z*)-octadecenoic acid (ricinoleic acid), and 9(*Z*)-octadecenoic acid (oleic acid) were from Sigma (St. Louis, MO). Methoprene was from Chem Service (West Chester, PA). 2-BA was from Fluka (Ronkonkoma, NY). (D)10-Hydroxyoctadecanoic acid (10-HOA), 10-oxooctadecanoic acid (10-OOA), and 12-oxooctadecanoic acid (12-OOA) were bioconversion products purified (>95%) in our laboratory. (D,L)8-hydroxyoctadecanoic acid (8-HOA) and (D,L)13-hydroxyoctadecanoic acid (13-HOA) were gifts from Dr. Gerhard Knothe (NCAUR, Peoria, IL)(3,4). (D)14-Hydroxyeicosanoic acid (14-HEA)

[1]Presented in part at the 37th West Central States Biochemistry Conference Annual Meeting, Columbia, Missouri, Oct. 1994.

*To whom correspondence should be addressed at NCAUR, USDA, ARS, 1815 N. University St., Peoria, IL 61604.

Copyright © 1996 by AOCS Press

[prepared according to the method of Smith *et al.* (5)] was a gift from Dr. Kenneth D. Carlson (NCAUR). Yeast extract and dehydrated skim milk were from Difco Laboratories (Detroit, MI).

Microorganisms. Isolation and identification of B-14864 and FUI-2 were as reported previously (2).

Medium preparation. The 1% YE medium contained 1% yeast extract, 0.2% KH_2PO_4, 0.4% Na_2HPO_4, 0.2% $(NH_4)_2SO_4$, 0.01% $CaCl_2 \cdot H_2O$, and 0.001% $FeSO_4 \cdot 7H_2O$, pH 7.2. Medium was sterilized by filtration.

Conversion reactions with B-14864 or FUI-2. Cells of either B-14864 or FUI-2 in 100 mL of 1% YE medium in 500 mL Erlenmeyer flasks were grown aerobically at 30°C on rotary shaker (250 rpm) until OD_{560} reached 2.0. Aliquots of 4 or 5 mL of the culture medium were each transferred to 50-mL sterile conical tubes and the conversion reactions were set up as follow: (i) 10-HOA, 10-OOA, 12-HOA, 12-OOA, or 14-HEA (in 100% ethanol) was added to each of the 5-mL cultures of either B-14864 or FUI-2. Except for 14-HEA, the final concentration of each substrates were 0.2% (vol/vol). Due to poor solubility of 14-HEA in 100% ethanol, the final concentration of 14-HEA was about 0.03%; and (ii) 8-HOA, 13-HOA (in 100% ethanol) was added to each of the 4-mL cultures of B-14864 to a final concentration of 0.1% (wt/vol). In all cases the final concentration of the ethanol was 2%. Incubation was continued for 36 h. Samples of 0.5 mL were taken at various times for gas chromatography (GC) and GC/mass spectrometry (GC/MS) analysis. Cultures containing 2% ethanol served as controls.

Inhibition studies. Cells of B-14864 were grown in 1% YE medium until OD_{560} reached 2.0. An aliquot of 5 mL of culture medium was transferred to 50-mL conical tubes for inhibition studies. Various amounts of oligomycin (final conc. 12.5, 50, 100, and 250 μg/mL culture), 2-BA [final conc. 0.02, 0.04, 0.2, and 0.4% (vol/vol)], or sodium azide [final conc. 0.02, 0.04, 0.1, and 0.2%, (wt/vol)], was added to each 5-mL culture medium. Each culture medium containing inhibitor was allowed to grow for 30 min prior to the addition of 12-HOA. After incubation for two days, samples (0.5 mL) were taken for GC and GC/MS analyses. Cultures without inhibitor containing either 12-HOA or no 12-HOA served as controls. The viability of the cells were determined by plating the diluted culture on nutrient agar plates. The plates were incubated at 37°C overnight and the number of viable cells were counted.

Mass spectra. GC/MS was performed as previously reported (2). Methyl esters of various conversion products were chromatographed with a Hewlett-Packard (Avondale, PA) model HP 5890A series II gas chromatograph equipped with a flame-ionization detector. Esters were separated on a SPB-1 column (15 m × 0.32 mm i.d. and 0.25 μm thickness; Supelco Co., Bellefonte, PA) either isothermally (220°C) or temperature-programmed (3 min at 100°C, 100 to 270°C at 10°C/min and 4 min at 270°C). Peak areas were determined with a Hewlett-Packard 3396A electronic integrator. Methyl palmitate served as internal standard for quantitative analyses. Electron impact mass spectra of methyl esters and trimethylsilyl (TMS) ether derivatives were obtained on a Hewlett-Packard 5890 gas chromatograph (equipped with DB-5MS capillary column, 15 m × 0.25 mm i.d.; J&W Scientific Co., Folsum, CA) coupled to a Hewlett-Packard 5970 mass-selective detector with programmed temperature as above. The mass spectrum of methyl 4-oxododecanoate had fragmentation ions at *m/z* 197(M–31, 9%), 141(12%), 130(82%), 115(46%), 98(130–32, 100%), and 88 (87 + H, 17%). The *m/z* 141, 115, and 88 resulted from α-cleavage of the sigma bond to the left and right of the oxo-group (6). The mass spectrum of methyl 4-oxodecanoate is identical to the literature value (7). The mass spectrum of methyl 6-trimethylsilyl-oxydodecanoate gave prominent fragment ions at *m/z* 217 (42%) and 187 (64%), resulting from α-cleavage of the sigma bond on the left and right of the TMS group, respectively (8).

Insects. Driedfruit beetles (*Carprophilus hemipterus*) were reared on a pinto bean-based diet as described previously (9). Three-day-old pupae were topically treated with 0.2 μL of liquids using a 10 μL Hamilton syringe (Hamilton Co., Reno, NV) fitted with a Hamilton repeater. When the test compound was examined, any dilution occurred in acetone, and acetone was used as control. At least 20 pupae were used for each treatment. Insects were examined after seven days for mortality and normality of emerged adults.

RESULTS AND DISCUSSION

Identification of conversion products. γ-Lactones and 2-tetrahydrofuranyl acetic acid conversion products (methyl esters) were identified by means of the GC retention time on a DB-5MS capillary column. The typical mass ions detected were m/z 85 (furanone ring) (10) or *m/z* 143, 116(143–32) and 111 (methyl 2-tetrahydrofuranylacetate) (2,11).

When 8-HOA was used as substrate for biotransformation by B-14864, there were three prominent conversion products (Fig. 1). One was γ-tetradecanolactone (retention time, 11.8 min); the remaining two were stereoisomers of 5-*n*-decyl-tetrahydrofuran-2-acetic acid (retention times, 12.9 and 13.0 min). When 10-HOA was used as substrate for biotransformation by B-14864 or FUI-2, there were two prominent conversion products. One was γ-dodecanolactone (retention time, 9.4 min) and the other was 5-*n*-octyl-tetrahydrofuran-2-acetic acid (retention time, 10.8 min). When 12-HOA was used as substrate for biotransformation by B-14864 or FUI-2, the prominent conversion products were γ-decanolactone (retention time, 7.0 min) and 5-HTFA (retention time, 8.7 min) (Fig. 1). Another product, 6-hydroxydodecanoic acid (6-HDA)(retention time, 10.3 min) was present in the conversion products of FUI-2 (1.5% yield), but not in the conversion products of B-14864. When 13-HOA was used as substrate for biotransformation by B-14864, γ-lactones and 2-tetrahydrofuranylacetic fatty acid derivatives were not detected. With 14-HEA as substrate for biotransformation by B-14864 or FUI-2, the major conversion products detected were γ-decanolactone (retention time, 6.9 min) and 5-HTFA (retention time, 8.6 min) (Fig. 1).

Substrate		Conversion Product			
Name	Structure	Name	Structure	Name	Structure
	$H_3C-(CH_2)_n-C(OH)(H)-(CH_2)_{n_1}-C(=O)OH$		$H_3C-(CH_2)_n-CH$ (O, C=O, CH_2-CH_2 ring)		$H_3C-(CH_2)_n-CH$ (O, CH_2-CH_2 ring) $CH-CH_2-C(=O)-OH$
8-Hydroxy-octadecanoic acid (8-HOA)	$n = 9$, $n_1 = 6$	γ-Tetradecano-lactone	$n = 9$	5-*n*-decyl tetrahydro-furan-2-acetic acid (5-DTFA)	$n = 9$
10-Hydroxy-octadecanoic acid (10-HOA)	$n = 7$, $n_1 = 8$	γ-Dodecano-lactone	$n = 7$	5-*n*-octyl tetrahydro-furan-2-acetic acid (5-OTFA)	$n = 7$
12-Hydroxy-octadecanoic acid (12-HOA)	$n = 5$, $n_1 = 10$	γ-Decano-lactone	$n = 5$	5-*n*-hexyl tetrahydro-furan-2-acetic acid (5-HTFA)	$n = 5$
13-Hydroxy-octadecanoic acid (13-HOA)	$n = 4$, $n_1 = 11$	None	None	None	None
14-Hydroxy-eicosanoic acid (14-HEA)	$n = 5$, $n_1 = 12$	γ-Decano-lactone	$n = 5$	5-*n*-hexyl tetrahydro-furan-2-acetic acid (5-HTFA)	$n = 5$

FIG. 1. Names and chemical structures of substrates and prominent conversion products.

When monounsaturated hydroxyfatty acids (ricinoleic and ricinelaidic acid) and oleic acid were used as substrate for biotransformation by B-14864 or FUI-2, we did not detect the presence of 2-tetrahydrofuranylacetic acid derivatives in the conversion products (Fig. 2). When 10-OOA was used as substrate for biotransformation by B-14864 or FUI-2, the prominent conversion product was 4-oxododecanoic acid (retention time, 9.6 min) (Fig. 3). When 12-OOA was used as substrate, the prominent conversion product was 4-oxodecanoic acid (retention time, 7.2 min).

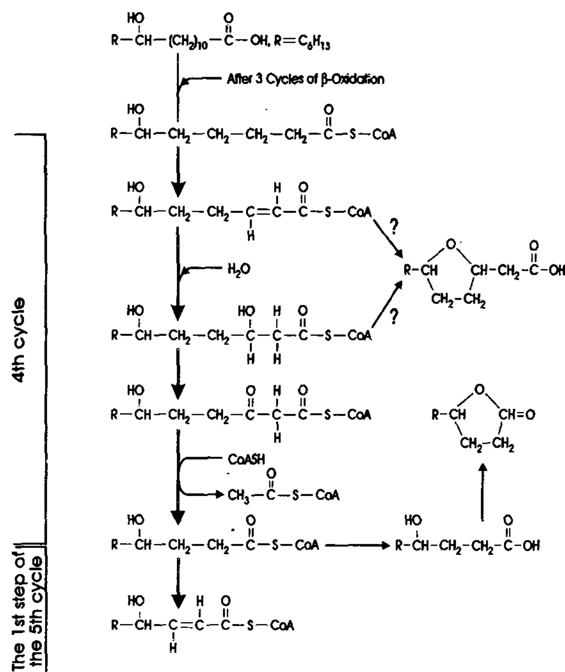
Mechanism for the formation of 5-HTFA from 12-HOA by B-14864. Several pieces of evidence support the formation of 5-HTFA from 12-HOA by B-14864 through the fatty acid β-oxidaton pathway. First, B-14864 transformed 8-HOA, 10-HOA, 12-HOA, and 14-HEA to γ-lactones and 2-tetrahydrofuranylacetic acid derivatives. As the hydroxyl group in the starting material becomes more distant from the carboxyl group, the products become shorter due to the removal of carbon from the carboxylic end of the starting materials. Removing two carbon units one at a time from carboxylic end of the molecules during fatty acid degradation *via* the β-oxidation pathway would shorten the carbon length of the substrate and provide metabolic intermediates which could form the conversion products (Scheme 1).

Substrate		Conversion Product	
Name	Structure	Structure	Structure
	$H_3C-(CH_2)_n-C(R)(H)-CH_2-CH=CH-(CH_2)_{n_1}-C(=O)OH$	$H_3C-(CH_2)_n-CH$ (O, C=O, CH_2-CH_2 ring)	$H_3C-(CH_2)_n-CH$ (O, CH_2-CH_2 ring) $CH-CH_2-C(=O)-OH$
12-Hydroxy-9(*Z*)-octa-decenoic acid	R = OH $n = 5$, $n_1 = 7$	None	None
12-Hydroxy-9(*E*)-octa-decenoic acid	R = OH $n = 5$, $n_1 = 7$	None	None
9(*Z*)-Octa-decenoic acid	R = H $n = 5$, $n_1 = 7$	None	None

FIG. 2. Names and chemical structures of substrates.

Substrate		Conversion Product	
Name	Structure	Name	Structure
	$H_3C-(CH_2)_n-C(=O)-(CH_2)_{n_1}-COOH$		$H_3C-(CH_2)_n-C(=O)-(CH_2)_2-COOH$
10-Oxo-octadecanoic acid (10-OOA)	$n = 7$, $n_1 = 8$	4-Oxo-dodecanoic acid	$n = 7$
12-Oxo-octadecanoic acid (12-OOA)	$n = 5$, $n_1 = 10$	4-Oxo-decanoic acid	$n = 5$

FIG. 3. Names and chemical structures of substrates and prominent conversion products.



SCHEME 1

Secondly, nonenzymatic formation of 5-methyltetrahydrofuran-2-acetic acid from 6-hydroxy-2(*E*)-heptenoic acid by acid catalysis (12), for tetrahydrofuran fatty acid derivatives under low pH (13) or by Pd(II) activation of hydroxyalkenes (14), and for 5-HTFA from R(−)-3-hydroxy-5(*Z*)-dodecenoic acid under extremely low pH (11) have been reported. These starting materials share the common feature of containing either a hydroxy group and a double bond separated by 1 or 2 methylene groups, or two hydroxy groups separated by 2 methylenes.

During fatty acid β-oxidation of saturated monohydroxyfatty acids, the metabolic intermediates have similar structures, as mentioned above. These metabolic intermediates may become the precursors for synthesis of tetrahydrofuran fatty acid derivatives or may be further oxidized by fatty acid β-oxidation.

These observations may also explain why 13-HOA gave no detectable tetrahydrofuran fatty acid derivatives in the conversion products. During the fourth cycle of fatty acid β-oxidation of 13-HOA, the metabolic intermediates should either contain a hydroxy group and a double bond or two hydroxy groups separated by three methylene units. The additional methylene group in this intermediate may not provide the necessary molecular structure for enzymatic reaction(s) to form 2-tetrahydrofuranyl acetic acid derivatives. Chemical synthesis of a tetrahydropyran derivative and not a tetrahydrofuran derivative from the hydroxyalkenes having a hydroxy group and a double bond separated by three methylenes has been reported (14). This result indicates that it is impossible to form a five- or six-membered cyclic ether ring simply by chemical means under our experimental conditions.

Third, the metabolic intermediates, 4-oxodecanoic acid and 4-oxododecanoic acid were detected in the conversion products. 4-Oxododecanoic acid was the metabolic intermediate during the fourth cycle of fatty acid β-oxidation of 10-OOA, and 4-oxodecanoic acid was the metabolic intermediate during the fifth cycle of fatty acid β-oxidation of 12-OOA. The length of the n-alkyl side chain is identical both in the substrate and conversion product. These results not only confirmed that fatty acid β-oxidation occurred but also indicated the importance of the hydroxyl group in order to form the tetrahydrofuran ring.

Fourth, the metabolic intermediate, 6-HDA, was detected from the conversion products of 12-HOA by FUI-2, but not by B-14864. We do not know why B-14864 could not transform 12-HOA to 6-HDA, and there is no explanation why no similar metabolic intermediate accumulated when 8-HOA, 10-HOA, and 14-HOA were substrates for FUI-2. Fifth, inhibitors which specifically inhibit the enzymes for fatty acid β-oxidation inhibited the formation of 5-HTFA by cells.

Effect of inhibitors. Transformation of 12-HOA to 5-HTFA by B-14864 was carried out in the presence or absence of oligomycin, 2-BA or sodium azide. Oligomycin is an ATPase inhibitor. The synthesis of ATP is coupled to the oxidative phosphorylation system. When the activity of ATPase is inhibited, so is the activity of oxidative phosphorylation and fatty acid β-oxidation. With low concentrations of oligomycin (12.5 and 50 μg/L), ATPase activity was not completely inhibited and some degree of fatty acid β-oxidation occurred which resulted in production of small amounts of 5-HTFA (8 and 3% yield, respectively). As the concentration increased

to 100 and 250 μg/mL, the ATPase activity was fully inactivated, resulting in no production of γ-decanolactone and 5-HTFA. However, there were 58% (2.9×10^8/mL culture) viable cells in culture medium. A sub-lethal dose of oligomycin inhibited the production of 4-hydroxydecanoic acid and 5-HTFA from ricinoleic acid by *Rhodotorula glutinis* and *Sporobolomyces odorus* (15). Oligomycin at concentrations from 50 to 100 μg/mL of culture has been widely used to inhibit microorganism growth. Sodium azide is an oxidase inhibitor in the oxidative phosphorylation system. As the activity of oxidase is inhibited, so is the activity of oxidative phosphorylation and fatty acid β-oxidation system. In the presence of low concentration of sodium azide (0.02%), cells produced a small amount of 5-HTFA (2% yield). However, as the concentration increased from 0.04 to 0.2%, there was no detectable 5-HTFA, even with 75% (4.8×10^8/mL) viable cell. Sodium azide at concentrations from 0.03 to 0.05% (wt/vol) has been widely used to inhibit microorganism growth.

2-BA is a specific inhibitor of the 3-ketoacyl-CoA thiolase of fatty acid β-oxidation enzyme complex. When thiolase activity is inhibited, so is fatty acid β-oxidation. In the presence of low concentration of 2-BA (0.02 to 0.4%), cells did not produce 5-HTFA, even though there were 70% (4.2×10^8/mL culture) viable cells after incubation for two days. Ten μM of 2-BA completely inactivates thiolase activity of rat liver mitochondria (16).

These results indicated that 2-tetrahydrofuranylacetic acid derivatives were produced through fatty acid β-oxidation pathway. The results also suggest that enzymes are probably involved in the cyclization of fatty acid β-oxidation intermediates to 2-tetrahydrofuranylacetic acid derivatives. This concept was further supported by the results obtained with ricinoleic, ricinelaidic, and oleic acids as substrates. In theory, tetrahydrofuran derivatives should be formed if the cyclization reaction does not require high temperature and low pH. However, we did not detect the presence of 2-tetrahydrofuranylacetic acid derivatives in their conversion products.

Biological activity test. Initial tests with 1 mg/mL acetone solutions of methoprene, a juvenile hormone (JH) analog, produced 15% pupal–adult intermediates after seven days under the conditions normally used to rear the insects, as indicated by tanning (browning) of wing covers and abdomens without molting into adults. Thus, this insect is suitable for demonstrating JH activity of our test compound. At 1 and 10% concentration, 5-HTFA showed no effect on the pupae that was different from that on the insects treated with solvent alone. However, when used undiluted (0.2 μL per pupae), only 20% of the treated pupae emerged as normal adults, as compared to 100% of control insects. The remainder of the insects showed various forms of developmental abnormalities, including tanning or molting into "adults" that still retained pupal characteristics (such as undeveloped wings). These effects suggest that 5-HTFA has JH-like activity.

ACKNOWLEDGMENTS

This research was supported in part by the Department of Chemistry, Western Illinois University (WIU), and the Specific Cooperative Agreement 58-5114-0-1024 between WIU and Agricultural Research Service, U.S. Department of Agriculture. We thank Drs. Knothe and Carlson for supplying 8-hydroxyoctadecanoic acid, 13-hydroxyoctadecanoic acid, and 14-hydroxyeicosanoic acid.

REFERENCES

1. Miller, M.W., in *The Pfizer Handbook of Microbial Metabolites*, edited by M.W. Miller, McGraw-Hill Book Company, Inc., 1967, pp. 501–507.
2. Huang, J.-K., K.C. Keudell, J. Zhao, W.E. Klopfenstein, L. Wen, M.O. Bagby, A.C. Lanser, R.D. Plattner, R.E. Peterson, T.P. Abbott, and D. Weisleder, Microbial Transformation of 12-Hydroxyoctadecanoic Acid to 5-*n*-Hexyl-Tetrahydrofuran-2-Acetic Acid, *J. Am. Oil Chem. Soc. 72*:323–326 (1995).
3. Knothe, G., R.O. Dunn, and M.O. Bagby, Surface Tension Studies on Novel Allylic Mono- and Dihydroxy Fatty Compounds. A Method to Distinguish Erythro/Threo Diastereomers, *Ibid. 72*:43–47 (1995).
4. Knothe, G., M.O. Bagby, D. Weisleder, and R.E. Peterson, Allylic Mono- and Di-Hydroxylation of Isolated Double Bonds with Selenium Dioxide-*tert*-Butyl Hydroperoxide. NMR Characterization of Long-Chain Enols, Allylic and Saturated 1,4-Diols, and Enones, *J. Chem. Soc. Perkin Trans. 2*:1661–1669 (1994).
5. Smith, C.R., Jr., T.L. Wilson, T.K. Miwa, H. Zobel, R.L. Lohmar, and I.A. Wolff, Lesquerolic Acid. A New Hydroxy Acid from Lesquerella Seed Oil, *J. Org. Chem. 26*:2903–2905 (1961).
6. Ryhage, R., and E. Stenhagen, Mass Spectrometric Studies. VI. Methyl Esters of Normal Chain Oxo, Hydroxy, Methoxy, and Epoxyacids, *Arkiv. Kemi. 15*:545–574 (1960).
7. Pfordt, V.J., and G. Spiteller, ω-Formyl- und 4-Oxocarbonsauren im Blutplasma, *Angew. Chem. 91*:328–329 (1979).
8. Kleiman, R., and G.F. Spencer, Gas Chromatography–Mass Spectrometry of Methyl Esters of Unsaturated Oxygenated Fatty Acids, *J. Am. Oil Chem. Soc. 50*:31–38 (1973).
9. Dowd, P.F., A Labor-Saving Method for Rearing the Driedfruit Beetle (*Coleoptera:Nitidulidae*) on Pinto Bean-Based Diet, *J. Econ. Entomol. 80*:1351–1353 (1987).
10. Vorderwulbecke, T., M. Epple, H.K. Cammenga, S. Peterson, and K. Kieslich, Racemic Lactones from Butterfat: an Advanced Approach That Includes Stereo-Differentiation, *J. Am. Oil Chem. Soc. 69*:797–801 (1992).
11. Weisshaar, R., and F. Lingens, The Lipopolysaccharide of a Chloridazon-Degrading Bacterium, *Eur. J. Biochem. 137*:155–161 (1983).
12. Whittaker, M., C.R. McArthur, and C.C. Leznoff, Asymmetric Synthesis Towards (3*Z*,6*R*)-3-Methyl-6-Isopropenyl-3,9-Decadien-1-yl Acetate, a Component of the California Red Scale Pheromone, *Can. J. Chem. 63*:2844–2852 (1985).
13. Gunstone, F.D., Some New Chemical Properties of Unsaturated C_{18} Acids, *Acc. Chem. Res. 9*:34–40 (1976).
14. Semmelhack, M.F., and C. Bodurow, Intramolecular Alkoxypalladation/Carbonylation of Alkenes, *J. Am. Chem. Soc. 106*:1496–1498 (1984).
15. Maume, K.A., and P.S.J. Cheetham, The Production of γ-Decanolactone by Fermentation of Castor Oil, *Biocatalysis 5*:79–97 (1991).
16. Raaka, B.M., and J.M. Lowenstein, Inhibition of Fatty Acid Oxidation by 2-Bromooctanoate. Evidence of the Enzymic Formation of 2-Bromo-3-Ketooctanoyl Coenzyme A and the Inhibition of 3-Ketothiolase, *J. Biol. Chem. 254*:6755–6762 (1979).

[Received November 27, 1995; accepted May 25, 1996]