7,10-Dihydroxy-8(E)-Octadecenoic Acid: Stereochemistry and a Novel Derivative, 7,10-Dihydroxyoctadecanoic Acid[1]

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7,10-Dihydroxy-8(E)-octadecenoic acid (obtained by bioconversion of oleic acid with the bacterial strain PR3) was hydrogenated with hydrazine hydrate under air in ethanolic solution to give a novel compound, 7,10-dihydroxyoctadecanoic acid. The absolute configuration of both compounds was determined with the aid of circular dichroism to be R,R. A different purification method for 7,10-dihydroxy-8(E)-octadecenoic acid is described.

KEY WORDS: Absolute configuration, circular dichroism, 7,10-dihydroxy-8(E)-octadecenoic acid, 7,10-dihydroxyoctadecanoic acid, diimide, hydrazine, hydrogenation, specific optical rotation.

Bioconversion reactions of fatty acids are of considerable interest in the production of novel fatty acids. The new materials could find applications such as lubricants or additives in cosmetics (1,2). Recently, a novel compound, 7,10-dihydroxy-8(E)-octadecenoic acid (DHOE), arising from the bioconversion of oleic acid with the bacterial strain PR3, has been reported (3,4). Other authors obtained DHOE from olive oil by conversion with *Pseudomonas* strain 42A2 (5). Similar hydroxylations have been reported (6). The production of DHOE, however, is remarkable because it involves hydroxylation at two carbon atoms as well as migration of the double bond.

In this paper, we report the hydrogenation of the double bond of DHOE, which yields a novel compound, 7,10-dihydroxyoctadecanoic acid (DHOA). The absolute configuration of both DHOE and DHOA was determined with the aid of circular dichroism.

EXPERIMENTAL PROCEDURES

Materials and methods. DHOE was obtained by bioconversion of oleic acid with strain PR3 (3,4). The reaction was conducted with 10 mL of oleic acid and a culture size of 1 L. All other components were used in corresponding amounts calculated from prior literature (3).

Melting points were determined with a Fisher-Johns apparatus (Fisher Scientific, Pittsburgh, PA) and are uncorrected. Optical rotation measurements were performed with a Perkin-Elmer 241 polarimeter (Perkin-Elmer, Norwalk, CT) and circular dichroism investigations with a Jasco J600. Fourier transform infrared (FTIR) spectra (KBr) were recorded either on a Perkin-Elmer 1750 spectrometer coupled to a Perkin-Elmer 7300 computer or on a Mattson Polaris spectrometer (Madison, WI). Proton and ^{13}C nuclear magnetic resonance (NMR) spectra were determined in CD_3OD with a Bruker WM-300 spectrometer (Burlington, Ontario, Canada) operating at frequencies of 300 MHz and 75.5 MHz, respectively. For GC-MS, a Hewlett-Packard (Palo Alto, CA) 5890 GC (gas chromatograph) coupled to a Hewlett-Packard 5970 mass-selective detector were used. A J&W Scientific (Folsom, CA) DB-1 column heated to 270°C (initial temperature 120°C, initial hold time 2 min, then 20°C/min to 200°C, then 10°C/min to 270°C) was employed. Retention time of trisilylated DHOA is approximately 11 min. Silylation was carried out at room temperature by adding a Supelco (Bellefonte, PA) Sylon BTZ mixture containing trimethylchlorosilane, N,O-bis(trimethylsilyl)acetamide and n-trimethylsilylimidazole to the solid DHOA. Both hydroxy groups and the acid group are silylated by this procedure. Chemical ionization (positive ionization; isobutane) mass spectra were obtained on a Finnigan TSQ mass spectrometer (Finnigan Corp., Sunnyvale, CA).

The semi-preparative high-performance liquid chromatography (HPLC) system was comprised of a DuPont Instruments (Wilmington, DE) chromatographic pump, Rheodyne Inc. (Cotati, CA) Model 7125 injector fitted with a 5-mL injection loop, Rainin Instrument Co., Inc. (Woburn, MA) Dynamax-60A silica column (25 cm × 21.4 mm I.D.), Waters Chromatography (Millipore Corp., Milford, MA) Model 403 refractive index detector, Isco, Inc. (Lincoln, NE) V^4 variable wavelength detector and a Houston Instruments (Houston, TX) Omniscribe recorder.

Preparative separations were performed on PrepPak-500/Silica columns (30 cm × 57 cm I.D.) with a Waters Delta Prep 3000 chromatograph and detectors as described above. Fractions were collected manually into 500-mL flasks.

Chromatographic conditions. All HPLC runs were conducted isocratically. Preparative (1–5 g) quantities of extracts were dissolved in elution solvent, injected and pumped through the solvent delivery system. Flow-rates were 8 and 50 mL/min for the 21.4 and 57 mm I.D. columns, respectively. Effluents were monitored serially, first at 254 nm (0.2 to 2.0 AUFS) and then with the refractive index detector.

Purification of DHOE. The crude fermentation reaction mixture was subjected to a different purification procedure from that reported (3). The fermentation mixture was acidified to pH 2 with conc. HCl and then extracted three times with 300 mL ether. The major portion of unreacted oleic acid was removed from the combined extracts by partitioning between an acetonitrile:hexane:water:methylene chloride (50:50:20:10) system. The top, less polar fraction contained the oleic acid. DHOE was in the lower, more polar fraction. Thin-layer chromatography (TLC) [methylene chloride:methanol (90:10), spots visualized with iodine vapor and then by spraying with 50% H_2SO_4 and baking at 120°C for 5 min] showed that a significant excess of oleic acid remained in these fractions, although less than prior to solvent system treatment. Column chromatography (silica gel 100/200 mesh, Alltech Associates, Deerfield, IL) with a methylene chloride:methanol (92:8) system removes more oleic acid. TLC indicated that DHOE was in excess of oleic acid. The still

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yellowish oily material was further purified, depending on the quantity, with the aid of either preparative or semi-preparative HPLC with the instrumentation described above and with a solvent system varying between 92:8 and 94:6 methylene chloride:methanol. HPLC runs were repeated as necessary until desired purity (>99%) was obtained.

Hydrogenation of DHOE. DHOE (129 mg, 0.41 mmol) was placed in a three-necked flask and dissolved in 13 mL ethanol, and 2.5 mL hydrazine hydrate (64% solution in water; obtained from Eastman Kodak Co., Rochester, NY) was added. A gentle stream of air was bubbled through the solution, which was heated at 60°C for 24 hr. Then, the reaction was worked up by adding water and acidifying to approximately pH 2 with conc. HCl. Extraction with ether and subsequent evaporation of the solvent afforded 102 mg (78%) of white solid with a melting point of 94-95°C. Gas chromatographic analysis of the silylated material confirmed that the product was DHOA with a purity >99%. The DHOA was analyzed without further purification.

RESULTS AND DISCUSSION

The DHOE obtained by bioconversion of oleic acid was subjected to a modified purification procedure by using ether extraction and chromatographic procedures (details are given in the Experimental Section). Generally, yields of DHOE from the bioconversion reactions were inconsistent and lower than that reported previously (3). Work is currently in progress to improve the consistency and yields of the bioconversion reactions.

DHOE was converted into its saturated derivative, DHOA, by hydrogenation with hydrazine hydrate in ethanolic solution in the presence of air (Fig. 1). Diimide is the active hydrogenating species in this reaction and results in syn addition to the double bond (7).

DHOA was characterized by the usual spectroscopic techniques. Positive ion (PI) chemical ionization (CI) mass spectrometry was carried out on DHOA, and its tris(trimethylsilyl) derivative was subjected to GC-MS with electron ionization (EI). In the CI mass spectrum (Fig. 2a), DHOA exhibits M^+ at *m/z* 317 (56%), corresponding to protonation of the unfragmented material. Protonation to give $(M + 1)^+$ and liberation of H_2O is common in the PI CI mass spectra of compounds like alcohols (8).

In the investigation of unsaturated fatty acids, CI MS has mostly been used to determine the position of unsaturation. DHOA exhibits three other strong peaks in CI MS at large *m/z*, namely 299 (100%), 281 (33%) and 263 (23%). These peaks correspond to successive losses of H_2O. Similar ionic series have been reported for polyhydroxy fatty acids, thus allowing the determination of the number of OH groups (9). Numerous other fragments in the PI CI mass spectrum of DHOA can be explained by cleavage at the carbon atoms carrying the hydroxyl groups and loss of water. The peak at *m/z* 185 corresponds to cleavage of the C10-C11 bond, and loss of H_2O gives rise to the peak at *m/z* 167. Cleavage of the C7-C8 bond yields *m/z* 145 and *m/z* 127 (145 − H_2O). Splitting of the C6-C7 bond results in two low-intensity ions, *m/z* 201 and *m/z* 115; however, loss of H_2O from these ions gives fragments of higher intensity at *m/z* 183 and *m/z* 97.

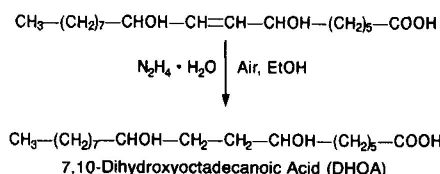

FIG. 1. Hydrogenation of 7,10-dihydroxy-8(E)-octadecenoic acid (DHOE).

The EI mass spectrum of the tris(trimethylsilyl) derivative (Fig. 2b) displays base peaks at either *m/z* 73 or *m/z* 329 when replicating GC/MS runs. The highest *m/z* are found at 517 and 518 and correspond to $(M–CH_3)^+$ and $(M+1–CH_3)^+$. Characteristic peaks for this derivative are the ions at *m/z* 329 (85.5% in Fig. 1a), 289 (34.0%), 255 (40.0%) and 215 (24.9%). The ions at *m/z* 329 and 255, resulting from cleavage of the C10-C11 and C6-C7 bonds, correspond to those at *m/z* 419 (2.9%) and 345 (6.7%) with loss of TMSOH (*m/z* 90). The peaks at *m/z* 289 and 215 arise from cleavage of the C7-C8 and C9-C10 bonds, respectively. Comparison of these mass spectrum data to those of the tris(trimethylsilyl) derivative of DHOE showed, as expected, that the peaks at *m/z* 517, 329 and 255 occurred two mass units higher than in DHOE (*m/z* 515, 327 and 253). The EI mass spectrum of the trisilylated material coincides with the CI MS, however, giving more intense fragments from chain cleavage.

Proton NMR of DHOA in CD_3OD exhibited the following peaks: 4.89 (s, br, OH), 3.52 (s, br, -CH-O), 2.28 (t, CH_2 at C2), 1.61-1.30 (m, CH_2), 0.90 (CH_3). ^{13}C-NMR showed the following peaks: 177.6 (COOH), 72.4 (C-OH at C7), 72.3 (C-OH at C10), 38.5, 38.3, 34.9, 34.4, 33.1, 30.9, 30.8, 30.4, 30.3, 26.8, 26.5, 26.1, 23.7 (various CH_2), 14.5 (CH_3). A 2D-NMR spectrum showing the ^{1}H-NMR in the range 3.60–0.80 ppm and the ^{13}C-NMR in the range 75–14 ppm is depicted in Figure 3.

Some characteristic IR absorptions of DHOA were detected at 3214 [ν (OH), br], 2925, 2852 [ν (CH)], 1713 [ν (C=O)], 1466, 1439, 1300, 1069, 1026, 907 cm^{-1}.

The specific optical rotation values of DHOE and DHOA were determined as follows: DHOE $[\alpha]_D^{26} = +14°$ (methanol), $[\alpha]_D^{28} = +8°$ (chloroform); DHOA $[\alpha]_D^{28} = -0.15°$ (methanol).

To complete the characterization of both DHOE and DHOA, we were interested in establishing their absolute configuration. Since the hydrogenation reaction did not involve any changes of the chiral centers at C7 and C10, DHOE and DHOA should possess identical absolute configurations.

Circular dichroism (CD) was employed to determine the absolute configuration of DHOE and DHOA. Corresponding to its positive optical rotation, DHOE shows one positive Cotton effect at 187.8 nm ($\theta = 27500$, MeOH, 1.52 mg/mL) resp. 186.6 nm ($\theta = 22700$, CF_3CH_2OH, 11.27 mg/mL). Also corresponding to optical rotation, DHOA has a negative maximum at 240.2 nm and a positive maximum at 224.7 nm ($\theta = -26$ resp. 62, tert.-BuOH, 13.05 mg/mL). These findings imply that both DHOE and DHOA have R,R-configuration. The results coincide with those reported in previous literature where various saturated and unsaturated hydroxy fatty acids had been found to possess D configuration. For example, dimorphecolic,

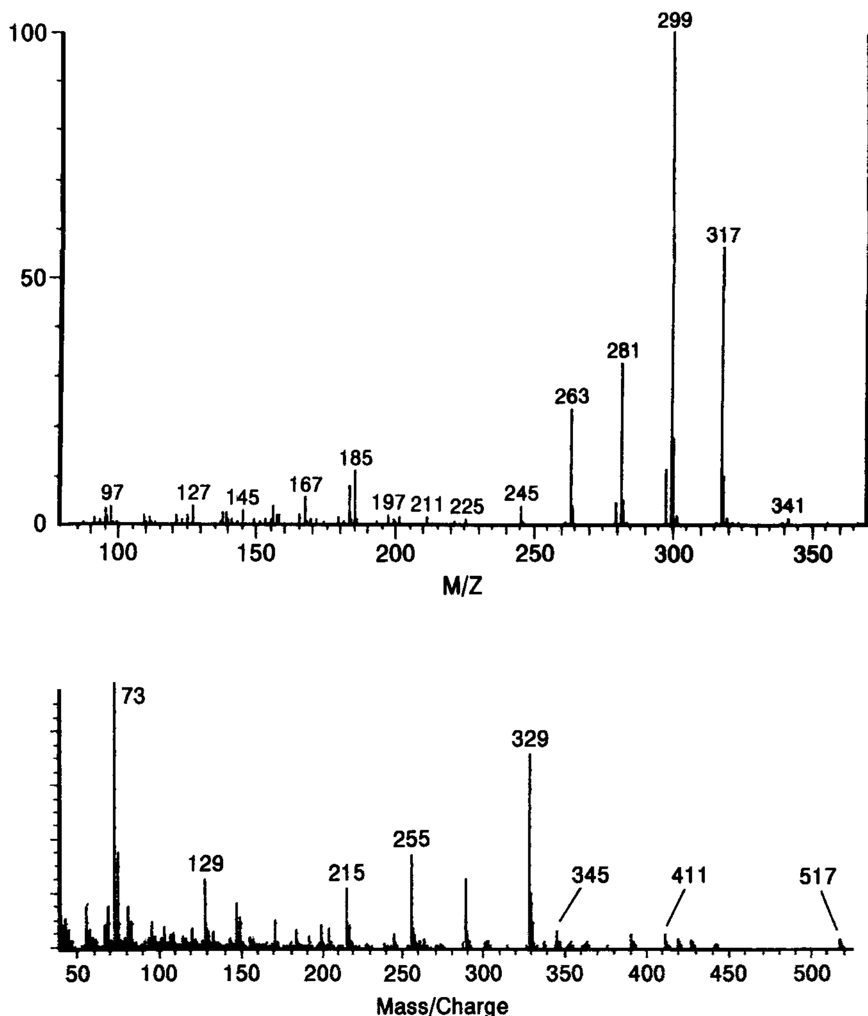

FIG. 2. A, PI CI mass spectrum of DHOA; and B, EI MS of trisilylated DHOA as it was obtained from a GC/MS analysis.

lesquerolic and other acids, from optical rotatory dispersion (ORD) studies in the region 250–600 nm, showed plain negative ORD curves of the corresponding saturated methyl esters. The unsaturated derivatives usually exhibited negative plain curves up to 250 nm in ORD, thus showing the D configuration (10). The CD spectra of DHOE and DHOA show similar behavior, as shown in Figures 4 and 5.

R configuration (in older literature this usually conforms to D configuration but not always) is apparently the preferred configuration in saturated and unsaturated hydroxy fatty acids (11–16). Four unsaturated hydroxy fatty acids, among them 10-hydroxy-8-octadecenoic acid (HOE), also exhibited R configuration, which was determined by CD of benzoate derivatives (17). The assignment of R,R is also supported by Brewster's rule (18) and other stereochemical analyses (19,20), such as the olefin octant rule.

10-Hydroxy-8-octadecenoic acid, which is also formed in the same bioconversion reaction as DHOE (21), has been found in naturally occurring systems (17,22,23). Both racemic DHOE and HOE have been proposed as products occurring in isomer mixtures formed by hydroxylation of oleic acid *via* bromination (24). Oxidative acetoxylation of methyl oleate yielded the diacetoxylated methyl ester of racemic DHOE and the acetoxylated methyl ester of racemic HOE in an isomer mixture (25). The acetoxylated compounds were also hydrogenated, thus giving the diacetoxylated methyl ester of racemic DHOA in an isomer mixture (25). Other syntheses of HOE have been reported (26–29). If HOE is a precursor of DHOE, as suggested by Hou (21), and if it has the same configuration as the previously reported HOE (17), this would be further confirmation of R configuration at C10 of DHOE. X-ray crystallography is being investigated to confirm the absolute configuration. These results will be published separately.

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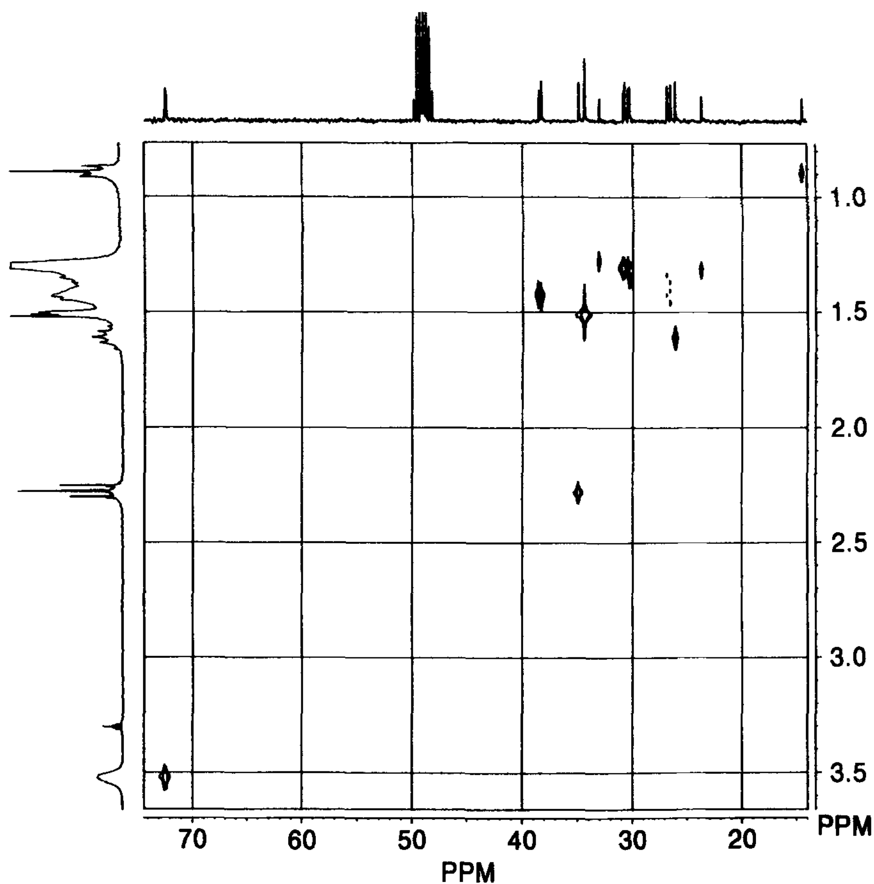

FIG. 3. 2D-NMR of DHOA (solvent: CD_3OD). The ^1H-NMR is shown on the left and the ^{13}C-NMR across the top of the 2D-NMR.

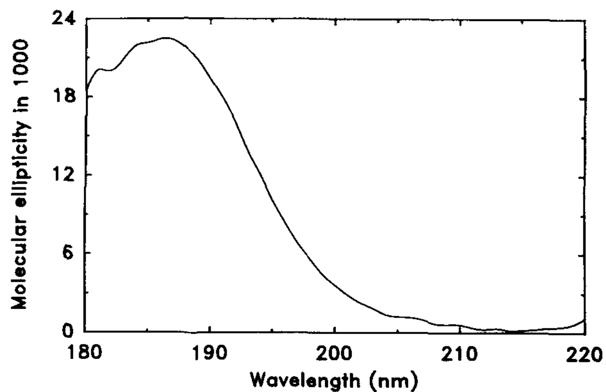

FIG. 4. Circular dichroism of DHOE (solvent, CF_3CH_2OH; conc., 11.27 mg/mL; cell, 0.01121 mm).

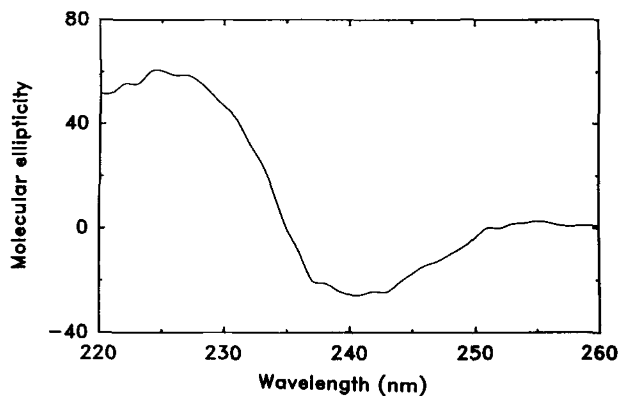

FIG. 5. Circular dichroism of DHOA (solvent, tert.-BuOH; conc., 13.05 mg/mL; cell, 0.106 cm).

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