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One-Step Production of a Biologically Active Novel Furan Fatty Acid from 7,10-Dihydroxy-8(*E*)-octadecenoic Acid

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ABSTRACT: Furan fatty acids (F-acids) gain special attention because they are known to play important roles in biological systems including humans. Specifically, F-acids are known to have strong antioxidant activitis such as radical scavenging activity. Although widely distributed in most biological systems, F-acids are trace components and their biosynthesis is complicated and quite different by sources. On the basis of biochemical study, they are considered to be an essential nutritional factor for mammals and should be provided through the diet. Hence, several studies reported the chemical synthesis of F-acids using chemical catalysts. However, chemical synthesis required complicated multiple steps. In this study was developed a simple one-step synthesis of a novel F-acid, 7,10-epoxyoctadeca-7,9-dienoic acid (EODA), from a dihydroxyl fatty acid, 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD), by heat treatment. The structure of EODA was confirmed by GC-MS, NMR, and FTIR analyses, and maximum production yield under the reaction conditions of 90 °C and 24 h reached 80%.

KEYWORDS: 7,10-epoxyoctadeca-7,9-dienoic acid, heat treatment, 7,10-dihydroxy-8(*E*)-octadecenoic acid, furan fatty acid

INTRODUCTION

Furan fatty acids (F-acids) are a large group of fatty acids characterized by a furan ring, which carries at one α-position an unbranched fatty acid chain with 9, 11, or 13 carbon atoms and at the other α-position a short straight-chain alkyl group with 3 or 5 carbon atoms.[1] Mostly two β-positions of the furan ring are substituted by either one or two methyl residues or other groups. F-Acid without any substitution on either β-position of the furan ring was also found in the seed oil of *Exocarpus cupressiformis*.[2] F-Acids are widely distributed in nature as trace components of plants, fishes, amphibians, reptiles, microorganisms, and mammals including humans.[1,3−7]

Although the biological role of F-acids in the biological system is not fully understood, it has been pointed out that F-acids can be involved in various important biological functions, acting as antioxidants, antitumorals, and antithrombotics.[8−10] In some fishes F-acids comprise up to 25% of the acids in the liver lipids and accumulated during the spawning season, indicating possible correlation between F-acids and the fertilization process.[11] The correlation between the consumption of fish rich in F-acid and protection against coronary heart disease mortlaity has been confirmed in several studies.[12] F-Acids have also been reported to have inhibitory effects on blood platelet aggregation[9] and to have potential antitumor activity.[8] F-Acids were found to prevent the oxidation of linoleic acid[13] and act as antioxidants in plants.[14] Some studies demonstrated that F-acids underwent oxidation by ring-opening to form dioxoenes[15,16] in the presence of linoleic acid as cosubstrate, demonstrating that the F-acid acted as a radical scavenger.[17,18]

The biosynthesis of F-acids is complicated and quite different depending on the source. The biogenetic precursor of most F-acids is known to be linoleic acid.[19] It was recognized that plants synthesized the basic skeleton of F-acids from different sources.[20] However, study with the radiolabeled feeding to fish indicated that fish synthesized neither the alkyl side chain nor the furan ring of F-acids.[1] Therefore, F-acids in fish were considered to originate from the diet, especially algae. Consequently, F-acids are introduced into the human body through the diet like vegetables and fishes. Diet-derived F-acids are incorporated into the tissue and blood of mammals, especially into phospholipids,[21] where they might act as radical scavengers resulting in inhibition of blood paltelet aggregation.[9]

These reports indicated that F-acid could be an essential nutritional factor for mammals and could be used as an active component of functional food. However, no matter what the biological sources of F-acids were, the biosynthesis of F-acids required multistep reactions due to the formation of the furan ring and the different alkyl substituents. Accordingly, chemical synthesis of F-acids required complicated multistep reactions and chemical catalysts, causing difficulties and high costs for industrial application.[22]

Recently we have produced 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD) from vegetable oil containing oleic acid by microbial conversion.[23] DOD is a dihydroxy monoenoic C_{18} fatty acid uniquely carrying two hydroxyl groups at carbons 7 and 10 and a *trans* double bond between carbons 8 and 9. On the basis of its unique structural feature, it is possible to modify DOD molecules by intra- or intermolecular interaction via chemical or physical ways. In our constant efforts to modify DOD for biological and industrial applications, we developed a simple way to produce a novel biologically active F-acid from DOD through one-step heat treatment.

MATERIALS AND METHODS

Materials. Olive oil (extra virgin grade) was purchased from a local market in Korea. Heptadecanoic acid (C17:0) was purchased from

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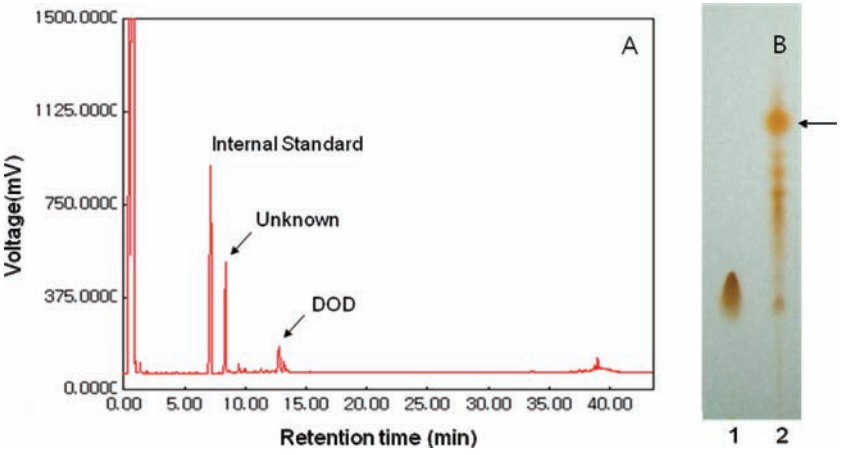

Figure 1. Analysis of the crude extract obtained from conversion of DOD by heat treatment. Analysis was carried out by GC (A) and TLC (B). The major unknown compound in GC analysis is indicated by the arrow in TLC analysis. Lanes: 1, standard DOD; 2, crude extract of the heat-treated DOD. Other experimental conditions were as given under Materials and Methods.

Nu-Chek Prep (Elysian, MN). A mixture of trimethylsilylimidazole (TMSI) and pyridine (1:4, v/v) was purchased from Supelco (Bellefonte, PA). Thin-layer precoated Kieselgel 60F_{254} plates were obtained from EM Science (Cherry Hill, NJ). Silica gel, Davisil, grade 635, 60−100 mesh, 60A, 99^{+}%, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless mentioned elsewhere. All other chemicals were of reagent grade and were used without further purification.

Production of DOD. DOD was produced according to our previous paper.[23] In brief, olive oil (1%, v/v) was added as a substrate to a 24-h-old culture of *Pseudomonas aeruginosa* PR3, which was cultivated aerobically at 28 °C and 200 rpm in a shaking incubator followed by an additional 72 h of incubation. Crude DOD extract obtained by extraction of the culture with an equal volume of ethyl acetate was applied to the silica gel column (1.5 cm i.d. × 30 cm) for purification. Fractionation was conducted with 2 column volumes of the solvent mixture with varied ratios of ethyl acetate over hexane.

Conversion of DOD by Heat Treatment. Conversion of DOD by heat treatment was carried out in 4 mL glass vials containing 10 mg of DOD and 500 μL of hexane as solvent. The mixture was incubated at 90 °C for 24 h on a heating block (Barnstead/Thermolyne Type 176000 Dri-Bath). At the end of the treatment, solvent was evaporated using nitrogen flushing, and the reaction product was dissolved in the mixture of chloroform and methanol (1:1, v/v). For the study of time course production, vials containing 10 mg of DOD were heated at 90 °C and withdrawn for analysis after a given time.

Analytical Methods. Reaction products were analyzed by TLC and quantified by GC analysis with heptadecanoic acid being an internal standard. The TLC analysis was developed in a solvent system (toluene/1,4-dioxane/acetic acid, 79:14:7, v/v/v), and the spots were visualized by spraying the plate with 50% sulfuric acid followed by heating at 95 °C for 10 min. For GC analysis, the sample methylated with diazomethane for 5 min at room temperature was analyzed with an ACME 6100 series gas chromatography system (Younlin Co., Korea) equipped with a flame ionization detector and a capillary column (SPB-1, 15 m × 0.32 mm i.d., 0.25 μm thickness, Supelco Inc., Bellefonte, PA). GC was run with temperature gradients of 20 °C/min from 100 to 150 °C, 5 °C/min from 150 to 200 °C, and then 0.5 °C/min from 200 to 210 °C followed by a 10 min hold at 300 °C (nitrogen gas flow rate = 0.67 mL/min). Injector and detector temperatures were held at 270 and 280 °C, respectively.

The chemical structure of the purified target product was determined by GC-MS, NMR, and FTIR. Electron impact (EI) mass spectra were obtained with a Hewlett-Packard 5890 GC (Avondale, PA) coupled to a Hewlett-Packard 5972 series mass selective detector. The column outlet was connected directly to the ion source. Separation was carried out in a methylsilicone column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with a temperature gradient of 20 °C/min from 70 to 170 °C, a 1 min hold at 170, and a gradient of 5 °C/min to 250 °C followed by a 15 min hold (helium flow rate = 0.67 mL/min). ^{1}H NMR and ^{13}C NMR spectra were determined in deuterated chloroform with a Varian 400 spectrometer (Billerica, MA), operated at a frequency of 400 and 100 MHz, respectively. FTIR analysis of the purified compound was run as films on KBr on a Perkin-Elmer infrared Fourier transform model 1750 spectrometer (Perkin-Elmer, Oakbrook, IL).

Antioxidant activity was analyzed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to previous studies.[24] Briefly, 50 μL of sample solution in DMSO was added to 200 μL of 200 μM DPPH radical solution in a 96-well plate. L-Ascorbic acid and α-tocopherol were used as positive controls. After 30 min of incubation at 37 °C, the absorbance at 515 nm was measured. DPPH free radical scavenging activities were calculated using the following equation: radical scavenging activity (%) = $[1 - (A_{sample} - A_{blank})/(A_{control} - A_{blank})] \times 100$. DMSO was used as a control.

RESULTS AND DISCUSSION

Production and Isolation of a Major Product. Heat treatment of DOD in hexane at 90 °C for 24 h yielded a mixture of several products including one major product analyzed by TLC (R_f = 7.2, Figure 1B) and GC (peak retention time = 8.2−8.4 min, Figure 1A). This major product was purified using a silica gel column. The target compound was obtained from the fraction of hexane/ethyl acetate (8:2, v/v). The purified product with white crystal-like powder was identified as a single major peak with 96% purity by GC (Figure 2A) and revealed as one major spot on TLC analysis (Figure 2B).

Structure Determination. The purified target compound was applied to GC-MS, FTIR, and NMR analysis for structure determination. The electron impact GC-MS spectrum and the corresponding proposed structure of the methylated product are shown in Figure 3. The mass spectra of the purified product were characterized by six major peaks in the fragmentogram. Intense peaks at m/z 193 and 209 represented the ions formed by β-cleavage to the furanoid ring toward the methyl end and the methylated carboxyl end, respectively. β-Cleavages at both sides

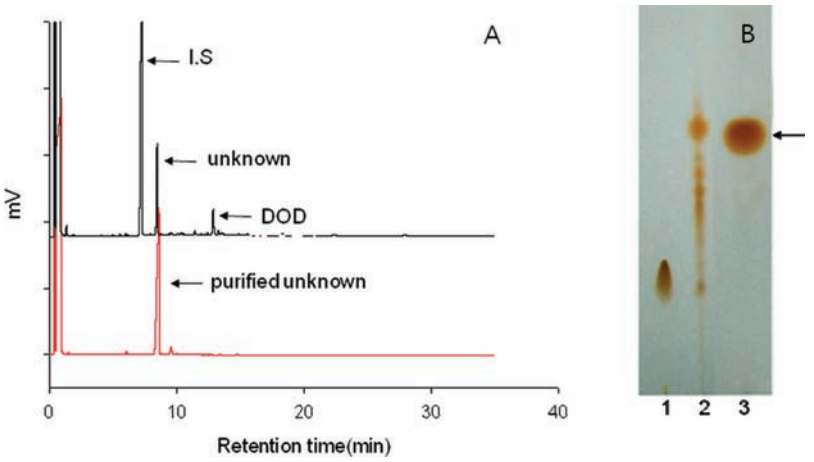

Figure 2. Analysis of the crude extract and the purified unknown compound obtained from conversion of DOD by heat treatment. Analysis was carried out by GC (A) and TLC (B). Upper and lower GC chromatograms represent crude extract and purified samples, respectively. The major unknown compound in GC analysis is indicated by the arrow in TLC analysis. Lanes: 1, standard DOD; 2, crude extract of the heat-treated DOD; 3; purified unknown compound. Other experimental conditions were as given under Materials and Methods.

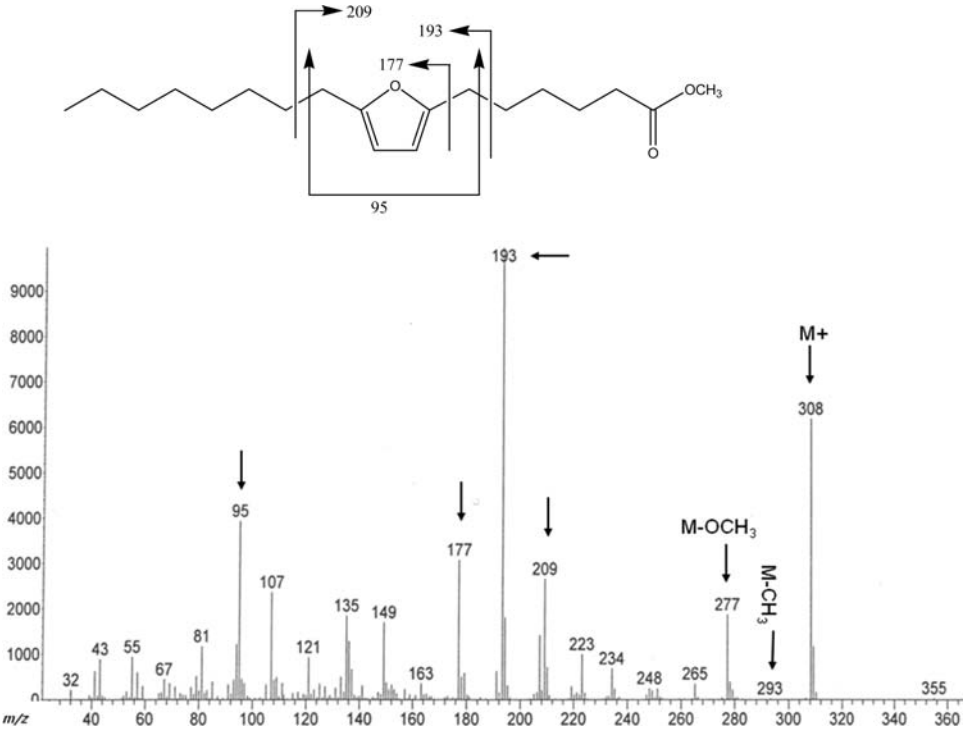

Figure 3. Electron impact mass spetrum of the methylated unknown compound in Figure 2. Major fragments are indicated by the arrow. Analytical conditions are explained under Materials and Methods.

of the furan ring yielded a furan fragment at m/z 95. Evidence of the loss of a methoxy group was seen at m/z 277. This result was in close agreement with that of the chemically synthesized 9,12-epoxyoctadeca-9,11-dienoic acid, except the location of the furan ring being two carbons away from the carboxyl group.[25] FTIR analysis presented several characteristic absorptions at 1710 cm^{-1} (carbonyl), 780 cm^{-1} (out of plane δCH), 1250 cm^{-1} (in plane δCH), and 1567 cm^{-1} (C═C, furan) (Figure 4). The overall absorption pattern was in close agreement with data reported by other workers on a synthetic furan molecule.[26] NMR analysis confirmed the elucidated structure of the purified product. Resonance signals (ppm) and corresponding molecular assignments were given as follows: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (2H, CH_2COOH), 5.84 (s, 2H, furan), 2.57 (t, 4H, J = 7.3 Hz, 2 × CH_2-furan), 0.89 (t, 3H, CH_3), 1.28−1.61 (m, 18H, 9 × $-CH_2-$); ^{13}C NMR (100 MHz, $CDCl_3$) confirmed the presence of a furan ring at 104.81, 105.02, 154.09, and 154.81 (C7, C8, C9, and C10, respectively), $-CH_3$ at 14.11 (C18), and carboxyl carbon at 178.64 (C1). Other carbons were 33.78 (C2), 24.45 (C3), 27.22 (C4), 29.71 (C5), 33.78 (C6), 31.87 (C11), 29.35 (C12), 27.84 (C13), 28.58 (C14), 29.24 (C15), 31.94 (C16), and 22.67 (C17). The data from GC-MS, FTIR, and NMR

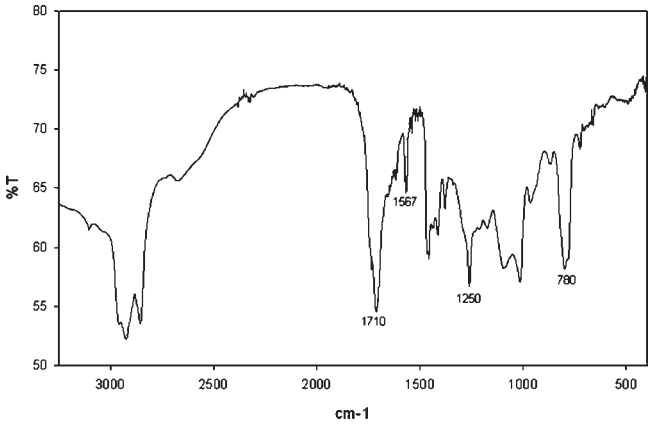

Figure 4. FTIR analysis of the purified 7,10-EODA. Analytical conditions are explained under Materials and Methods.

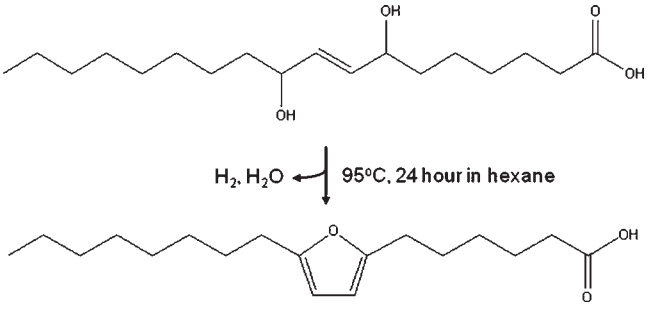

Figure 5. Schematic pathway for conversion of DOD by heat treatment leading to the formation of 7,10-EODA.

analyses confirmed that the purified product was 7,10-epoxyoctadeca-7,9-dienoic acid with the molecular weight being 294 (Figure 5). On the basis of the structure, this compound was named 7,10-EODA.

Chemical synthesis of F-acid without a substituted group was first reported by Lie Ken Jie et al. in that some isomeric C_{18} furan-containing fatty acids were chemically synthesized from furan through a complicated multistep procedure and several catalysts.[26] Alaiz et al. also reported that 9,12-epoxyoctadeca-9,11-dienoic acid was synthesized from recinoleic acid through several chemical steps with chemical catalysts.[22] However, in this study no chemical catalysts were used. Instead, a single heat treatment step was enough to produce a novel furan fatty acid from DOD. This is the first report of the one-step synthesis of a novel furan fatty acid from DOD by heat treatment. We validated our claim that EODA was a newly synthesized furan fatty acid by database search (NIST MS Search 2.0 and Cambridge Soft Chem Office ver. 5) compared to other F-acids, and no information was found on this compound.

Time Course Production and Antioxidant Activity. Time course production of 7,10-EODA was studied for 96 h at 90 °C. As shown in Figure 6, production of 7,10-EODA increased proportionally with time up to 48 h and reached a plateau thereafter. Maximum production yield under these conditions represented 82% of DOD.

Because F-acids have been reported to contain antioxidant activities, the antioxidant activity of 7,10-EODA was determined using the DPPH assay represented as radical scavenging activity

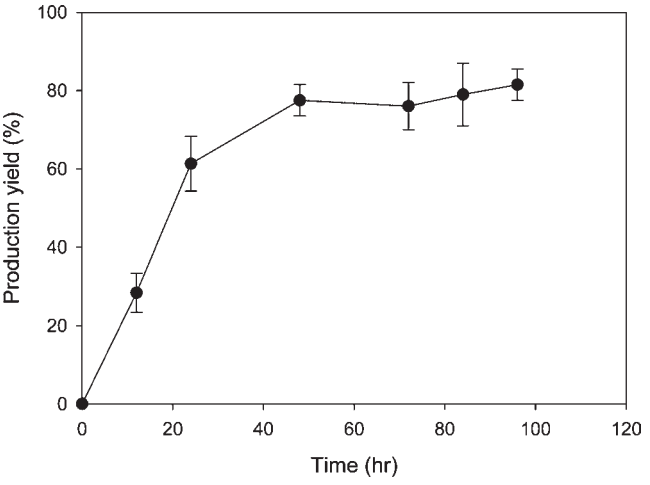

Figure 6. Time course production of 7,10-EODA. Experimental conditions were as given under Materials and Methods.

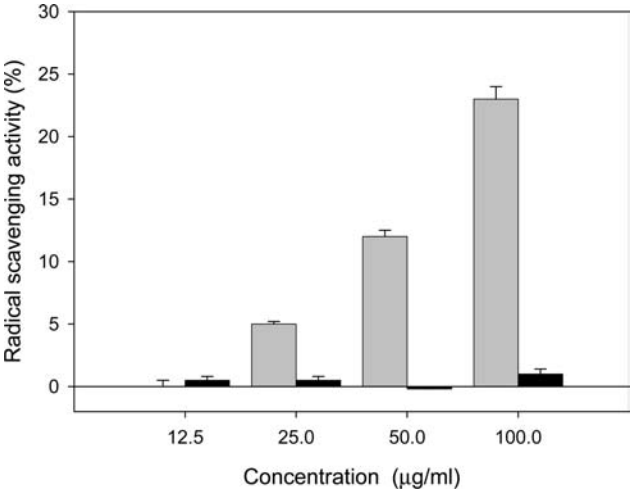

Figure 7. Radical scavenging activity of the purified 7,10-EODA. Black and gray bars represent 7,10-EODA and DOD, respectively. Analytical conditions are explained under Materials and Methods.

and compared to that of DOD (Figure 7). The radical scavenging activity of 7,10-EODA increased dose-dependently, presenting 23% at the highest concentration (100 μg/mL) tested, whereas DOD did not show any activity. Although the activity was relatively low when compared to that of α-tocopherol or ascorbic acid, 7,10-EODA showed a conclusive radical scavenging activity in a dose-dependent manner. This finding confirmed previous assumptions that F-acids contained antioxidant activity.[10] Furan fatty acids are potent strong scavengers of hydroxyl radicals, inihibit erythrocyte hemolysis induced by singlet oxygen, and are found exclusively at the *sn*-1 position of phosphatidylcholine in salmon.[27] Diet-derived F-acids are incorporated into the tissue and blood of mammals, especially into phospholipids, where they partly substitute for PUFA.[28] In plants and algae, the great tendency of F-acids to undergo oxidation suggests that these compounds can be used as radical scavengers to defend against the deleterious effect of UV radation. On the basis of these biochemical and biological studies of F-acids, it is natural to consider F-acids as critically important antioxidant materials for

mammals including humans. Hence, new findings of a simple way to cost-effectively produce F-acid are meaningful.

Taken together, this study presented a simple way to produce a 7,10-EODA from a dihydroxyl fatty acid precursor. Considering the difiiculties in purifying natural F-acids because of easy attack by peroxyl radicals and the complicated multistep procedure for chemical synthesis, this paper provides useful information to produce a biologically active F-acid in a cost-effective manner on a large scale.

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