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Original Article

Optimal Production of a Novel Furan Fatty Acid from 7,10-dihydroxy-8(*E*)-octadecenoic Acid by Heat Treatment

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Abstract

As a specialty oil, furan fatty acids have gained special attentions since they are known to play important roles in biological systems including human. Although several studies reported chemical synthesis of furan fatty acids, their synthesis consisted of complicated chemical multistep with chemical catalysts. Recently, a simple one-step heat treatment method was developed to produce a novel furan fatty acid, 7,10-epoxy-octadeca-7,9-dienoic acid (7,10-EODA) from a dihydroxyl fatty acid 7,10-dihydroxy-8(E)-octadecenoic acid (DOD). In this report we studied about optimization of environmental conditions for the maximum production of 7,10-EODA from DOD by heat treatment. Production of 7,10-EODA was maximized at over 85℃ for at least over 48 hour in hexane. Solvent volume for maximum production should be over 300 mL per 10 mg DOD.

Keywords : 7,10-epoxy-octadeca-7,9-dienoic acid, Heat treatment, 7,10-dihydroxy-8(E)-octadecenoic acid, Furan fatty acid.

Introduction

Furan, a small cyclic ether with aromatic character, was reported to be present in a number of foods, with the highest levels being found in coffee (Maga 1979). This has been followed by a series of food surveys of heat-processed foods and studies into its formation, toxicity and analysis. Furan is carcinogenic in rats and mice and has been classified as 'possibly carcinogenic to humans' (International Agency for Research on Cancer, 1995). However, 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(Glass et al. 1975). In most cases, two β-positions of the furan ring are substituted by either one or two methyl residues or other group. However F-acid without any substitutions on both β-positions of the furan ring was also found in the seed oil of *Exocarpus cupressiformis* (Morris et al. 1966). F-acids are widely and commonly distributed in nature as trace components of plants, fishes, amphibians, reptiles, microorganisms and mammals including human (Glass et al. 1975; Hannemann et al. 1989; Glass et al. 1974; Gunstone et al. 1978; Ishii et al. 1988; Ota and Takagi, 1992). The biological role of F-acids in the biological systemis still unclear, but it has been pointed out that F-acids can be involved in various important biological functions acting as antioxidant, antitumoral and antithrombotic (Graft et al. 1984; Ishii et al. 1989; Okada et al. 1996).

Biosynthesis of F-acids are complicated and quite different from sources. The biogenetic precursor of the most F-acids is known to be linoleic acid. However, it was recognized that plants synthesized the basic skeleton of F-acids from different sources (Scheinkonig et al. 1995). Yet regardless of the biological sources of F-acids were, biosynthesis of F-acids required multistep reactions due to the formation of furan ring and the different alkyl substituents. Accordingly, chemical synthesis of F-acids required complicated multistep reactions and chemical catalysts creating difficulties and high costs for industrial production.

Recently, a novel F-acid, 7,10-epoxyoctadeca-7,9-dienoic acid (7,10-EODA), was produced from 7,10-dihydroxy-8(*E*) octadecenoic acid (DOD) using simple heat treatment (Joel et al. 2011). DOD was produced from vegetable oil conatining oleic acid by microbial conversion (Chang et al. 2007). DOD is a unique dihydroxy monoenoic C_{18} fatty acid uniquely carrying two hydroxyl groups at carbon 7 and 10 and a *trans* double bond between carbon 8 and 9. Accordingly, this study was focused on optimization of environmental conditions for production of 7,10-EODA.

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Materials and Methods

Materials

Olive oil (extra virgin grade) was purchased from local market in Korea. Heptadecanoic acid (C17:0) was purchased from Nu-Chek Prep (Elysian MN, USA). A mixture of trimethylsilylimidazole (TMSI) and pyridine (1:4, v/v) was purchased from Supelco (Bellefonte, PA. USA). Thin-layer precoated Kieselgel 60F_{254} plates were obtained from EM Science (Cherry Hill, NJ, USA). Silica gel, Davisil^{TM}, grade 635, 60-100 mesh, 60A, 99+% and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless mentioned elsewhere. All other chemicals were reagent grade and were used without further purification.

Production of DOD

7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD) was produced according to previous report (Chang et al. 2007). In brief, olive oil (1%, v/v) was added as a substrate to the 24 h-old culture of *Pseudomonas aeruginosa* PR3 which was cultivated aerobically at 28℃, 200 rpm in shaking incubator followed by an additional 72 h incubation. Crude DOD extract obtained by extraction of the culture with an equal volume of ethyl acetatewas applied to the silica-gel column (1.5 cm I.D. x 30 cm) for purification. Fractionation was conducted with two column volumes of the solvent mixture with varied ratio of ethyl acetate over hexane.

Production of EODA from DOD by heat treatment

Standard conversion of DOD by heat treatment was carried out in 4 mL glass vial containing 10 mg DOD and 500 μL hexane as solvent. The mixture was incubated at 90℃ for 24 hours 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 optimization, standard conditions were modified according to individual treatment.

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℃ for 10 minutes. For GC analysis, the sample methylated with diazomethane for 5 min at room temperature was analyzed with ACME 6100 Series Gas Chromatography System (Younlin Co., Korea) equipped with a flame-ionization detector and a capillary column (SPB-1TM, 15 m x 0.32 mm i.d., 0.25 μm thickness, Supelco Inc., Bellefonte, PA, USA). GC was run with a temperature gradient of 20℃/min from 100 to 150℃, 5°C/min from 150 to 200℃, and then 0.5℃/min from 200 to 210℃ followed by holding for 10 min at 300℃ (nitrogen gas flow rate = 0.67 mL/min). Injector and detector temperatures were held at 270 and 280°C, respectively.

Results and Discussion

A novel F-acid, 7,10- EODA, was produced from 7,10-dihydroxy-8(*E*) octadecenoic acid (DOD) through simple heat treatment (Joel et al. 2011). 7,10-EODA was uniquely produced by the application of heat in hexane. Synthesis of 7,10-EODA can be assumed that the precursor compound DOD containing two hydroxyl group at the C7 and C10 position in the carbon chain and a double bond between C8 and C9 undergo cyclization followed by the incorporation of one oxygen in the pentyl ring and removal of one molecule of water. The pentyl ring carries one α-position an unbranched FA chain with 7 carbon atoms and in the other α-position a straight chain alkyl group with 8 carbon atoms, thus forming a F-acid with 18 carbon atoms (Figure 1).

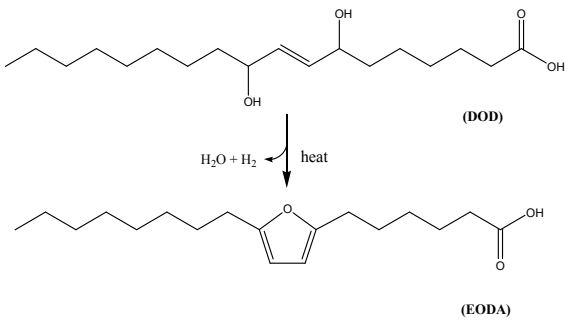

Figure 1. Schematic pathway for one-step synthesis of a novel 7,10-epoxy-octadeca-7,9-dienoic acid from DOD by heat treatment.

Effect of different solvent on 7,10-EODA production

Although 7,10-EODA was successfully produced by heat in hexane, it was highly necessary to determine the effect of different organic solvents on 7,10-EODA production since solubility of DOD and remover of water molecule from the process coud be important factor for 7,10-EODA production. Therefore five polar and four nonpolar solvents were tested to check the effect of solvents on 7,10-EODA production.

Among nine solvets tested, acetone, 1,4-dioxane, cyclohexane and hexane showed 7,10-EODA production (Figure 2). However the size of 7,10-EODA spot with hexane was bigger than those of any other solvents. Based on this result, further experiments for optimization of 7,10-EODA production were carried out with hexane.

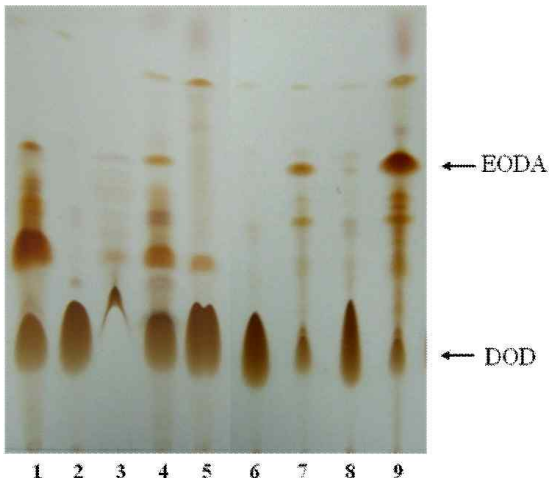

Figure 2. Effect of solvent on 7,10-EODA production.

Lane 1; acetone, lane 2; chloroform, lane 3; DMSO, lane 4; 1,4-dioxane, lane 5; acetonitril, lane 6; benzene, lane 7; ccyclohexane, lane 8; toluene, lane 9; hexane.

Effect of reaction temperature and time on 7,10-EODA production

Previously 7,10-EODA production was performed at 90℃ for 96 hours and production of 7,10-EODA increased proportionally with time up to 48 hours and reached plateau thereafter (Joel et al. 2011). In this study, we tried to determine the effect of reaction temperature and time on 7,10-EODA production. Under standard condition, varied reaction temperatures (65-9 5℃) were applied and then time-coursed 7,10-EODA productions were monitored. As shown in Figure 3, production of 7,10-EODA was highly dependant on temperature and time. Considerable yield could be obtained when DOD in hexane was heated at over 75℃ across reaction time but 7,10-EODA production was negligible below 70℃. Over 85℃, 7,10-EODA production was maximized with production yield ranging from 70 to 80%. Moreover, prolonging the reactiom time was inconguent to temperature increase. As such, 7,10-EODA formation started from 12 h in a fashionable manner and maximized towards 48 h to 96 h.

Effect of solvent volume on 7,10-EODA production

The effect of solvent volume over recatant on 7,10-EODA production was determined. Varying the volume of solvent with fixed amount of DOD (10 mg) could also affect 7,10-EODA production, wherein, an increasing yield was observed when the solvent volume increased and 7,10-EODA production was maximized at over 300 μL per 10 mg DOD (Figure 4). Therefore optimal condition of solvent volume was determined to be at least 300 μL per 10 mg DOD.

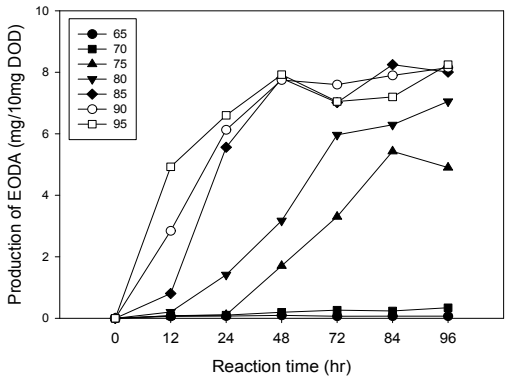

Figure 3. Effect of reaction temperature and time on 7,10-EODA production.

Reaction was conducted in 4 mL glass vial containing 10 mg DOD and 500 μL hexane at different temperatures.

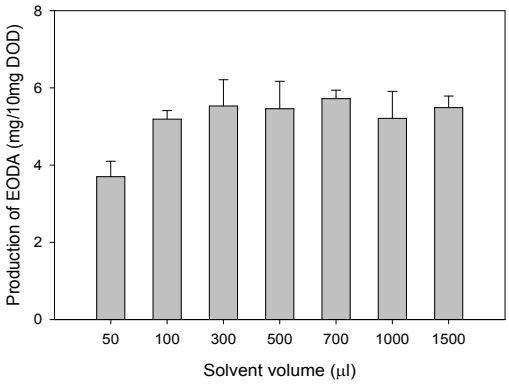

Figure 4. Effect of solvent volume on 7,10-EODA production.

Reaction was conducted in 4 mL glass vial containing 10 mg DOD and varied amount of hexane at 90oC for 24 hours.

Effect of temperature on stability of 7,10-EODA

Since F-acid was considered to be potent strong scavenger of hydroxyl radicals inihibiting erythrocyte hemolysis induced by singlet oxygen (White et al. 2005), the antioxidant activity of 7,10-EODA was determined using DPPH assay represented as radical scavenging activity and compared to that of DOD (Joel et al. 2011). Radical scavenging activity of 7,10-EODA increased dose-dependantly presenting 23 % at the highest concentration (100 mg/mL) tested while DOD did not show

any activity. Although the activity was relatively low when compared to that of a-tocopherol or ascorbic acid, 7,10-EODA showed a conclusive radical scavenging activity in a dose-dependent manner. These results suggested that 7,10-EODA could be auto-oxidized resulting in conformational changes in structure. Therefore we tested the effect of temperature on long-term stability of 7,10-EODA by changing storage temperature (Figure 5). As shown in the figure, residual amount of 7,10-EODA was changed dramatically according to temperature. At 4℃, residual amount of 7,10-EODA dropped down to 15% of the original amount of 7,10-EODA after1 month. When stored at -25oC, residual amount of 7,10-EODA was maintained over 60% after three month, confirming that 7,10-EODA was relatively stable when stored at -25℃.

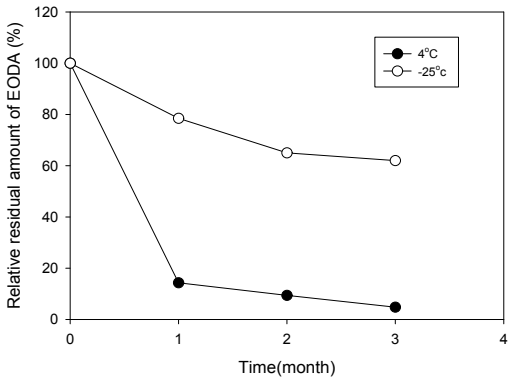

Figure 5. Effect of storage temperature on stability of 7,10-EODA production.

Vials with 10 mg of DOD were stored at different temperature and the residual amount of 7,10-EODA were determined after a given time.

Chemical synthesis of furan fatty acid without substituted group was first reported by Lie Ken Jie *et al.* in that some isomeric C18furan-containing fatty acids were chemically synthesized from furan through complicated several steps and catalysts (Lie Ken Jie and Lam 1978). Alaiz et al. also reported that 9,12-epoxy-octadeca-9,11-dienoic acid was synthesized from recinoleic acid through several chemical steps with chemical catalysts (Alaiz et al. 1988). However, in this report, no chemical catalysts were used. Instead single heat treatment step was enough to produce a novel furan fatty acid from a hydroxyl fatty acid precursor with maximum production yield being over 80% through optimization of environmental conditions.

Acknowledgements

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