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Chemo-Enzymatic Synthesis of 11-Hydroxyundecanoic Acid and 1,11-Undecanedioic Acid from Ricinoleic Acid

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Abstract

A practical chemoenzymatic synthetic method for 11-hydroxyundecanoic acid and 1,11-undecanedioic acid from ricinoleic acid (12-hydroxyoleic acid) was investigated. Biotransformation of ricinoleic acid into the ester (**3**) via 12-ketooleic acid (**2**) was driven by recombinant *Escherichia coli* cells expressing an alcohol dehydrogenase from *Micrococcus luteus* and the Baeyer–Villiger monooxygenase from *Pseudomonas putida* KT2440. The carbon-carbon double bond of the ester (**3**) was chemically reduced, and the ester bond was hydrolyzed to afford n-heptanoic acid (**5**) and 11-hydroxyundecanoic acid (**7**), which were converted into other related derivatives. For example, 11-hydroxyundecanoic acid was transformed into 1,11-undecanedioic acid (**8**) under fairly mild reaction conditions. Whole-cell biotransformation at high cell density (i.e., 20 g dry cells/L) allowed the final ester product concentration and volumetric productivity to reach 53 mM and 6.6 mM/h, respectively. Overall molar yield of 1,11-undecanedioic acid from ricinoleic acid was 55% based on the biotransformation and chemical transformation conversion yields of 84% and 65%, respectively.

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Introduction

Medium chain (C7 to C13) α,ω-dicarboxylic acids and ω-aminocarboxylic acids are widely used as building blocks and/or intermediates for producing plastics (e.g., polyamides and polyesters), pharmaceuticals, plasticizers, lubricants, and hydraulic fluids [1-5]. For example, 11-aminoundecanoic acid is used to synthesize polyamide 11, which has outstanding chemical, thermal, and impact resistance over a wide range of flexibilities compared to that of other high performing and engineered plastics [2].

However, the medium chain α,ω-dicarboxylic acids and ω-aminocarboxylic acids are usually produced chemically under harsh conditions requiring high temperature and pressure, strong acids (e.g., H_2SO_4 and HNO_3), and/or toxic oxidants (e.g., ozone) [1, 3]. 11-Aminoundecanoic acid is produced from ricinoleic acid methyl ester via cracking at high temperature of 450 to 500^{o}C and adding hydrogen bromide followed by nucleophilic substitution with ammonia [2, 6].

A variety of emerging ideas to tackle the problems in production of such chemicals have been reported. Milder methods based on e.g., ruthenium catalysis in combination with peracetic acid to replace ozonolysis, or the metathesis reaction of unsaturated fatty acids to yield linear diacids have been investigated [7, 8]. 11-Aminoundecanoic acid has been synthesized from vernolic (*cis*-12,13-epoxy-*cis*-9-octadecenoic) acid via a reaction sequence that includes the formation of 12-oxododecanoic acid oxime [2]. Biotransformation routes using *Candida tropicalis* have been also reported [1]. Sebacic acid and azelaic acid were produced from hydrocarbons via fermentation of *C. tropicalis* [5]. However, these processes can be constrained by the limitations in the availability of the substrates used and/or low product yields.

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Microbial synthesis of medium chain (e.g., C8–C14) fatty acids, ω-hydroxycarboxylic acids and α,ω-dicarboxylic acids from sugars and glycerol was also investigated [9-12]. For instance, the expression of the *Pseudomonas putida* alkane monooxygenase system, encoded by *alkBGT*, in combination with all β-oxidation reversal enzymes in *Escherichia coli* allowed to produce 6-hydroxyhexanoic acid, 8-hydroxyoctanoic acid, and 10-hydroxydecanoic acid from glycerol [9]. The medium chain ω-hydroxycarboxylic acids were further oxidized into the corresponding α,ω-dicarboxylic acids by coexpression of potential alcohol and aldehyde dehydrogenases, *chnD* and *chnE* from *Acinetobacter* sp. strain SE19. However, the medium chain carboxylic acids were produced to a mixture at a rather low concentration in the medium.

Biotransformation of renewable fatty acids and/or plant oils into C9–C13 ω-hydroxycarboxylic acids, ω-aminocarboxylic acids, and α,ω-dicarboxylic acids has been recently reported [13-16]. For example, 11-aminoundecanoic acid and 1,11-undecanedioic acid have been produced from ricinoleic acid; the C12-hydroxyl group of ricinoleic acid was oxidized to the ester (**3**) via 12-ketooleic acid (**2**) in a serial reaction with the alcohol dehydrogenase (ADH) of *Micrococcus luteus* and the Baeyer–Villiger monooxygenase (BVMO) of *P. putida* KT2440 (Scheme 1) [13, 14]. The ester (**3**) was hydrolyzed by an esterase to yield n-heptanoic acid and (*Z*)-11-hydroxyundec-9-enoic acid. (*Z*)-11-Hydroxyundec-9-enoic acid was then further converted to 1,11-undecanedioic acid by an ADH of *P. putida* GPo1 and a reductase of *E. coli*. (*Z*)-11-Hydroxyundec-9-enoic acid was transformed into 11-aminoundecanoic acid via oxidation followed by amination with the ADH of *P. putida* GPo1 and ω-transaminase of *Silicibacter pomeroyi*. However, the final product concentration and productivity of the biosynthetic routes remains very low because of high toxicity of the

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reaction intermediates and final products (e.g., n-heptanoic acid, (*Z*)-11-hydroxyundec-9-enoic acid) to the microbial host cells (i.e., *E. coli*) [17] and low stability of the BVMOs [18-22].

One of the approaches to bypass such product toxicity problem could be to use a chemo-enzymatic process [23, 24]. Here, a chemo-enzymatic catalytic process to synthesize 11-hydroxyundecanoic acid (**7**) and 1,11-undecanedioic acid (**8**) from ricinoleic acid was investigated. Ricinoleic acid was transformed into ester (**3**) by recombinant *E. coli* cells expressing the ADH from *M. luteus* and the BVMO from *P. putida* KT2440 (Scheme 1). Then, the carbon-carbon double bond of ester (**3**) was chemically reduced and the ester bond was hydrolyzed to n-heptanoic acid (**5**) and 11-hydroxyundecanoic acid (**7**) (Scheme 2), which can be converted into other related derivatives, including 11-aminoundecanoic acid [14]. 11-Hydroxyundecanoic acid was also oxidized to 1,11-undecanedioic acid (**8**) under fairly mild reaction conditions.

Materials and Methods

Microbial strains and culture media

Recombinant *E. coli* BL21(DE3) pACYC-ADH and pJOE-BVMO were grown in Luria-Bertani medium (5 g/L yeast extract, 10 g/L tryptone, and 10 g/L NaCl) supplemented with chloramphenicol and ampicillin for seed cultivation as described in our previous study [13, 14]. Riesenberg medium [25] supplemented with 10 g/L glucose and the appropriate antibiotics was used for the main cultivation and biotransformation. The Riesenberg medium consisted of 4 g/L $(NH_4)_2HPO_4$, 13.5 g/L KH_2PO_4, 1.7 g/L citric acid, 1.4 g/L $MgSO_4$, and 10 mL/L trace metal solution (10 g/L $FeSO_4$, 2.25 g/L $ZnSO_4$, 1.0 g/L $CuSO_4$, 0.5 g/L $MnSO_4$, 0.23 g/L

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105 $Na_2B_4O_7$, 2.0 g/L $CaCl_2$, and 0.1 g/L $(NH_4)_6Mo_7O_{24}$). Recombinant gene expression was
106 induced by adding 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and/or 2 g/L
107 rhamnose to the culture broth.

108

109 **Biotransformation in a flask and bioreactor**

110 Biotransformation was carried out as reported previously [17]. In brief, biotransformation was
111 initiated at the stationary growth phase, usually 8 h after inducing gene expression with 0.1
112 mM IPTG and/or 2 g/L rhamnose. After changing the culture broth pH to 8.0 and increasing
113 temperature to 35℃, 15–60 mM ricinoleic acid and 0.5 g/L Tween80 were added to the
114 culture broth containing 3–20 g dry cells/L. Culture and biotransformation were performed in
115 a 250 mL flask (working volume: 20 mL) in a shaking incubator (200 rpm). The bioreactor
116 experiment was conducted in a 1 L scale reactor (Biotron, Bucheon, Korea). Agitation speed
117 and aeration rate were 400–1000 rpm and 1 vvm, respectively, to avoid oxygen limitation
118 during culture and biotransformation.

119

120 **Product analysis by gas chromatography/mass spectrometry (GC/MS)**

121 The concentrations of the remaining fatty acids and accumulating carboxylic acids in the
122 medium (e.g., ricinoleic acid (**1**), 12-ketooleic acid (**2**), and ester (**3**)) were determined as
123 described previously[16, 17]. The reaction medium was mixed with an equal volume of ethyl
124 acetate containing 0.1 or 0.5 g/L methyl palmitate as an internal standard. The organic phase
125 was harvested after vigorous vortexing and subjected to derivatization with *N*-methyl-*N*-

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(trimethylsilyl) trifluoroacetamide. The trimethylsilyl derivatives were analyzed using a Thermo Ultra Trace GC system connected to an ion trap mass detector (Thermo ITQ1100 GC-ion Trap MS, Thermo Scientific, Indianapolis, IN, USA). The derivatives were separated on a non-polar capillary column (30 m length, 0.25 μm film thickness, HP-5MS, Agilent Technologies, Palo Alto, CA, USA). A linear temperature gradient was programmed as 90°C, 5°C/min to 280°C. Injection port temperature was 230°C. Mass spectra were obtained by electron impact ionization at 70 eV. Scan spectra were obtained within the range of 100–600 m/z. Selected ion monitoring was used for detection and the fragmentation analysis of the reaction products.

Isolation of ester intermediate (3) after biotransformation

The ester intermediate was isolated by simple extraction with ethyl acetate several times. The combined organic layer was washed with saline, dried over sodium sulfate, filtered, and evaporated *in vacuo*. The crude ester was purified by column chromatography (silica gel) eluting with 20% ethyl acetate-hexane. The isolation/purification yield of ester (**3**) was > 80%, based on the observed concentration of the ester after biotransformation.

(*Z*)-11-(Heptanoyloxy)undec-9-enoic acid (**3**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.67-5.46 (m, 2H), 4.60 (d, J = 6.0 Hz, 2H), 2.36-2.27 (m, 4H), 2.12-2.02 (m, 2H), 1.65-1.58 (m, 4H), 1.35-1.21 (m, 14H), 0.86 (m, 3H): ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.2, 172.0, 133.4, 121.6, 58.3, 32.5, 32.2, 29.5, 27.4, 27.2, 27.1, 27.0, 26.9, 25.6, 23.0, 22.7, 20.6, 12.1 (see the supporting information, Fig. S1).

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Step-by-step chemical conversion of ester intermediate (3) to undecanedioic acid (8)

11-(Heptanoyloxy) undecanoic acid (**6**)

Ester (**3**) (1.0 g, 3.2 mmol) was taken in methanol (10 mL) and moist Raney-Ni (0.4 g) was added after to this solution soaking it in filter paper. The heterogeneous mixture was subjected to hydrogenation under hydrogen (1 atm) for 8 h. The reaction mixture was filtered through Celite and washed several times with methanol. The filtrate was evaporated to afford saturated ester (**6**) (0.95 g, 95%) as a yellowish semi-solid, which determined to be sufficiently pure (as per ^{1}H-NMR). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (t, J = 6.6 Hz, 2H), 2.31-2.26 (m, 4H), 1.61-1.59 (m, 6H), 1.28-1.25 (m, 18H), 0.87 (t, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.09 (2C), 64.38, 34.41, 31.45, 29.71, 29.40, 29.31, 29.18, 29.03, 28.81, 28.61, 25.89, 24.97, 24.65, 22.47, 14.01 (see the supporting information, Fig. S2).

11-Hydroxyundecanoic acid (**7**)

Saturated ester (**6**) (950 mg, 3.02 mmol) was treated with NaOH (1 N) in MeOH-H_2O (4/1, 25 mL) at 60°C for 2.5 h. The reaction mixture was cooled, and pH was adjusted to 2 by slowly adding aq. HCl (6 N) under ice-cold conditions. The reaction mixture was evaporated to reduce the volume to about 5 mL (until water started to evaporate). The residual solvent was saturated with NaCl and extracted with ethyl acetate (3 × 30 mL). The combined organic extract was washed with saline (20 ml) and water (20 ml), dried over anhydrous Na_2SO_4, filtered, and evaporated. The crude product was purified by silica gel column chromatography using 50% ethyl acetate-hexane as the eluent to afford 11-hydroxyundecanoic acid (**7**) (550 mg, 90%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.63 (t, J = 6.6 Hz, 2H), 2.32 (t, J = 7.5 Hz, 2H), 1.64-1.51 (m, 4H), 1.35-1.25 (m, 12H); ^{13}C

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NMR (75 MHz, $CDCl_3$) δ 179.47, 62.96, 34.01, 32.60, 29.41, 29.28, 29.25, 29.12, 28.96, 25.63, 24.63 (see the supporting information, Fig. S3)

Undecanedioic acid (**8**)

A stock solution of H_5IO_6/CrO_3 was prepared by dissolving periodic acid (H_5IO_6) (11.4 g, 50 mmol) and CrO_3 (23 mg, 0.23 mmol) in wet acetonitrile (75% CH_3CN/water) to a volume of 114 mL (complete dissolution typically required 1–2 h). The H_5IO_6/CrO_3 stock solution (13 mL, 2.5 eq. of H_5IO_6, and 0.01 eq. of CrO_3) was added to a solution of compound **7** (470 mg, 2.33 mmol) in wet acetonitrile (12 mL, 75% CH_3CN/water) while maintaining the reaction temperature at 0–5 °C for 30 min. The mixture was aged at 0°C for 45 min. The reaction was quenched by adding an aqueous solution of Na_2HPO_4 (0.7 g in 7 ml H_2O). Ethyl acetate (20 mL) was added and stirred. The organic layer was separated. The aqueous phase was saturated with NaCl and extracted further with 10% MeOH-ethyl acetate (4 × 20 ml). The combined organic layer was washed with saline (15 ml), 5% $NaHSO_3$ (15 mL), and saline (15 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give the crude dicarboxylic acid, which was further purified by recrystallization from ethyl acetate-hexane to yield undecanedioic acid (**8**) (450 mg, 90%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.17 (t, J = 6.6 Hz, 4H), 1.49-1.44 (m, 4H), 1.23 (m, 10H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.90 (2C), 34.09 (2C), 29.23, 29.14 (2C), 28.98 (2C), 24.92 (2C) (see the supporting information, Fig. S4).

Direct chemical conversion of ester intermediate (3) to undecanedioic acid (8)

The final product, undecanedioic acid (**8**) was synthesized directly without column

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chromatography through three-step chemical reactions of the crude ester. Crude ester (**3**) (about 5.3 g, calculated amount of the ester: 4.0 g), which was obtained by centrifugation, filtration, and extraction procedures using the reaction medium (volume: 300 mL, measured concentration of the ester: 42.75 mM) after biotransformation, was subjected to hydrogenation, hydrolysis, and oxidation as described above. Undecanedioic acid (**8**) (1.77 g, 65% yield based on the observed concentration of the crude ester) was isolated by recrystallization at the final step.

Results and Discussion

Biocatalyst engineering for biotransformation of ricinoleic acid into ester 3

Whole-cell biotransformation of ricinoleic acid (**1**) into 11-hydroxyundec-9-enoic acid (**4**) and n-heptanoic acid (**5**) (Scheme 1) was investigated in our previous study [17]. As a result, the biotransformation performance of the biocatalyst (i.e., recombinant *E. coli* BL21(DE3) pACYC-ADH-BVMO, pCOLA-PFEI expressing the ADH of *M. luteus*, the BVMO of *P. putida* KT2440, and the esterase of *P. fluorescens* WI SIK) was limited by the toxic effect of the final products on the host cells. Therefore, we examined biotransformation of ricinoleic acid into ester **3** which is much less toxic to the cells (see the supporting information, Fig. S5), which could be chemically converted into undecanedioic acid (Scheme 1 and 2).

Biotransformation productivity of the recombinant *E. coli* pACYC-ADH-BVMO was limited by low BVMO activity compared to ADH activity [17]. Therefore, the BVMO was

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expressed with pJOE-BVMO, which allows for a higher copy number than that of the pACYC-duet vector [21]. Biotransformation with recombinant *E. coli* BL21(DE3) pACYC-ADH, pJOE-BVMO was initiated at the early stationary growth phase by adding 15 mM ricinoleic acid and 0.5 g/L Tween80 to the culture broth. The starting material was converted into ester **3** via 12-ketooctadeca-9-enoic acid (**2**) at a rate of 1.7 mM/h (0.43 g/L/h) at $t < 6$ h (Fig. 1). The final product concentration and product yield was > 11 mM and 73%, respectively. The biotransformation rate was ca. 50% greater compared to biotransformation of ricinoleic acid to 11-hydroxyundec-9-enoic acid and n-heptanoic acid [17]. The high biotransformation rate and low concentration of reaction intermediate **2** in the medium suggested that the combination of pACYC-ADH, pJOE-BVMO would be close to optimal for expressing catalytic enzymes for whole-cell biotransformation.

Biotransformation at high cell density

The next step was to increase final product concentration and volumetric productivity. This was accomplished by enhancing the substrate and biocatalyst concentrations in the reaction medium. The biotransformation was carried out after fed-batch cultivation in a 1 L scale bioreactor. When the recombinant *E. coli* BL21 pACYC-ADH, pJOE-BVMO was grown to 10 g dry cells/L, ricinoleic acid was added to a final concentration of 30 mM in the culture broth (Fig. 2A). The reaction dynamics were similar to those of the whole-cell biotransformation with 15 mM ricinoleic acid; the ester compound was stoichiometrically produced from ricinoleic acid via 12-ketooctadeca-9-enoic acid (**2**). However, the final product concentration and volumetric productivity increased to 25 mM and 6.2 mM/h,

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respectively, which were 2.3- and 3.6-fold higher than the values obtained in the experiment shown in Fig. 1 (Table 1). The volumetric productivity was ca. two-fold greater than that of recombinant *E. coli* pACYC-ADH- BVMO, pCOLA-PFEI during biotransformation of ricinoleic acid into 11-hydroxyundec-9-enoic acid and n-heptanoic acid under comparable conditions [17].

Further increases in substrate and biocatalyst concentrations in the reaction medium to 63 mM and 20 g dry cells/L led to a final product concentration and volumetric productivity of 53 mM (conversion yield, 84%) and 6.6 mM/h, respectively (Fig. 2B and Table 1). This result indicates that the ester product was efficiently produced via whole-cell biocatalysis at high cell density.

Isolation of ester intermediate (3) after biotransformation

We used extraction, centrifugation, and filtration techniques to remove the cell mass and isolate ester intermediate (**3**) after biotransformation. The ester intermediate was isolated by simple extraction with ethyl acetate (2–3 times). The combined organic layer was washed with saline, dried over sodium sulfate, evaporated, and chromatographed to afford the pure ester intermediate. Ester (**3**) was recovered as a 90–95% crude mixture and was purified/isolated to > 80% by column chromatography based on the observed concentration of the ester intermediate after the biotransformation.

Chemical conversion of the ester intermediate to undecanedioic acid

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We developed a three-step chemical reaction including hydrogenation of the alkene, hydrolysis of the ester, and oxidation of the alcohol to convert ester intermediate (**3**) to undecanedioic acid (**8**) (Scheme 2). Step-by-step reactions were examined initially to optimize each step. At first, the alkene moiety was easily reduced using Raney-Ni under a hydrogen atmosphere (1 atm) to afford the saturated ester 11-(heptanoyloxy)undecanoic acid (**6**) as a semi-solid at almost quantitative yield. Particularly, the Raney-Ni catalyst was very effective and may be very economical for large-scale reactions, compared to other metal catalysts. Next, the ester was successfully hydrolyzed with sodium hydroxide in a co-solvent of methanol and water (4/1) at 60°C to provide 11-hydroxyundecanoic acid (**7**) as a semi-solid with about a 90% yield. Oxidation of the terminal hydroxyl group to carboxylic acid was the key step; thus, several oxidation conditions were examined. Among them, periodic acid in the presence of a catalytic amount of chromium trioxide yielded the best conversion efficiency, lowest cost, and mildness of the reaction conditions [26]. 11-Hydroxyundecanoic acid (**7**) was treated with a stock solution of periodic acid (2.5 eq.) and chromium trioxide (0.01 eq.) in 75% aq. acetonitrile at 0°C to afford about 90% undecanedioic acid (**8**) after recrystallization from ethyl acetate-hexane. The overall yield was about 80% over the three step-by-step syntheses. All intermediates were fully confirmed by their NMR spectra.

A three-step reaction will be needed for large-scale preparations without any purification procedures. Thus, we determined the optimal conditions without the column purification steps. Ester intermediate (**3**) was prepared as a mixture of the ester and some other fatty acids by simple centrifugation and filtration without column purification. After hydrogenation of the mixture as described above, the methanol was partially evaporated. Then, the ester-containing solution was treated with aq. sodium hydroxide (resulting

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concentration, 1 N) at 60°C for 2.5 h. After pH was adjusted to 2 by slow adding aq. HCl (6 N) at 0°C, the reaction mixture was evaporated to remove the low-boiling point solvents and extracted with ethyl acetate. Finally, the ω-hydroxy acid-containing mixture was treated with periodic acid (2.5 eq.) and chromium trioxide (0.01 eq.) in 75% aq. acetonitrile at 0°C. After simple extraction with ethyl acetate, the desired product, 1,11-undecanedioic acid (**8**) was purified by recrystallization from ethyl acetate-hexane. Based on the concentration of the ester intermediate from the biotransformation, the overall yield of undecanedioic acid was > 65% after isolating the ester and the three chemical reactions, which was reasonable and efficient after considering the purification yield of the ester and the step-by-step chemical yields over the three steps. Particularly, use of methanol was minimized and the time-consuming, laborious and cost-consuming column purification steps were not needed. In addition, the final product, 1,11-undecanedioic acid, was obtained is a highly pure form by recrystallization. We easily prepared 1–10 g of 1,11-undecanedioic acid (**8**) in the laboratory using these procedures, which could be applicable to future industrial preparations.

Feasibility of the ricinoleic acid transformation process

One of the key factors to influence feasibility of the ricinoleic acid transformation process for industrial application may include final product concentration and volumetric productivity of the biotransformation process, overall product yield, and cost of the reagents used for bio/chemical transformations. Although cost of the reagents used for bio/chemical transformations is difficult to estimate at the moment, the final product concentration and volumetric productivity of the biotransformation can be compared between the complete

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biological transformation and the chemo-enzymatic transformation processes. As discussed previously [17], the final product concentration and volumetric productivity of the complete biological transformation process appeared to be limited in the range of 20 mM and 3 mM/h, respectively, because of high toxicity of the reaction intermediates and final products (e.g., n-heptanoic acid, 11-hydroxyundec-9-enoic acid) to the host cells (see the supporting information, Fig. S5A). On the other hand, the final product concentration and volumetric productivity of the chemo-enzymatic transformation process reached 53 mM and 6.6 mM/h, respectively (Fig. 2B and Table 1). The final product concentration and volumetric productivity of the chemo-enzymatic transformation process may further increase due to low toxicity of the ester product (**3**) (see the supporting information, Fig. S5). Indeed, engineering of the catalytic enzymes (e.g., BVMO) allowed the recombinant *E. coli* cells expressing the ADH of *M. luteus* and the engineered BVMO of *P. putida* KT2440 to reach the final ester (**3**) concentration and the volumetric productivity of over 100 mM in the reaction medium and 20 mM/h, respectively (unpublished data). This result indicates that cost effectiveness of the chemo-enzymatic process could be larger than the complete biological transformation process.

Comparison to the complete chemical process

The key step and the most striking difference between the chemo-enzymatic and the complete chemical processes for cracking ricinoleic acid (C18) into C7 and C11 products would be cleavage method of the C11-C12 bond of ricinoleic acid. This is driven by the BVMO enzyme of *P. putida* KT2440 under physiological condition at the chemo-enzymatic process, whereas conducted under high temperature over 450°C in case of the complete chemical

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process [6]. The following step, oxidation of the cleavage products (i.e., 11-hydroxyundec-9-enoic acid and methyl undecylenate at the chemo-enzymatic and complete chemical processes, respectively) into 1,11-undecanedioic acid would be comparable in terms of cost effectiveness. Thereby, it was assumed that the chemo-enzymatic process could be less energy consuming and more environment-friendly compared to the currently operating chemical process.

Feasibility of the chemo-enzymatic transformation process could be also enhanced by using castor oil instead of ricinoleic acid as the starting material. Ricinoleic acid accounts for over 80% of the fatty acid constituents of castor oil, which is cheaper than ricinoleic acid and commercially available. If a lipase is included into the reaction medium containing castor oil, the plant oil will be hydrolyzed generating ricinoleic acid, oleic acid, and glycerol. Ricinoleic acid will be converted into the ester product (**3**), whereas glycerol and oleic acid will be consumed as energy sources by metabolic enzymes of *E. coli*.

Conclusion

A practical chemo-enzymatic synthesis of 11-hydroxyundecanoic acid (**7**) and 1,11-undecanedioic acid (**8**) from ricinoleic acid was examined. Biotransformation of ricinoleic acid into ester (**3**) was driven by recombinant *E. coli* expressing the ADH from *M. luteus* and the BVMO from *P. putida* KT2440. Ester (**3**) was chemically converted to 11-hydroxyundecanoic acid (**7**) and then to 1,11-undecanedioic acid (**8**) under fairly mild reaction conditions. The whole-cell biotransformation at high cell density (i.e., 20 g dry cells/L) led to a final ester concentration and volumetric productivity of 53 mM and 6.6

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mM/h, respectively. The overall molar yield of 1,11-undecanedioic acid from ricinoleic acid was 55% based on the biotransformation and chemical transformation yields of 84% and 65%, respectively. This method can be further applied to produce 9-hydroxynonanoic acid and 1,9-nonanedioic acid (azelaic acid) from oleic acid because 9-(nonanoyloxy)nonanoic acid can be produced from oleic acid using recombinant *E. coli* expressing the C9 double bond hydratase of *Stenotrophomonas nitritireducens*, the ADH from *M. luteus*, and the BVMO from *P. putida* KT2440 [14]. This study will contribute to development of sustainable processes for the production of medium chain fatty acids from renewable long chain fatty acids.

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407 Table 1. Biocatalytic performance of the recombinant *Escherichia coli*-based biocatalyst

	Reaction at batch cultivation	Reaction at fed-batch cultivation 1	Reaction at fed-batch cultivation 2	Reaction at fed-batch cultivation 3 [c]
Substrate concentration (mM)	15	30	63	30
Biocatalyst concentration (g dry cells/L)	3	10	20	10
Final product concentration (mM)	11	25	53	21
Volumetric productivity (mM/h) [a]	1.7	6.2	6.6	3.2
Product yield (%) [b]	73	83	84	70

408 [a] Volumetric productivity was calculated based on the product concentration, which was
409 determined by gas chromatography/liquid chromatography (GC/MS), and biotransformation
410 time, which was measured when > 90% of the starting material was converted to the
411 products.

412 [b] Product yield was calculated based on substrate depletion and product concentration, which
413 were determined by GC/MS.

414 [c] Recombinant *E. coli* pACYC-ADH-BVMO, pCOLA-PFEI was used to biotransformation
415 of ricinoleic acid to 11-hydroxyundec-9-enoic acid. The result was adopted from our
416 previous study [17].

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Scheme Legends

Scheme 1. Designed biotransformation pathway. Ricinoleic acid (**1**) is enzymatically converted into ester **3** (see the Results section for details), which can be hydrolyzed into *n*-heptanoic acid and (*Z*)-11-hydroxyundec-9-enoic acid (**4**) [13]. Adopted from our previous study [13].

Scheme 2. Chemical conversion of ester intermediate (**3**) to undecanedioic acid (**8**)

Figure Legends

Figure 1. Time course of the biotransformation of ricinoleic acid (**1**) by recombinant *Escherichia coli* BL21(DE3) pACYC-ADH, pJOE-BVMO, expressing the alcohol dehydrogenase (ADH) from *Micrococcus luteus* and the BVMO from *Pseudomonas putida* KT2440. The biotransformation was initiated at the stationary growth phase (cell density: 3 g dry cells/L) in Riesenberg medium by adding 15 mM ricinoleic acid and 0.5 g/L Tween80 to the culture broth. Symbols indicate concentrations of ricinoleic acid (**1**) (△), 10-ketooleic acid (**2**) (▽), and **3** (■).

Figure 2. Biotransformation of ricinoleic acid by *Escherichia coli* pACYC-ADH, pJOE-BVMO at high cell density. Biotransformation was initiated by adding (A) 30 mM ricinoleic acid and 0.5 g/L Tween80 to the culture broth after fed-batch cultivation to a cell density of 10 g dry cells/L or (B) 63 mM ricinoleic acid and 0.5 g/L Tween80 to the culture broth after fed-batch cultivation to a cell density of 20 g dry cells/L. Symbols indicate concentrations of ricinoleic acid (**1**) (△), 10-ketooleic acid (**2**) (▽), and **3** (■).

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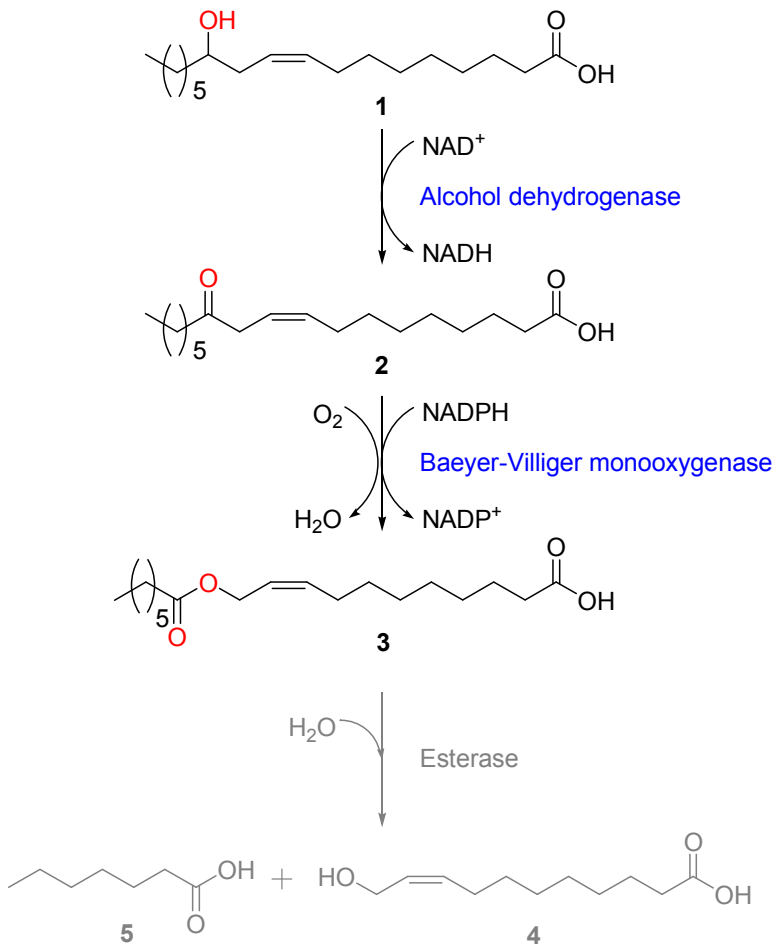
Scheme 1



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444 **Scheme 2**

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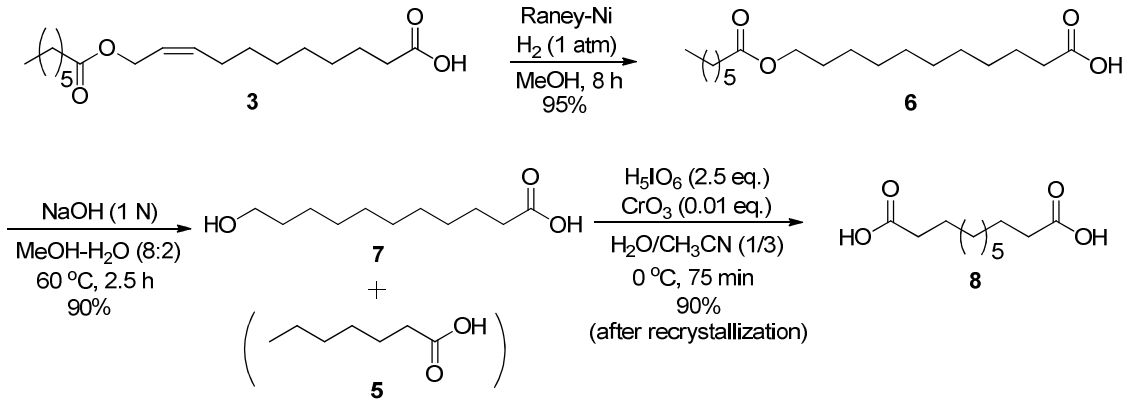

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454 **Fig. 1**

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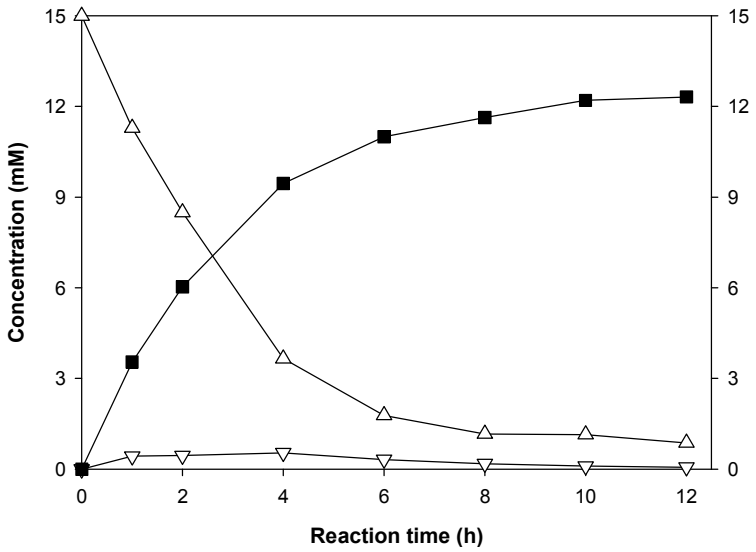

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460 **Fig. 2**

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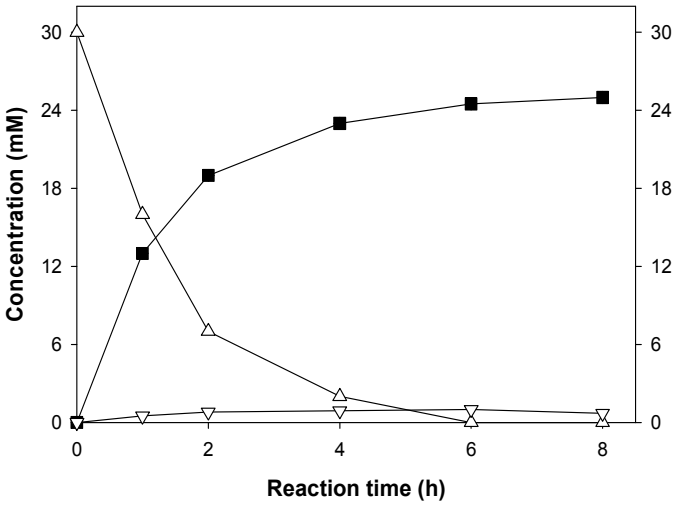
462 **A**



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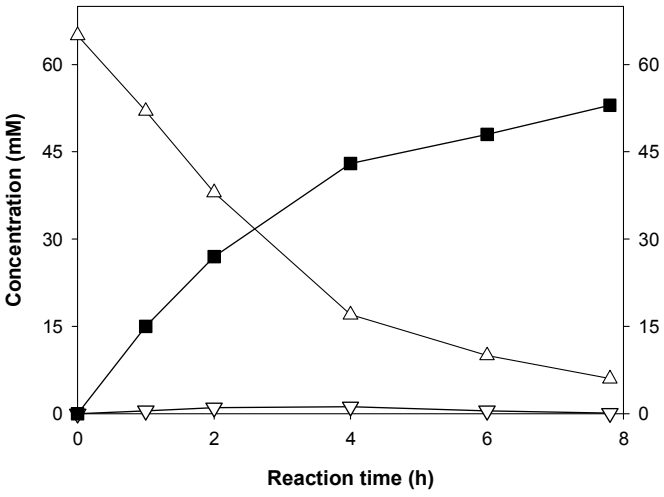
465 **B**



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