An improved synthesis of 1-monoolein

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Abstract. The synthesis of 1-monoolein has been carried out through a two-step reaction: transesterification of ethyl oleate and 1,2-acetonide glycerol in the presence of sodium carbonate as a catalyst, and followed by deprotection using an Amberlyst-15 catalyst in ethanol. The transesterification reaction of ethyl oleate could produce 1,2-acetonide-3-oleoyl glycerol as a yellow liquid with a yield of 74%. Meanwhile, the deprotection of the intermediate compound could afford 1-monoolein as an unstable white soft solid in a yield of 59% and melting point at 35-37 °C.

1. Introduction

Monoacylglycerols or monoglycerides are a class of lipids with the hydrophilic and lipophilic functional group present in a molecule [1]. Based on their structure, monoglycerides are a non-ionic surfactant that potentially being used as an emulsifier, stabilizer, plasticizer, and conditioner. Monoglycerides could be produced conventionally from glycerol and vegetable oils or animal fats through chemical or enzymatic reactions. Industrial production of monoglyceride mainly involves a glycerolysis reaction of fats or vegetable oils that requires a high temperature reaction (220-250 °C), nitrogen atmosphere system, an inorganic alkaline catalyst, and a high vacuum distillation for purification [2]. Although the reaction requires high energy consumption, it produces monoglyceride in a lower quality, with a dark-colored and charred-smell compound, and also in a low yield.

Monoglycerides have antimicrobial activity. Monolaurin compounds, like 1-monolaurin and 2-monolaurin, have been reported to have antibacterial, anti-fungal, and antiviral activity [3-7]. Other monoglycerides such as 1-monocaprin and 1-monomyristin also have shown antibacterial and antifungal properties [8-10]. On the other hand, monoolein exhibits a high antioxidant and anti-atherosclerosis activity [11,12]. Therefore, the synthesis of monoolein as an unsaturated monoglyceride has been of great interest to researchers.

Wang et al. [13] have reported the synthesis of 1-monoolein through esterification reaction of oleic acid and 1,2-acetonide glycerol, to produce an intermediate compound 1,2-acetonide-3-oleoyl glycerol, in the presence of Novozym-435 lipase enzyme as a catalyst. In their work, the deprotection
reaction of 1,2-acetonide-3-oleoyl glycerol using Amberlyst-15 in methanol produced 1-monoolein with a yield of 72.8%. However, *Novozym*-435 lipase enzyme catalyst is expensive and not economically recommended. Additionally, utilization of methanol in a deprotection reaction could produce a toxic 1-monoolein, and the esterification of oleic acid with 1,2-acetonide glycerol could give a low yield product because it is a reversible reaction. Jumina *et al.* [14] have successfully synthesized unsaturated 1-monolinolein via the protected glycerol as 1,2-O-isopropylidene glycerol (1,2-acetonide glycerol). Based on their studies, we proposed that the same method can also be applied to produce 1-monoolein from ethyl oleate.

In this work, 1-monoolein was firstly prepared through transesterification of ethyl oleate with 1,2-acetonide glycerol in the presence of sodium carbonate as a catalyst to produce 1,2-acetonide-3-oleoyl glycerol. The deprotection reaction of 1,2-acetonide-3-oleoyl glycerol was then conducted using Amberlyst-15 in ethanol to obtain 1-monoolein. Transesterification is preferred than esterification reaction because it is a direct reaction so that 1,2-acetonide-3-oleoyl glycerol could be afforded in a higher yield. Meanwhile, sodium carbonate catalyst is available abundantly, and utilization of ethanol as a solvent in a deprotection reaction enables to give a non-toxic 1-monoolein.

2. Experimental

2.1 Materials

Chemicals used in this experiment were ethyl oleate, 1,2-acetonide glycerol, sodium carbonate (Na₂CO₃), anhydrous sodium sulfate (Na₂SO₄), sodium hydrogen carbonate (NaHCO₃), Amberlyst-15, dichloromethane, n-hexane, chloroform, acetic, methanol, ethanol, and distilled water. All chemicals, except distilled water that was obtained from the Laboratory of Fundamental Chemistry Universitas Gadjah Mada, were purchased from E. Merck in analytical grade and were used without any further purification.

2.2 Instrumentation

Instruments used in this research were laboratory glassware and analytical mass balance (Mettler AT200). The ¹H— and ¹³C—NMR spectra were recorded at 500 MHz (¹H) and 125 MHz (¹³C) (JEOL JNM-MY spectrometer) using tetramethylsilane (TMS) as an internal standard. The chromatogram and mass spectra were measured on a Mariner Liquid Chromatography-Mass Spectrometry (LC-MS) using C-18 column (15 × 1 mm) and an injection volume of 2 μL. The mobile phase used was methanol with a flow rate of 0.1 mL/min.

2.3 Synthesis of 1,2-acetonide-3-oleoyl glycerol

Amount of 0.005 mol ethyl oleate (1.55 g) and 0.03 mol of 1,2-acetonide glycerol (3.96 g) was placed into three-neck round-bottomed flask that equipped with thermometer and condenser. To the mixture was added 5% Na₂CO₃ (w/v from the total of reactants) and then refluxed with stirring at 140 °C for 24 hours. After completion of the reaction, the mixture was dissolved in n-hexane and washed with distilled water. The organic layer was then dried using anhydrous Na₂SO₄ and evaporated to remove the solvent. The product of 1,2-acetonide-3-oleoyl glycerol with the highest purity was analyzed using GC-MS (Gas Chromatography-Mass Spectra).

2.4 Synthesis of 1-monoolein

Amount of 1,2-acetonide-3-oleoyl glycerol (0.0029 mol; MW: 396 g/mol; 1.14 g) was mixed with 0.43 g of Amberlyst-15 in ethanol (15 mL) and stirred at room temperature for 24 hours. After completion of the reaction, the mixture was evaporated to remove the ethanol and extracted using dichloromethane. The product was then analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS) to identify the formation of the target compound.

Purification of 1-monoolein was carried out by Preparative Thin Layer Chromatography (Prep. TLC) by dissolving 0.15 g of crude 1-monoolein in acetone and placed it on the TLC plate (20×20
cm). The separation was conducted using a mixed solvent of chloroform: acetone: methanol (9.5:0.45:0.05) as the eluent. The purification product was collected based on the visualization under a UV light lamp (265 nm). After it was dissolved in acetone, the product was evaporated and recrystallized from n-hexane to afford 1-monoolein. Characterizations of the purified 1-monoolein were conducted by $^1$H– and $^{13}$C–NMR Spectrometry.

3. Result and Discussion

3.1 Synthesis of 1,2-acetonide-3-oleoyl glycerol

Monoolein is a monoacylglycerol from oleic acid, which can be present as 1-monoolein and 2-monoolein. If the oleoyl group is attached to C number 2 (C2) from the glycerol framework, it will produce 2-monoolein, and if it is attached to C number 1 (C1) atoms, it will give 1-monoolein compound. Monoolein compounds are known to exhibit strong antioxidant activity as well as anti-arteriosclerotic. It also has a high nutritional function for humans.

There were some disadvantages in the synthesis of 1-monoolein from oleic acid that was performed by Wang et al. [13]. In their work, the Novozym-435 enzyme was used in the esterification reaction of oleic acid and 1,2-acetonide glycerol, which was costly. Some efforts are still needed in order to find a less costly synthesis pathway of 1-monoolein. One of the reaction pathways that is expected to provide a solution to this problem is the utilization of transesterification reactions. The transesterification is a direct reaction compared with esterification, which is an equilibrium reaction. Unidirectional or irreversible reactions are easier to handle because they require simpler reaction systems and produce more products. Therefore, in this work, the synthesis of 1-monoolein will be conducted through transesterification reaction of ethyl oleate and 1,2-acetonide glycerol using Na$_2$CO$_3$ as a catalyst. The utilization of Na$_2$CO$_3$ base catalysts was proposed to reduce the cost in the synthesis of 1-monoolein compared with Novozym-435 enzyme.

In this study, the transesterification reaction was carried out at 120 °C for 18 hours, with a 6:1 mol ratio between 1,2-acetonide glycerol and ethyl oleate. The color of the mixture was observed to turn to brownish when the reaction was stopped. Isolation of the 1,2-acetonide-3-oleoyl glycerol from the mixture was carried out in n-hexane and afforded the desired compound as a pale yellow liquid with a yield of 74%. The results of the analysis of 1,2-acetonide-3-oleoyl glycerol using GC-MS are presented in Figure 1 and Figure 2.

![Figure 1. Chromatogram of 1,2-acetonide-3-oleoyl glycerol](image_url)

Based on the chromatogram of the prepared 1,2-acetonide oleoyl glycerol in Figure 1, a single peak is present at a retention time of 75.44 minutes. The 1,2-acetonide-3-oleoyl glycerol compound relatively has more polar properties because of the double bond in the oleoyl chain. The mass
spectrum of the compound was presented in Figure 2 and showed a fragment with m/z=395, which was formed due to the release of 1 proton from the 1,2-acetonide-3-oleyl glycerol compound. The molecular ion of 1,2-acetonide-3-oleyl glycerol compound with m/z=396 did not appear because it was less stable. Fragments with m/z=257 appeared due to fragment release with the molecular formula C_{10}H_{19}* (m/z=139). The base peak that appears at m/z=183 was formed from the release of C_{3}H_{6}O_{2}* fragments (m/z=74).

![Figure 2. Mass spectra of 1,2-acetonide-3-oleyl glycerol](image)

### 3.2 Synthesis of 1-monoolein

The deprotection reaction by stirring of 1,2-acetonide-3-oleyl glycerol using Amberlyst-15 in ethanol at room temperature for 18 hours could give 1-monoolein compound and some side products such as oleic acid and ethyl oleate. Amberlyst-15 is a heterogeneous catalyst that can be separated from the reaction product through the filtration process. The final reaction product was then dissolved in dichloromethane due to the pH of the product solution was 4. The dichloromethane phase was then washed with 5% NaHCO_{3} solution (w/v) to reach neutral pH. After the evaporation, 1-monoolein was afforded as a soft white solid with a yield of 59%. The elucidation of the obtained 1-monoolein from the dichloromethane phase was conducted by LC-MS (Figure 3 and Figure 4).

![Figure 3. Chromatogram of 1-monoolein](image)

The chromatogram in Figure 3 showed the presence of 2 peaks with different retention times (R_{t}). The stationary phase used in the liquid chromatography was nonpolar (reversed-phase), so the polar compounds will pass through the column and eluted first. Based on this fact, it was proposed that the peak with a retention time of 2.8 minutes was 1-monoolein compound and will be eluted through a column first. Meanwhile, the peaks at a retention time of 5.1 minutes were predicted to be impurities. The relative content of the compound with an R_{t} of 2.8 minutes was 62.83%.
Figure 4. Mass spectra of a peak $R_t = 2.8$ min

The mass spectra in Figure 4 indicate the presence of 3 peaks of fragments (m/z) i.e. $M + H = 357.58; M + Na = 379.36; \text{ and } 2M + Na = 735.77$. These fragments referred to 1-monoolein compound with a molecular weight of 256 g/mol.

The TLC analysis showed that the two compounds in the chromatogram (Figure 3) could be separated using a mixture eluent of chloroform-acetone-methanol (0.95: 0.45: 0.005). The same solvent ratio was eventually used for the purification of 1-monoolein product by preparative-TLC, which gave a soft white solid (unstable) with 59% of yield and melting point around 35-37 °C.

The proton $^1H$-NMR (500 MHz, CDCl$_3$) spectrum of 1-monoolein was displayed in Figure 5. Characterization of each proton from the obtained compound was (δ ppm): 0.87 (3H, t, CH$_3$ of C$_{18}$), 1.23-1.29 (26H, m, CH$_2$ of C$_{4-8}$ and C$_{11-17}$), 1.58-1.62 (2H, m, CH$_2$ of C$_3$), 2.00 (2H, dd, CH$_2$ of -(OH)CH$_2$OCO-), 2.27 (2H, t, CH$_2$ of -CO-CH$_2$-CH$_2$), 2.61-2.75 (1H, m, -CH$_2$(OH)CH$_2$CH$_2$-), 4.11 (2H, dd, -(OH)CH$_2$OCO-), 5.33 (2H, s, (-CH=CH-)), 7.26 (2H, s, OH).
Figure 5. The $^1$H–NMR spectra of 1-monoolein

Peak A (0.87 ppm, 3H, triplet) was ascribed as the protons of the methyl group ($\text{-CH}_3$) at $C_{18}$. Peak B (1.23-1.29 ppm, 26H, multiplet) appeared as the resonance of methylene protons ($\text{-CH}_2$-$\text{CH}_2$) at $C_4$-$C_8$ and $C_{11}$-$C_{17}$ atoms from the oleoyl groups. Meanwhile, peak C (1.58-1.62 ppm, 2H, multiplet) was proposed as the methylene protons of the $C_3$ atom of the oleoyl group.

Peak D (2.00 ppm, 2H, $dd$) was interpreted as the resonance of two methylene protons of $\text{-}$(OH)CH-$\text{-CH}_2$-$\text{OCO}$, while peak E (2.27 ppm, 2H, triplet) was the peak of methylene proton of $\text{-CO-CH}_2$-$\text{CH}_2$- group. The multiplet peak F (1H) appeared from the resonance of methine proton of $\text{-CH}_2$-(OH)CH-$\text{CH}_2$- . Peak G (4.11 ppm, 2H, $dd$) as a doublet of doublet (dd) signal assigned as two methylene protons from $\text{-}$(OH)CH-$\text{-CH}_2$-$\text{OCO}$- group. A singlet peak H (5.33 ppm, 2H, singlet) and I (7.26 ppm, 2H, singlet) were interpreted as the proton signal of alkene ($\text{-CH=CH-}$) and hydroxy ($\text{-OH}$) group, respectively.

The $^{13}$C–NMR (125 MHz, CDCl$_3$) spectra of 1-monoolein in Figure 6 showed 14 carbon atom types with a different chemical environment. Generally, the electronic environment of each carbon atom can be presented as (6 ppm): 14.25 (C18), 22.70 (C17), 24.98 (C16), 27.17 (C15), 29.11 (C14, C13, C6, and C5), 29.18 (C7, C8, C11, and C12), 29.34 (C4), 29.69 (C3), 29.77 (C2), 31.92 ((OH)$\text{CH}_2$-$\text{CH}_2$-$\text{OCO}$), 34.37 ((OH)CH-$\text{-CH}_2$-$\text{OCO}$), 60.13 ((OH)CH-$\text{-CH}_2$-$\text{OCO}$), 129.98 ($\text{-CH}_2$-$\text{CH}=$-CH-$\text{CH}_2$), 173.86 (C=O).
Figure 6. The $^{13}$C NMR spectra of 1-monoolein

Peak A at a chemical shift ($\delta$) of 14.25 ppm was due to the methyl group (-CH$_2$-CH$_3$) at C$_{18}$, while peak B at 22.70 ppm was a signal carbon C$_{17}$ of the oleoyl group. Peak C and D that appeared at 24.98 and 27.17 ppm were assigned as the signal of atom C$_{16}$ and C$_{15}$ of the oleoyl group. Peak E at 29.11 ppm was interpreted as the signal of carbon C$_{14}$, C$_{13}$, C$_6$, and C$_3$ from the oleoyl group, while peak F at 29.18 ppm corresponded with atom C$_4$ on the same group. The signal of atom C$_7$, C$_8$, C$_{11}$, and C$_{12}$ (-CH$_2$-CH$_2$-CH=CH-CH$_2$-CH$_2$-) of the oleoyl group can be assigned as peak G at a chemical shift of 29.34 ppm. Peak H and I at 29.69 and 29.77 ppm respectively appeared as the signal of atom C$_3$ and C$_2$ of the oleoyl group.

Peak J and K appeared in a more downfield area (31.92 and 34.37 ppm) due to the signal of C atom at (OH)CH$_2$-(OH)CH-CH$_2$ and -(OH)CH-CH$_2$-OCO-, respectively. Peak L at 60.13 ppm was ascribed from C atom of -(OH)CH-CH$_2$-OCO-, while peak M at 129.98 ppm was alkene carbon of -CH$_2$-CH=CH-CH$_2$-. Peak N at the most downfield environment (173.86 ppm) was inferred as the C atom of the carbonyl group (-CO-).

This work showed that 1-monoolein could be successfully prepared through transesterification of 1,2-acetonide glycerol with ethyl oleate, followed by a deprotection reaction using Amberlyst-15. This reaction allows a low-cost and straightforward synthesis of 1-monoolein compared with the previously reported method by Wang et al. [13]. In this work, sodium carbonate was used as a catalyst in the transesterification reaction, instead of costly Novozym-435 enzyme. Moreover, the transesterification reaction of ethyl oleate could give a higher yield of 1-monoolein than the esterification of oleic acid by Wang et al. [13] because transesterification is a unidirectional reaction.

4. Conclusion

1-Monoolein could be produced from the reaction of ethyl oleate as a raw material with 1,2-acetonide glycerol (1:6 ratio mole) in the presence of sodium carbonate as a catalyst to obtain intermediate 1,2-acetonide-oleoyl glycerol as a pale yellow liquid (74%). Deprotection reaction of 1,2-acetonide-oleoyl glycerol using an Amberlyst-15 catalyst in ethanol could afford 1-monoolein as a fine white solid with a yield of 59%.
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References
[1] Nitbani F O, Jumina, Siswanta D and Sholikhah E N 2015 Reaction path synthesis of monoacylglycerol from fat and oils Int. J. Pharm. Sci. Rev. Res. 35(1) 126–36
[2] Zhong N, Li L, Xu X, Cheong L-Z, Li B, Hu S-Q and Zhao X 2009 An efficient binary solvent mixture for monoacylglycerol synthesis by enzymatic glycerolysis J. Am. Oil. Chem. Soc. 86 783–9
[3] Nitbani F O, Jumina, Siswanta D, Sholikhah E N and Fitria Astuti D 2018 Synthesis and antibacterial activity of 1-monolaurin Orient. J. Chem. 34(2) 863–67
[4] Nitbani F O, Jumina, Siswanta D, Sholikhah E N and Fitria Astuti D 2016 Synthesis and antibacterial activity of 2-monolaurin Orient. J. Chem. 32(6) 3113–20
[5] Krislee A, Fadly C, Nugrahaingsih D, Nuryastuti T, Nitbani F O, Jumina and Sholikhah E N 2019 The 1-Monolaurin inhibit growth and eradicate the biofilm formed by clinical isolates of Staphylococcus epidermidis BMC Proc. 13 (Suppl 11) 19 doi: 10.1186/s12919-019-0174-9
[6] Seleem D, Chen E, Benso B, Pardi V and Murata R M 2016 In vitro evaluation of antifungal activity of monolaurin against Candida albicans Biofilms Peer J. 4 e2148 doi: 10.7717/peerj.2148
[7] Thormar H and Hilmarsson H 2007 The role of microbicidal lipids in host defense against pathogens and their potential as therapeutic agents Chem. Phys. Lipids. 150(1) 1–11
[8] Nitbani F O, Jumina, Siswanta D and Sholikhah E N 2016 Synthesis and antibacterial activity test of 1-monocaprin Int. J. Pharm. Sci. Rev. Res. 39(16) 74–80
[9] Ma M, Wen X, Xie Y, Guo Z, Zhao R, Yu P, Gong D, Deng S and Zeng Z 2018 Antifungal activity and mechanism of monocaprin against food spoilage fungi Food Control. 84 561–8
[10] Jumina, Nurmla A, Fitria A, Pranowo D, Sholikhah E, Kurniaawan Y and Kuswandhi B 2018 Monomyristin and monopalmitin derivatives: Synthesis and evaluation as potential antibacterial and antifungal agents Molecules. 23(12) 3141
[11] Cho K-H, Hong J-H and Lee K-T 2010 Monoacylglycerol (MAG)-Oleic acid has stronger antioxidant, anti-atherosclerotic, and protein glycation inhibitory activities than MAG-Palmitic Acid J. Med. Food. 13(1) 99–107
[12] Feltes M M C, Villeneuve P, Baréa B, Barouh N, de Oliveira J V, de Oliveira D and Ninow J L 2012 Enzymatic production of Monoacylglycerols (MAG) and Diacylglycerols (DAG) from fish oil in a solvent-free system J. Am. Oil. Chem. Soc. 89(6) 1057–65
[13] Wang X, Jin Q, Wang T, Huang J and Wang X 2013 An improved method for the synthesis of 1-monoolein J. Mol. Catal. B. Enzym. 97 130–6
[14] Jumina J, Lavendi W, Singgih T, Triono S, Kurniawan Y S and Koketsu M 2019 Preparation of monoacylglycerol derivatives from Indonesian edible oil and their antimicrobial assay against Staphylococcus aureus and Escherichia coli Sci. Rep. 9(1) 1–8