Research Article

Synthesis and Antibacterial Activity Test of 1-Monocaprin

Febri Odel Nitbani[1,3*], Jumina[1], Dwi Siswanta[1], Eti Nurwening Sholikhah[2]
[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia.
[2]Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Gadjah Mada Yogyakarta, Indonesia.
[3]Department of Chemistry, Faculty of Science and Engeneering, Universitas Nusa Cendana, Kupang, Indonesia.
***Corresponding author's E-mail:** febri_nitbani@yahoo.com

Accepted on: 03-05-2016; **Finalized on:** 30-06-2016.

ABSTRACT

An antibacterial compound, 1-monocaprin, has been successfully synthesized through a reaction pathway that involved the use of protected glycerol compound, 1,2-acetonide glycerol. It was found that 1,2-acetonide glycerol could be obtained through a reaction between glycerol and acetone with the use of para-toluene sulfonic acid as an acidic catalyst with 99.07% purity. A transesterification reaction without solvent between 1,2-acetonide glycerol and ethyl caprate with mole ratio of 8, using a base catalyst Na_2CO_3 was able to produce 1,2-acetonide-3-capryl glycerol. The product of this reaction was in the form of yellow viscous liquid with 87% purity. Deprotection reaction of 1,2-acetonide-3-capryl glycerol with Amberlyst-15 in ethanol, followed by a purification step by using a preparative thin layer chromatography can result in the production of 100% white solid 1-monocaprin. Further study showed that 1-monocaprin compound was capable of inhibiting the growth of *Bacillus cereus*, *Salmonella thypimurium* and *Escherichia coli* bacteria at a concentration of 1000 µg/mL and *Staphylococus aureus* at a concentration of 500 µg/mL.

Keywords: 1-Monocaprin, Synthesis, Antibacterial Activity.

INTRODUCTION

Lipid compounds which consist of an acyl group of fatty acid and two hydroxyl groups have been widely known as mono acyl glycerol or monoglyceride. Due to the presence of both hydrophilic and hydrophobic functional groups in its chemical structure, mono acyl glycerol can be utilized either as a non-ionic or as an amphiphilic surfactant[1,2]. Until recently, mono acyl glycerol is the most popular compound utilized as an emulsifier in food, cosmetic and pharmacology industries[3-8]. Monoglyceride containing medium chain fatty acids has been reported to play important role in medical and nutritional application such as in treating a number of medical ailments, which are due to the damage of lipid metabolism. This compound has also been reported to be useful to stimulate baby growth and physiological development of baby[9].

Recent research has shown that monoglyceride of capric acid known chemically as 1-monocaprin and commercially as GRAS food additive[6], has been used as antimicrobial agent against food-borne infection bacteria such as *Campylobacter jejuni, Salmonella* spp., and *Escherichia coli*[10] and antiviral agent against HIV[6]. It has also been described that capric acid and its ester derivate such as ethyl capric have been successfully used in the production of 1-monocaprin and 2-monocaprin. Either capric acid or ethyl capric can be synthesized from different kind of vegetable oils such as crude coconut oil through a high-pressure hydrolysis reaction or transesterification. Capric acid content, at the end of this reaction, can be determined through a base-catalyzed transesterification reaction of coconut oil in the form of methyl capric. In Indonesia, it is generally known that capric acid content in the form of methyl capric in coconut oil is 9, 19%[11]. This compound can be separated from its mixture by using fractional distillation.

Usually, commercial mono acyl glycerol is produced through the glycerolysis reaction of vegetable oil or fats with the use of inorganic base as the catalyst at 200–260°C and is further purified through a molecular distillation technique. However, this method has several weaknesses where sometimes it results in the production of mono acyl glycerol, which has a very dark color with burning smell. This method also requires a very high energy because it takes place at high temperatures; therefore this method is not suitable to be applied in synthesizing heat-sensitive mono acyl glycerol[12]. In addition to this method, there have also been developed several other methods for synthesizing mono acyl glycerol such as enzymatic glycerolysis of vegetable oils and fats, transesterification of an ester with fatty acid, alcoholysis of vegetable oils or fats, enzymatic or chemical induced esterification of free fatty acids with glycerol, and transesterification of an ester fatty acid with 1,2-acetonide glycerol[13].

Recent report has shown that there is lack of study conducted to study about both synthesis and application of monocaprin as an antibacterial agent. It has just been reported that lipid acyl hydrolase (LAH) enzyme has been successfully applied as a catalyst in the esterification of capric acid with glycerol, where it produces more than 95% mole of monocaprin[14]. A new report has indicated

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

that the reaction between capric acid and glycerol at a molar ratio of 1 to 1, catalyzed by 9% (w/w) of lipase enzyme at 55°C can generate more than 60% molar fraction of monocaprin with 70% selectivity[3]. Different study on the esterification of capric acid and glycerol catalyzed by carboxylesterase enzyme isolated from *Calotropisprocera* R. Br. has also been successfully conducted[6]. This reaction was carried out in a reversed micellar system using (Bis (2-ethylhexyl) sodium sulfosuccinate) as the surfactant and isooctane as the solvent to homogenize the reaction system. As a result, 1-monocaprin can be produced with more than 80% yield.

Currently, there have also been reports on the weaknesses of enzymatic synthesis of monocaprine through esterification of capric acid and glycerol. Firstly, the formation of 1,2-dicaprin and also tricaprin in addition to monocaprin as a result of the migration of acyl groups at the other hydroxyl groups from glycerol. Secondly, it is difficult to obtain monocaprin with very high purity since the reaction between capric acid and glycerol is an equilibrium reaction. Thirdly, there is an additional requirement to make this reaction more efficient by homogenizing the reaction system through the addition of either surfactant or organic solvents.

In order to overcome these shortcomings, there has been developed a new method of synthesizing 1-monocaprin by the use of 1,2-acetonide glycerol. This compound is actually a typical of glycerol, which is protected at its two-hydroxyl groups bound to C_1 and C_2 through a reaction between glycerol and acetone in the presence of acidic catalyst. The transesterification of ethyl caprate with 1,2-acetonide glycerol can be done without any additional solvent and is catalyzed by a base catalyst to produce 1,2-acetonide-3-capryl glycerol. Further step to generate 1-monocaprin is done by deprotecting of 1,2-acetonide-3-capryl glycerol using Amberlyst-15 as the catalyst. The advantage of applying this method is that it can prevent the occurrence of acyl group migration and equilibrium of the reaction, hence resulting in the increase of monocaprin yield product. Moreover, the transesterification of ethyl capric with 1,2-acetonide glycerol can be completed without any additional solvents.

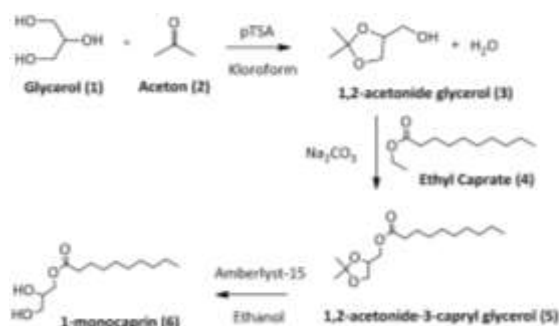

Figure 1: Reaction scheme synthesis of 1-monocaprin

Figure 1 shows the typical synthesis pathway of 1-monocaprin. The resulting 1-monocaprin is further tested its antibacterial activity against either gram positive or gram negative bacteria such as *Staphylococus aureus*, *Bacillus cereus, Salmonella thypimurium*, and *Eschericia coli*.

MATERIALS AND METHODS

Materials

Materials used in this research are ethyl caprate atau ethyl decanoate (Sigma Aldrich), glycerol, Na_2CO_3, para toluene sulfonic acid (pTSA), amberlyst-15, n-hexane, ethanol, dichloromethane, acetone, chloroform, DMSO, anhydrous sodium sulfate, agar nutrient, tetracycline, and some test bacteria such as *Staphylocous aureus*, *Salmonella thypimorium*, *Bacillus cereus, and Eschericia coli*.

Apparatus

There are several important tools used in this research including a set of reflux tools, silica gel plate for preparative TLC, evaporator, GC (Shimadzu), LC-MS (Mariner Biospectrometry), and NMR (JEOL ECS-400 ,400 Mhz).

Procedures

Synthesis of 1,2-acetonide glycerol

The synthesis of 1,2-acetonide glycerol in this research was based on the procedure developed by Yu with minor modification[15]. The 0.01 mole glycerol, 0.01 mole of acetone, 0.6 gram of pTSA were mixed in 50 mL chloroform in a three-neck flask which has already been connected with a condenser and equipped with a thermometer. The mixture was continuously refluxed for 6 hours after which its non-polar phase was neutralized by using $NaHCO_3$ solution (5% (w/v)) and the remaining chloroform solvent was separated from the mixture through evaporation. The resulting 1,2-acetonide glycerol was further purified by using fractional distillation technique where the purified fraction was collected at the temperature range of 78 to 80 °C.

Synthesis of 1,2-acetonide-3-capryl glycerol

A total of 0.005 mole of ethyl caprate (MW = 200,32 g/mol; 1,0016 g), 0.01 mol of 1,2-acetonide glycerol (MW = 130,43 g/mol; 1,3 g) and 5% (w/w) of Na_2CO_3 were mixed in a three neck flask which was connected with a condenser and equipped with a thermometer. After continuously refluxed at 140 °C for 24 hours, the mixture was then successively dissolved in n-hexane, washed with aquadest and dried with anhydrous sodium sulfate. The remaining solvents were then separated from the mixture by evaporation. Similar steps were also conducted to optimize the reaction by varying the mole ratio of 1,2-acetonide glycerol to ethyl capric from 4, 6 and 8. The product was further analyzed by using Gas Chromatography and the product with the highest percentage of 1,2-acetonide-3-capryl glycerol was analyzed using ^{1}H NMR dan^{13}C NMR Spectrometer.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Synthesis of 1-monocaprin

Initially, a total 0.0025 mole of 1,2-acetonide-3-capryl glycerol (BM = 286 g/mol; 0,71 g) was mixed 0.18 g of Amberlyst in a 10 mL of ethanol as the solvent. The reaction was allowed to complete at room temperature for 24 hours and the product was subsequently extracted with dichloromethane. After recrystallized in an n-hexane, crude 1-monocaprin was further analyzed by using thin layer chromatography. The mixture of n-hexane and ethyl acetate was used as an eluent while iodine vapour was used to visualize the spot. Briefly, the procedure of thin layer chromatography can be described as follow. 0.15 gram of the resulting 1-monocaprin was dissolved in a required volume of acetone and the solution was evenly dropped on the preparative silica plate. After completely eluted in a 20×20 chamber by the mixture of n-hexane and ethyl acetate, the preparative TLC plate was the visualized under a 265 nm UV lamp. The resulting dominant spot at the bottom of the plate was dried, dissolved in acetone and then filtrated. The filtrate was completely evaporated to separate the remaining solvent and the result was recrystallized with n-hexane. The 1-monocaprin product was finally analyzed by using LC-MS, ^{1}H NMR and ^{13}C NMR Spectrometer.

Antibacterial Activity Test

The antibacterial activity of the resulting 1-monocaprin was tested with double layer method. The initial step was to prepare both the solid and semi-solid triton soya agar (TSA), which were subsequently used as the media. Culture Liquid which has been cultivated for 24 hours and have at least 10^8 cell/mL was mixed with 1% of semi solid media at the temperature of 45°C. The mixture was further mixed with the solid media. Sterile paper discs (Ø= 6 mm) were placed on semi-solid TSA media, which had been previously solidified. Samples of each bacteria with variuous concentration were prepared in 20 µL and were dropped onto each sterile paper disc. Tetracycline and DMSO were respectively used as positive and negative controls. All the cultures then were incubated for 24 hours at 37 °C, and their developed clear zone were observed and measured as their inhibitory activity against target bacteria.

RESULTS AND DISCUSSION

Synthesis of 1,2-acetonide glycerol

It has been previously stated that 1,2-acetonide glycerol (**3**) or 1,2-*O*-isopropylidene glycerol is a typical of protected glycerol, which is popularly known in the synthesis of mono acyl glycerol.

This compound can be synthesized through the reaction of glycerol, which has three hydroxyl groups, and acetone, which has one group of carbonyl. This reaction can be accelerated by the use of pTSA as an acid catalyst. This reaction initially occurs through the protonation of the carbonyl group of acetone by a proton from the pTSA and followed by the hydroxyl group attack on the partial positive C of acetone to form hemicetal compound. This reaction continues to occur through the formation of ketal compound of 1,2-acetonide glycerol by the utilizing the other hydroxyl group that is attach to C_2 of the glycerol.

In this research, the synthesis of 1,2-acetonide glycerol was done through the reaction of glycerol and acetone (1:1) under a continuous heating and with the used 5% (w/w) pTSA as the catalyst in chloroform as the solvent. The use of chloroform in this reaction was intended to limit the amount of acetone required to actually react with 1 mole of glycerol in the formation of acetonide glycerol. This reaction was allowed to occur for 6 hours at the temperature similar to that of the solvent. The resulting product was then neutralized with sodium bicarbonate and the non-polar phase was separated. It was washed with aquadest until reaching neutral pH and the remaining chloroform was separated through evaporation. The product was purified by using fractional distillation and the distillate was collected at the temperature of 78-80°C where yellowish and viscous 1,2-acetonide glycerol was obtained with the yield of 66.7%.

Further analysis of the reaction product of 1,2-acetonide glycerol using IR Spectrometer (**supplementary data 1**) showed the appearance of its typical absorbance such as a wide absorbance with moderate intensity at wave number 3433.62 cm-1 which was due to the vibration of the hydroxyl group (-OH). Some other absorbance can also be found on the diagram such as those around 2900 and 2800 cm^{-1} which were due to the vibration of C-H sp3 and around 1373,32 and 1458,18 cm^{-1} which were due to the presence of $-CH_3$ and $-CH_2$. The absorbance in the area of 1049,28 cm^{-1} showed the span of C-O-C groups.

Figure 2 shows the result of analysis using Gas Chromatography-Mass Spectroscopy, which indicated a dominant peak at a retention time of 6.82 minute with a relative yield of 99.07%. This peak is estimated as the peak of 1,2-acetonide glycerol compound having a molecular weight of 132 g/mol.

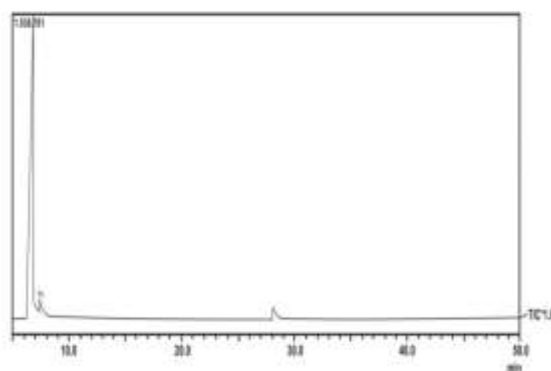

Figure 2: Chromatogram of 1,2-acetonide glycerol compound

Mass spectral data (**supplementary data 2**) indicated the emergence of several fragments at m/z = 117, 101, 72, 57 and the base peak at m/z = 43. These peaks are the result

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

of the fragmentation of 1,2-acetonide glycerol which show a 97% similarity with the fragmentation peaks of 1,2-acetonide glycerol from the data base. The combined results of IR spectroscopic data interpretation, chromatogram and mass spectra clearly revealed that the product of the reaction between glycerol and acetone in the presence of a pTSA catalyst in chloroform is 1,2-acetonide glycerol with a purity of 99.07%.

Synthesis of 1,2-acetonide-3-capryl glycerol

The synthesis of 1,2-acetonide-3-capryl glycerol (**5**) was conducted through a transesterification reaction of ethyl caprate (**4**) with 1,2-glycerol acetonide (**3**) utilizing Na_2CO_3 as the catalyst without any additional solvent. The use of ethyl caprate was preferred over methyl caprate in this reactant because ethyl caprate produces ethanol as its byproduct, which is considered to be less toxic to the main product compared to methanol which is produced when methyl caprate is used. The optimization of the synthesis of 1,2-acetonide-3-capryl glycerol (**5**) is done by varying the mole ratio between 1,2-acetonide glycerol and ethyl caprate such as 2,4,6 and 8. This process was done on the fixed amount of catalyst, temperature and reaction time. Based on Gas Chromatography data, it was found that compound **5** could be produced through this reaction path with optimum purity of 87% (**supplementary data 3**). The product of this reaction was in the form of yellow viscous liquid with a yield of 88.12%. This result was achieved when the mole ratio of 1,2-acetonide glycerol to ethyl caprate was 8 to 1, the amount of Na_2CO_3 catalyst was 5% (w/w) of the total reactants, the temperature was 140 °C and a reaction time was 24 hours.

Figure 3 shows the result of ^{1}H NMR analysis of compound **5**.

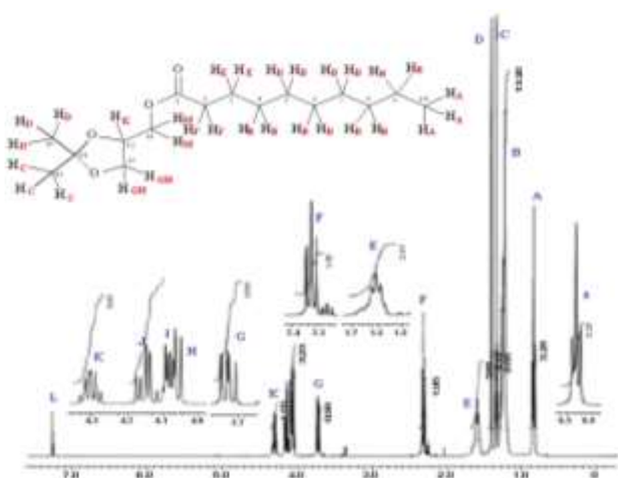

Figure 3: ^{1}H NMR spectra of 1,2-acetonide-3-capryl glycerol

It can be seen from the figure that A (δ_H of 0,865 ppm, 3H, triplet) which can be attributed to the chemical shift of methyl protons at -CH$_2$-**CH$_3$** group**.** The figure also shows a B peak (δ_H = 1,248 ppm, 12H, singlet) which confirmed the chemical shift of the combined methylene protons (-CH$_2$-**CH$_2$-)** from C_4 to C_9 of capryl group. C peak (δ_H = 1,362 ppm, 3H, singlet) and D peak (δ_H = 1,426 ppm, 3H, singlet) clearly confirmed a certain chemical shift, which was due to the presence of 2 methyl (-**CH$_3$**-C-) at C_{16} and C_{15} from acetonide group. The presence of E (δ_H = 1,575-1,667 ppm, 2H, multiplet) and F (δ_H = 2,333 ppm, 2H, triplet) peaks can be revealed by the chemical shift of methylene protons of C_3 (-CO-CH$_2$-**CH$_2$**-) and C_2 (-CO-**CH$_2$**-CH$_2$-) from capryl group. G peak (δ_H = 3,729 ppm, 1H, double of doublet) and H peak (δ_H = 4,069 ppm, 1H, double of doublet) peaks indicated the chemical shift of each two methylene protons (–(OH)CH-**CH$_2$**-O-CO-) at C_{13} of acetonide group. The chemical shift of 2 protons at C_{11} of **CH$_2$**-(OH)CH-CH$_2$- groups were clearly confirmed by the appearance of I peak (δ_H = 4,085 ppm, 1H, double of doublet) and J peak (δ_H = 4,159 ppm, 1H, double of doublet). The remaining K peak(δ_H = 4,279-4,338 ppm, 1H, multiplet) confirmed the presence of the chemical shift of protons at C_{12} of –CH$_2$-(OH)C**H**-CH$_2$- group. L peak (δ_H = 7,260 ppm, 1H, singlet) confirmed the presence of the proton of hydroxy group from unreacted 1,2-acetonide glycerol.

The result of further analysis using ^{13}C NMR spectrometer is shown on figure 4.

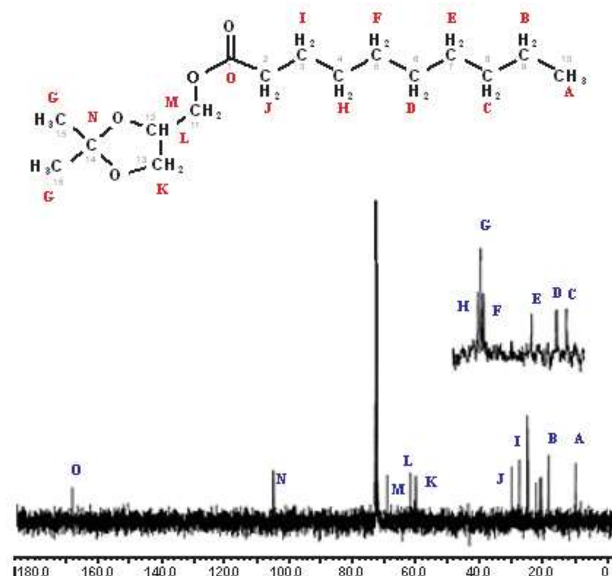

Figure 4: ^{13}C NMR spectra of 1,2-acetonida-3-capryl glycerol

It is seen that A peak can be found at δ = 9,221 ppm and represents a chemical shift of an upfield methyl group at C_{10}. Several other peaks including B, C, D, E, F, H and I can be found respectively at δ = 17,764; 19,976; 20,482; 21,769; 24,210; 24,505; 26,956; 29,206 ppm and were a respective chemical shifts of methylene carbon (-CH$_2$-**C**H$_2$-) of C_9, C_8, C_7, C_6, C_5, C_4, C_3 and C_2 of capryl group. The appearance of G peak at δ = 24,353 ppm confirmed the chemical shift of 2 C_{15} of 2 methyl groups from acetonide. K peak found at δ = 59,612 ppm revealed the chemical shift of C_{13} of (O)**C**H$_2$-(O)CH-CH$_2$- group. A chemical shift of C12 from-(O)**C**H-CH$_2$-OCO- group can be confirmed by the presence of peak at δ = 61,414 ppm. The appearance

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

of M peak at δ = 68,736 ppm revealed a downfield chemical shift of C_{11} from -(O)CH-**CH**$_2$-OCO- group which was due to the influence of carbonyl group. N peak which is found at δ = 104,920 ppm clearly indicates the chemical shift of C_{14} from $(CH_3)_2$-**C**$(O)_2$- group. Finally, O peak at δ = 169,792 ppm showed the chemical shift of carbonyl group (-**C**O-).

Synthesis of 1-monocaprin

The synthesis of 1-monocaprin (**6**) was conducted by deprotecting a 1,2-acetonide-3-capryl glycerol (**5**) with the use of Amberlyst-15 as the catalyst and ethanol as the solvent. The result indicated that 1-monocaprin produced through this reaction was in a white solid form with a yield of 88,67%. The result of Thin Layer Chromatography analysis using n-hexane and ethyl acetate in the ratio of 3 to 1 as a mobile phase and iodine vapor as the spot visualizer showed that the resulting 1-monocaprin products still contains several impurities such as capric acid and ethyl caprate. Both compounds may be formed during the deprotection of compounds 5 using Amberlyst-15 in ethanol. After the purification step of the resulting product using a mixture of n-hexane and ethyl acetate in the ration of 3 to 1, it was found that 1-monocaprin product was in a white solid form with the yield of 78,34%. Furthermore, the analysis result indicated that this product has a melting point at 53 °C.

The result of further analysis of compound 6 using Liquid Chromatography-Mass Spectroscopy (LC-MS) shown by chromatogram on figure 5 indicated a single peak at a retention time of 5.7 minutes. Mass spectra as shown in **the supplementary data 4** showed the emergence of two important fragments which are M+Na = 269.41 and 2M+Na = 515.83. These fragments clearly suggested the the formation of 1-monocaprin (**6**) with a molecular weight of of 246.34 g/mol as the product.

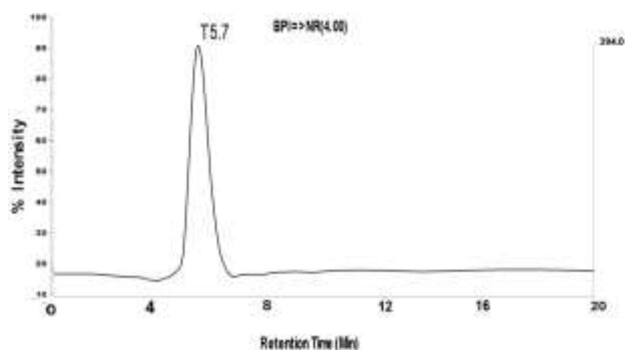

Figure 5: LC-MS chromatogram of 1-monocaprin

Figure 6 shows the result of ^1H NMR analysis of 1-monocaprin. As seen from figure 6, the ^1H NMR chromatogram showed several peaks, which indicated different kinds of chemical shifts. A peak (δ_H = 0,861 ppm, triplet, 3H) which confirmed the chemical shift of methyl protons (-C**H**$_3$**)** of C_{10}. B peak (δ_H = 1,267 ppm, singlet, 12H) represented the combined chemical shift of methylene (-CH_2-**CH**$_2$-) protons which were bound to C_4 – C_9 of capryl group. The appearance of C peak (δ_H = 1,576-1,649 ppm, 2H multiplet) which confirmed the chemical shift of methylene protons bound to C_3 of capryl group. D peak (δ_H = 2,336 ppm, 2H, triplet) clearly confirmed the chemical shift of methylene protons which were bound to C_2 of capryl group.

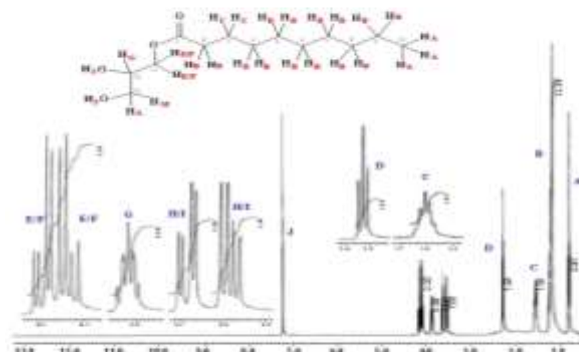

Figure 6: ^1H NMR spectra of 1-monocaprin

The appearance of E (δ_H = 4,133 ppm, 1H, double of doublet) and F (δ_H = 4,195 ppm, 1H, double of doublet) peaks represents the chemical shifts of methylene protons of C_{11} from –(OH)CH-**CH**$_2$-OCO-group. These protons showed a more downfield chemical shifts due to the attraction of the electrons by the ester carbonyl group. The appearance of G peak (δ_H = 3,893-3,944 ppm, 1H, multiplet) revealed the chemical shift of protons of C_{12} from –CH_2-(OH)C**H**-CH_2- group. The presence of H (δ_H = 3,584 ppm, 1H, double of doublet) and I (δ_H = 3,683 ppm, 1H, double of doublet) peaks were due to the chemical shifts of two methylene protons of C_{13} from –**CH**$_2$-(OH)CH-CH_2- group. Finally J peak (δ_H = 7,246 ppm, 2H, singlet) confirmed the chemical shift of hydroxy group proton (–O**H)**.

Figure 7 shows the chromatogram of ^{13}C NMR of compound **6**.

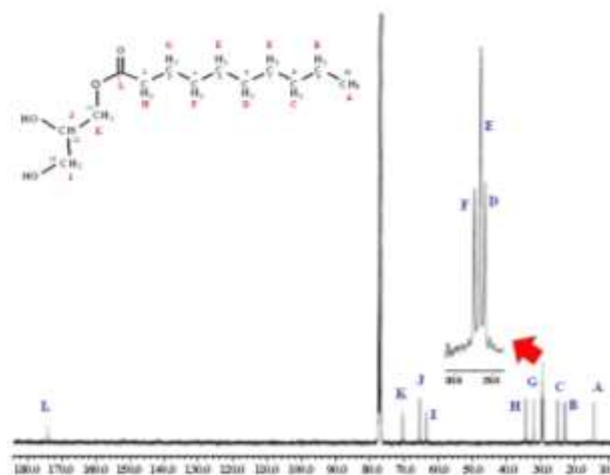

Figure 7: ^{13}C NMR spectra of 1-monocaprin

The result of ^{13}C NMR analysis also indicated several peaks, which revealed the presence of 1-monocaprine as a reaction product. As it is seen, A peak found at δ = 14,208 ppm clearly showed the carbon chemical shift of C_{10} methyl (-CH_2-**C**H_3) carbon. Differently, the appearance of consecutive B, C, D, E, F, G, and H peaks at δ = 22,751;

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

24,991; 29,330; 29,206; 29,330; 29,482; 31,993; and 34,240 ppm was due to the carbon chemical shift of methylene (-CH_2-**C**H_2-) carbon of C_9, C_8, C_7, C_6, C_5, C_4, C_3dan C_2 of capryl group. I peak, moreover, appeared at δ = 63,368 ppm clearly showed the chemical shift of C_{13} carbon of -**C**H_2-(OH)CH-CH_2-) group. J peak found at δ = 65,246 ppm revealed the chemical shift of C_{12} carbon of -(OH)**C**H-CH_2-OCO- group. Subsequently, the appearance of K peak at δ = 70,319 ppm was due to the more downfield chemical shift of methylene carbon of -(OH)CH-**C**H_2-OCO- group which was due to the chemical attraction of electron by carbonyl group. Subsequently, the presence of L peak at δ = 174,484 ppm was due to the chemical shift of carbon from the carbonyl (-**C**O-) group.

The combined result of LC-MS, ^{1}H NMR and ^{13}C NMR strongly suggested that the deprotection reaction product was 1-monocaprin with the yield of 100%. As such, this reaction pathway can be suggested as one of the reaction pathways for the synthesis of 1-monocaprin. This included the use of a protected glycerol compound such as 1,2-acetonide glycerol. The advantages of synthesizing 1-monocaprin through this proposed method is that it can result in the production of 1-monocaprin with high yield and purity. Moreover, this reaction can take place without any additional solvent and utilizing biodiesel by product from vegetable oil, which is glycerol as the main reactant.

Antibacterial Activity of 1-monocaprin

Chemically, 1-moncaprin (**6**) consists of two hydroxyl group and one acyl group from capric acid. The hydroxyl group are polar while the capryl group is non-polar, which provide the 1-monocaprin with a high potential to chemically interact with the bacterial cell walls either gram positive or negative bacteria by damaging their wall cell. Therefore the growth of the bacteria will be hampered after the destruction of bacterial cell wall. In this research, the resulting 1-monocaprin compound was tested its antibacterial activity against several gram positive bacteria including *Staphylococus aureus* and *Bacillus cereus* and some gram negative bacteria such as *Salmonella thypimurium* and *Eschericia coli.* All the bacteria used in this experiment were wild type which was obtained from isolation. The result of antibacterial activity test of 1-monocaprin against these bacteria is shown in table 1.

Table 1: Inhibitory Diameter Zone

Bacterial test	Diameter of inhibiting zone (mm)					
	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	Tetrasiklin 1000 µg/mL	DMSO
Staphylococcus aureus	25	8	-	-	35	-
Bacilluscereus	25	-	-	-	25	-
Salmonella thypimurium	10	-	-	-	25	-
Escherichia coli	10	-	-	-	23	-

The data in Table 1 indicate that 1-monocaprin is able to inhibit the growth of both gram-positive and gram-negative bacteria at a concentration of 1000 µg/mL. However at similar concentration, its activity against the test bacteria is still lower than that of the tetracycline.

It is shown that the ability of 1-monocaprin to inhibit gram positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus* is higher than that of the gram positive bacteria such as *Salmonella thypimurium* and *Escherichia coli*.

This trend can be attributed tothecell wall structure of gram-positive bacteria which consist of only two layers compared to that of the gram-negative bacteria.

Therefore, at the same concentration, it is much easier for 1-monocaprin to interact and damage the cell wall of gram-positive bacteria than that of the gram-negative bacteria.

It was also noticed that 1-monocaprin can inhibith the growth of *Staphylococcus aureus* at a concentration lower than 1000 µg/mL which is 500 µg/mL. Subsequently, it can be concluded that the 1-monocaprin is able to inhibit the growth of all the tested bacteria at a concentration of 1000 µg/mL and also able to inhibit the growth of *Staphylococcus aureus* at a concentration of 500 µg/mL.

CONCLUSION

1. 1,2-acetonide glycerol with a purity of 99.07% can be produced through the reaction of 1 mmol glycerol and 1 mmol acetone with the use of pTSA as the catalyst.
2. The reaction of 1 mmol of ethyl capric and 8 mmol of 1,2-acetonide glycerol with the use of Na_2CO_3 as the catalyst without any additional solvent can result in the production of 87% purity of 1,2-acetonide-3-capryl glycerol.
3. 1-monocaprin with the purity of 100% with the yield of 78,34% can be produced through the deprotection of 1,2-acetonide-3-capryl glycerol with the use of Amberlyst-15 as the catalyst and preparative thin layer chromatography as the purification techniques.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

4. 1-monocaprin is able to inhibit the growth of *Bacillus cereus, Salmonella thypimurium*, and *Eschericia coli* at a concentration of 1000 µg/mL and also able to inhibit the growth of *Staphylococcus aureus* at a concentration of 500 µg/mL.

Acknowledgement: The authors deeply thank to the Directorate of Research and Development, The Ministry of Research Technology and Higher Education, The Republic of Indonesia who has financially supported this research through PUPT Research Grant Program budget year 2016.

REFERENCES

1. Wang X, Jina Q, Wang T, Huang J, Wanga X, An improved method for the synthesis of 1-monoolein, *J. Mol. Catal. B: Enzym.,* 97, 2013, 130-136
2. Wang X, Li M, Wang T, Jin Q, Wang X, An improved method for the synthesis of 2-arachidonoylglycerol, Process Biochemistry, 49, 2014, 1415–1421
3. Langone MAP, Dee Abreu ME, Rezende MDC, Sant'anna GLJR, Enzymatic Synthesis of Medium ChainMonoglycerides in a Solvent-Free System, *Appl. Biochem. Biotechnol.*, 2002, Vols. 98–100
4. Pereira CCB, Silva MAP, Langone MAP, Enzymatic Synthesis of Monolaurin, *J. Appl. Biochem. Biotech.*, 114, 2004, 433-446.
5. Ferretti CA, Apesteguia CR, Cosimo JID, MgO-based catalysts for monoglyceride synthesis from methyl oleate and glycerol: Effect of Li promotion, Applied Catalysis A: General, 399, 2011, 146-153.
6. Park KM, Kwon OT, Ahn SM, Lee JH, Chang PS, Characterization and Optimization of carboxylesterase-catalyzed esterification between capric acid and glycerol for the production of 1-monocaprin in reversed micellar system, New Biotechnology, 27(1), 2010, 46-52.
7. Cetina DM, Giraldo GI, Orrego CE, Application of response surface design to solvent, temperature and lipase selection for optimal monoglyceride production, Journal of Molecular Catalysis B: Enzymatic, 72, 2011, 13-19.
8. Tai HP, Brunner G, Mono- and di-acylglycerol synthesis in CO2-expanded acetone, *J. of Supercritical Fluids*, 59, 2011, 87-91.
9. Nandi S, Gangopadhyay S, GhoshSProduction of Medium Chain Glycrides From Coconut and Palm Kernel Fatty Acid Distillates by Lipase-Catalyzed Reactions, *Enzyme Microb. Technol.,* 36, 2005, 725-728.
10. Thormar H, Hilmarsson H, Bergsson G, Stable Concentrated Emulsions of the 1-Monoglyceride of Capric Acid (Monocaprin) with Microbicidal Activities against the Food-Borne Bacteria *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli*, Appl. Environ. Microbiol., 72(1), 2006, 522–526.
11. Jumina, Siswanta D, Purnomo, S, Inovasi Teknologi Produksi Monolaurin dari Minyak Kelapa, Uji Aktivitasnya sebagai Immuno Stimulant, and *Scalling Up* Produksinya pada Reaktor Pilot Plant, *Laporan Hibah PUPT*, 2014, DP2M-DIKTI, Jakarta.
12. Naik MK, Naik SN, Mohanty S, Enzymatic glycerolysis for conversion of sunflower oil tofood based emulsifiers, *Catalysis Today*, 237, 2013, 145-149.
13. Nitbani FO, Jumina, Siswanta D, Sholikhah EN, Reaction Path Synthesis of Monoacylglycerol from Fat and Oils: A Review, *Int. J. Pharm. Sci. Rev. Res.*, November-Desember, 35(1), 2015, 126-136.
14. Macrae AR, Visicchio JE, Lanot A, Application of Potato Lipid Acyl Hydrolase for the Synthesis of Monoacylglycerols, *J. Am.Oil Chem. Soc.*, 75(11), 1998, 1489–1494.
15. Yu CC, Lee YS, Cheon BS, Lee SH, Synthesis of glycerol monostearate with high purity, *Bull. Korean Chem. Soc.*, 24(8), 2003, 1229-1231.

Source of Support: Nil, **Conflict of Interest:** None.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.