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**RESEARCH NOTE**

# Selective Synthesis of Monolaurin: a Preliminary Investigation

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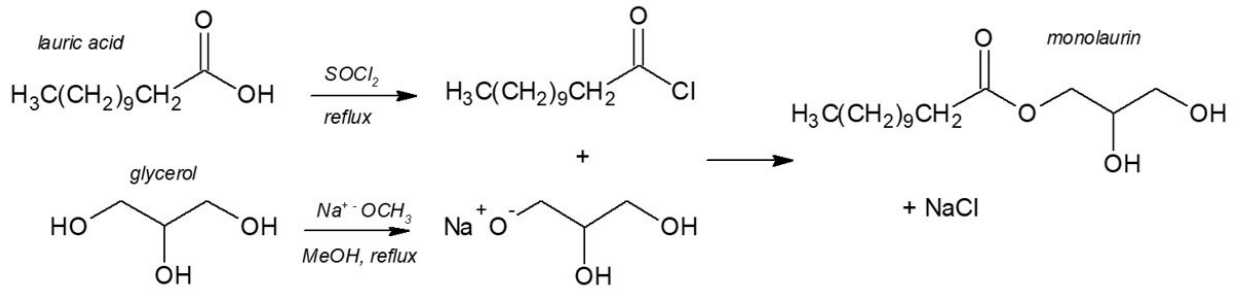
**Monolaurin (ML) is a monoacylglycerol (MAG) with important industrial and biomedical properties. It is commonly prepared in large quantities from the direct esterification of lauric acid (LA) and glycerol with chemical catalysts. However, this method also generates other glyceride by-products whose properties are inferior to ML. Enzymes have also been used to optimize selectivity, but enhancing scalability and throughput is always challenging. Successful selective convergent chemical synthesis of ML involving activated derivatives of LA and glycerol is demonstrated in this study. The present yield of 3.41% can be improved with careful process control.**

Keywords: monolaurin, monoacylglyceride, medium-chain fatty acid, selective synthesis, sodium glyceroxide

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Monolaurin (IUPAC: 2,3-dihydroxypropyl dodecanoate, also commonly called 1-monolaurin) is a monoacylglycerol (MAG) of lauric acid (LA), a medium-chain fatty acid (MCFA) with 12 carbons, which has important biomedical and industrial properties. LA was found to exhibit activity against some Gram-negative (Bergsson *et al.* 2001, 1999, 1998; Preuss *et al.* 2005) and most Gram-positive organisms (Batovska *et al.* 2009; Conley and Kabara 1973; Feldlaufer *et al.* 1993; Galbraith *et al.* 1971; Hilmarsson *et al.* 2006; Kabara *et al.* 1972). Furthermore, LA is active against viruses (Hilmarsson *et al.* 2006), fungi (Bergsson *et al.* 2001; Kabara *et al.* 1972), protozoa (Dohme *et al.* 2001), and algae (McGrattan *et al.* 1976). These findings were supplemented by studies that show that the optimum chain length for therapy against Gram-positive bacteria and yeasts is 12 carbons (Batovska *et al.* 2009; Kabara and Vrable 1977). The studies also revealed that MAGs, including ML, have higher activity than the free acid counterparts unlike di- and triglycerides, which do not have antimicrobial properties. It was hypothesized that the glycerol moiety of ML serves as a hydrophilic carrier that transfers LA through the bacterial cell membrane where it exhibits activity (Ruzin and Novick 2000). In the case of di- and triglycerides, the hydrophilicity due to glycerol is diminished, which directly affects their surfactant property.

Impure ML is abundantly obtained from the partial hydrolysis of glycerides in coconut oil and is commonly used as additives (*i.e.* surfactant) in commercial products. This process is relatively simple but requires exhaustive purification procedures (*e.g.* molecular distillation) because the hydrolyzed oil will contain a mixture of glycerides from fatty acids of different sizes. Higher purity is sought to elicit optimum antimicrobial and biomedical activities. Esterification of LA and glycerol limits the impurities to LA glycerides (2-ML, 1,2-dilaurin, 1,3-dilaurin, and 1,2,3-trilaurin) alone but suffers from poor selectivity, even with heterogeneous (Hermida *et al.* 2010; Hoo and Abdullah 2015; Kotwal *et al.* 2011; Setianto *et al.* 2017) or homogeneous (Nakamura *et al.* 2008) chemical catalysts involved at elevated temperature.

As an alternative, enzymatic esterification using lipases, which is much more selective towards ML, is also being employed. Lipases are ubiquitous in nature and are also easily available commercially (Singh and Mukhopadhyay 2012). In addition, lipases are stable at elevated temperatures and in organic solvents, highly enantioselective, and resistant to degradation (Gácser *et al.* 2007). Reports vary on the selectivity of lipases which also depended on the conditions applied (Freitas *et al.* 2007; Pereira *et al.* 2004), but the highest so far is 98% from a patented technology (Davies and Macrae 2003). Lipases can be used at milder conditions compared to chemical catalysts but at much longer reaction periods for millimolar yields only. In addition, it is important to mention that the most selective process available for ML (Davies and Macrae 2003) is not exclusively selective all; and, the removal glyceride byproducts even at 2% remains a challenging task. Clearly, the elevated cost of ultra-pure ML in the market reflects the difficulty of its preparation. Previous perspectives for the preparation of ML are favorably focused on the utilization of chemical and biological catalysts. In this study, an exclusively selective non-catalytic route is presented.

LA for synthesis, ≥99.0%, CN: 8.05333.1000, BN: S7400933 728, Merck, Made in Malaysia. Glycerol, > 99.5%, 2.5L, BN: 0903388, Ajax Finechem Pty Ltd. Methanol (MeOH), 99.9%, CN: LC1115-G4L, BN: 12030230, RCI Labscan Limited. Sodium rods (protective liquid: paraffin oil) for synthesis, ≥99.0%, CN: 8.22284.0250, BN: S6999884 731, Merck, Made in France. Thionyl chloride ($SOCl_2$), for synthesis,≥99%, CN: 8.08154.0100, LN: S6352654135, Merck Schuchardt, Germany.

A chunk of Na metal from the main container was cut into small pieces and soaked into about 20-mL hexane to remove the protective oil layer. After at least 5 min, the hexane was drained and then replaced with a new 20 mL. This process was repeated five times. Smaller pieces of Na were then added gently into an excess of MeOH in an Erlenmeyer flask under an ice bath and inside a fume hood. The reaction was allowed to proceed until no more gaseous product evolved. The mixture was then filtered to remove solid impurities. This mixture contains sodium methoxide (NaOMe). A measured amount of glycerol was added to this mixture, stirred, and heated at 60 °C for 30 min. White solid sodium glyceroxide (NaOGl) produced from the reaction was filtered and washed multiple times with small volumes of MeOH before drying at 120 ± 5 °C for 3 h to remove excess MeOH and to prevent its undesirable reaction with water vapor. It is preferable to use NaOGl immediately as it is very hygroscopic.

In a separate setup, a measured amount of LA was placed inside a three-necked round bottom flask with the center connected to a reflux assembly. A 10% stoichiometric excess of thionyl chloride ($SOCl_2$) was added to solid LA through one of the side necks of the round-bottom flask; the other neck held a thermometer. As soon as a liquid product is formed, magnetic stirring was gently applied. After about 3 h, the condenser was removed, heating was applied at 80 °C, vigorous stirring was commenced, and a stream of nitrogen gas ($N_2$) was blown into the mixture to remove excess $SOCl_2$ and gaseous acid products. The step lasted for 30 min. The yellowish, viscous liquid product, lauroyl chloride (LauCl), was then filtered with a Whatman filter paper to remove trace $SOCl_2$, which

reacts with the cellulosic material (Boehm 1958; Silva Filho *et al.* 2013).

After the preparation of the activated derivatives, measured amount of NaOGl and LauCl (1:1 mol) were mixed at room temperature and pressure for at least 30 min. The entire mixture containing the target material was diluted with HPLC grade MeOH, and the residues were filtered using a filter paper with a fine mesh size. The filtrate along with MeOH used to wash was collected into a 10.0-mL volumetric flask and diluted to the mark.

Qualitatively, ML was monitored against a standard by thin-layer chromatography (TLC). The stationary phase used in TLC was silica on aluminum support, and the mobile phase (MP) was a mixture of hexane (60%, v/v), diethyl ether (40%, v/v), and acetic acid (1%, v/v); iodine ($I_2$) vapor was used as the viewing agent (Pengon *et al.* 2012). Quantitatively, ML analysis was carried out using high-performance liquid chromatography (HPLC). The HPLC (Waters Alliance e2695 Separations Module) was equipped with a 2414 refractive index detector and an Empower processing software. The SP was of Atlantis octadecyl (dC18) material and the MP under isocratic elution mode consisted of 95% (*v/v)* acetonitrile, 5% (v/v) water, and 0.1% (v/v) trifluoroacetic acid flowing at 0.800 mL/min. Both column and detector temperatures were held at 30 ±2 °C. The injection volume was 20 µL for all the runs. The conditions implemented in this study was adapted with slight modification from a previous work (Lee *et al.* 2013). Five concentrations for each of LA and ML calibration standards were prepared to generate the calibration curve. Selectivity was both assessed from the TLC and HPLC data, but percent ML yield was computed from the concentration of ML obtained from HPLC.

The reaction of NaOGl and LauCl was exothermic and occurred immediately. An important thermodynamic aspect considered in the synthesis design is production of a salt, NaCl, together with ML. NaCl has a large energy of formation ($\Delta H_f^0 = 411.15 kJ/mol$), which served as the primary driving energy for the reaction in Figure 1.

TLC data for the reaction mixtures are summarized in Table 1, whereas complete profiles can be found in the supplementary information. There are only three spots associated to three components present in the product mixture and were detectable with $I_2$. The first one with the lowest retention factor ($R_F$) value of 0.03 was identified as ML by comparing it against a standard. The remaining spots were not easily identified by direct comparison to $R_F$ values of standards. The second spot with $R_F$ value of 0.25 resemble LA, but its identity was only ascertained after spiking the mixture with standard ML and LA, which produced a similar pattern to that of the product mixture. In contrast, ML and LA mixture did not produce the same retention profiles. The third component was an unreacted LauCl and the little difference of $R_F$ values of the reference ($R_F$ 0.80) and the component in the product ($R_F$ 0.74) may be due to matrix effect, which was also observed with LA. This simple experiment showed that the synthesis was successful and selective to ML.

**Table 1.** TLC profile of the components in the product mixture.

| Sample | Component(s) | $R_F$ range | Ave $R_F$ (SD) |
|---|---|---|---|
| ML std. | ML | 0.02-0.04 | 0.04 (0.01) |
| LA std. | LA | 0.38-0.45 | 0.42 (0.03) |
| LauCl | LauCl | 0.78-0.81 | 0.80 (0.02) |
| ML + LA | ML | 0.02-0.03 | 0.03 (0.03) |
| | LA | 0.43-0.45 | 0.44 (0.01) |
| ML + LA + product (spiked) | ML | 0.02-0.02 | 0.03 (0.01) |
| | LA | 0.25 | 0.25 (0.00) |
| | LauCl | 0.73-0.76 | 0.74 (0.01) |
| Product mixture | ML | 0.03-0.04 | 0.03 (0.01) |
| | LA | 0.24-0.25 | 0.25 (0.01) |
| | LauCl | 0.73-0.76 | 0.74 (0.02) |

[RF] retention factor; [SD] standard deviation; [SP] silica on aluminum; [MP] hexane (60%, v/v), diethyl ether (40%, v/v) and acetic acid (1%, v/v); detection reagent, iodine (I2); average of six spots.

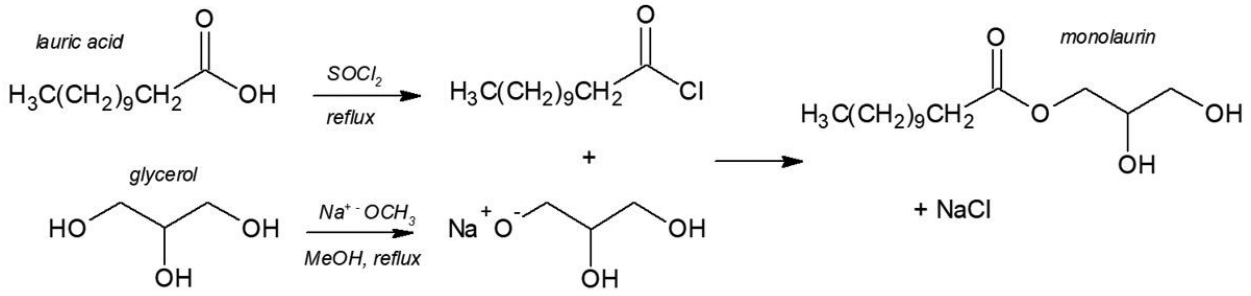


**Figure 1.** Synthesis route for ML. Mole to mole ratios as LA: SOCl2 (1:1.1); glycerol: NaOMe (approx. 1:2); LauCl: NaOGl (approx. 1:1).

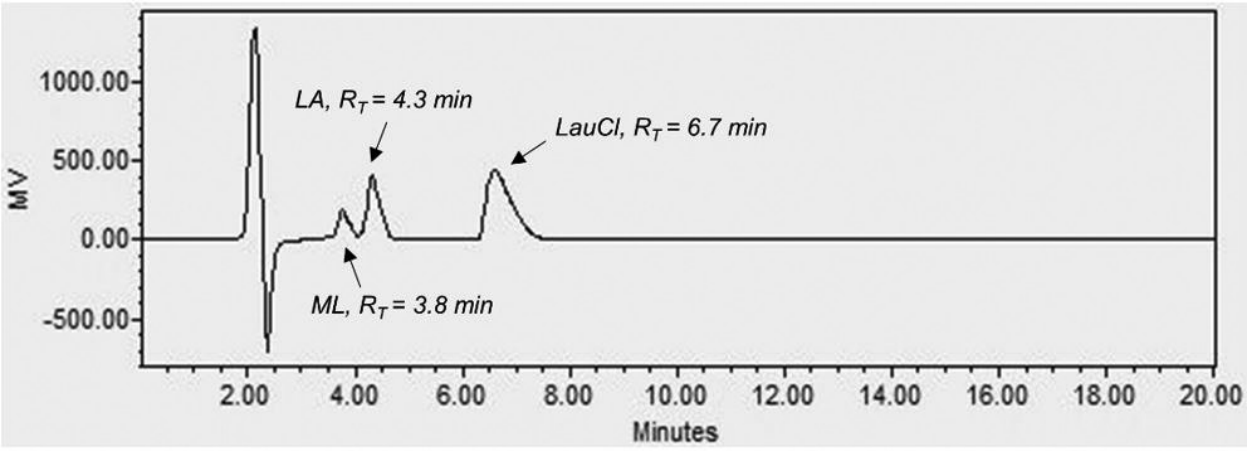


**Figure 2.** Chromatogram of the product mixture. SP as octadecyl (dC18); MP composition as 95% (v/v) acetonitrile, 5% (v/v) water, and 0.1% (v/v) trifluoroacetic acid; elution type as isocratic; flow rate as 0.800 mL/min; injection volume as 20 µL; column and detector temperature as 30± 2 °C.

Consistent with TLC, the results of HPLC show only three peaks in the chromatogram of the product mixture (Figure 2). The *x*-axis is a plot of time given in minutes, and the *y*-axis represents the electrical response of the chromatograph in millivolts (mV), which was converted from the refractive index units of the detector. The positive peak and the negative dip that appears at 2–2.25 min is an instrument response to the rapid change in pressure when the instrument changes from injection to running mode. This peak does not influence the analyte signal, but parameters were optimized so that analyte peaks will appear farther from this response. The first two peaks were easily identified by comparing $R_T$'s and trial spiking with calibration standards ML and LA. The first and second peaks are ML ($R_T$, 3.8 min) and LA ($R_T$, 4.3 min), respectively. The third peak which was retained longer ($R_T$, 6.7 min) was identified as LauCl after spiking the reactant into the product mixture. Chromatograms for the tests conducted are provided in the supplementary information.

It is clear from the chromatogram that there is no other peak associated with lesser polar di- and triglycerides up to 20 min. Scanning up to 40 min conducted during optimization produced also did not show these byproducts. TLC and HPLC results show that ML was selectively synthesized.

Quantification of LA and ML was done using calibration standards. The average concentrations of LA and ML in the 10.00-mL methanolic product mixture were 22.410 and 8.543 mg/mL, respectively. Using the computation below, the percentage ML yield was determined to be 3.41%. The reaction conditions were not optimized in this study, hence the low yield. An important aspect of the optimization that could significantly improve the yield is performing the reaction under "dry" condition because both reactants are very reactive to water.

**Table 2.** Quantification of LA and ML concentrations in the product mixture.

| **Component** | **LA** | | | **ML** | |
|---|---|---|---|---|---|
| Replicate | R1 | R2 | R3 | R1 | R2 |
| Peak area (PA) | 5800653 | 5880689 | 5914758 | 1845872 | 1819245 |
| Peak height (PH) | 422507 | 426247 | 428141 | 182900 | 174833 |
| Conc., mg/mL | 22.151 | 22.471 | 22.607 | 8.905 | 8.780 |
| Average, mg/mL | | 22.410 (± 0.23) | | 8.543 (± 0.09) | |

Injection volume as 20$\mu L$; dilution volume as 10 mL; reactant mol ratio as 1:1; target amount of ML as 2.50 g; volume of LauCl used as 2.10 mL; calibration information as linear plot of PA *vs.* conc.; LA equation of the line as $PA = 2.92\ x\ 10^5\ (\text{Conc}) - 8.19\ x\ 10^4$; R = 0.999908; $R^2$ = 0.999815; ML equation of the line as $PA = 2.13\ x\ 10^5\ (\text{Conc}) - 4.67\ x\ 10^4$; R = 0.999959; $R^2$ = 0.999919.

Computations:

$$g\ ML\ (actual)\ = 10.0mlx\frac{8.543mg}{1mL}MLx\frac{1g}{1000mg} = 0.0854g \quad (1)$$

$$g\ ML\ (theoretical) = 2.10mlx\frac{0.95gLauCl}{1mL}x\frac{1mol}{218.80g}x\frac{1mol\ ML}{1mol\ LauCl}x\frac{274.40g}{1mol} = 2.50g \quad (2)$$

$$\%\ Yield\ ML = \frac{0.0854g}{2.50\ g}x100\% = \mathbf{3.41}\% \quad (3)$$

A new way to prepare ML was developed using activated derivatives of LA and glycerol – namely, LauCl and NaOGl, respectively. The reaction was carried under neat condition and is both thermodynamically and kinetically favorable at room temperature and pressure. Unlike the methods reported previously, even the ones that involved chemical catalysts and enzymes, the present synthesis route does not produce other glyceride byproducts (*e.g.* diglycerides and triglycerides), which are difficult to separate. The yield obtained was modest (3.41%) because the process conditions were not optimized. The success of the strategy with ML implies that the same strategy is adaptable to the general preparation of MAGs for the optimal utilization of their industrial and biomedical properties.

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## STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest.

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