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# Antibacterial Study of the Medium Chain Fatty Acids and Their 1-Monoglycerides: Individual Effects and Synergistic Relationships

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Abstract

The antibacterial activity of the medium chain fatty acids and their 1-monoglycerides was evaluated towards several Gram-positive strains belonging to the genera *Staphylococcus*, *Corynebacterium*, *Bacillus*, *Listeria* and *Streptococcus*. The 1-monoglycerides were more active than the fatty acids with monolaurin being the most active compound. Interesting effects were observed when the streptococcal strain *Streptococcus pyogenes* was used as a test microorganism. First, blocking of the hydroxyl groups of the glycerol moiety of monolaurin led to a compound with remarkable antibacterial activity (MIC, 3.9 μg/ml). Secondly, synergistic relationships were observed between monolaurin and monocaprin as well as between monolaurin and the poorly active lauric acid when their two component mixtures were examined. The mixtures in which one of the components was 2-fold more predominant than the other one were much more active than the pure components taken individually. Moreover, the presence of the components in ratio 1:1 was disadvantageous. Synergistic relationships were also found between monolaurin and monomyristin towards *Staphylococcus aureus* 209 when monomyristin was in the same quantity as monolaurin or in shortage.

Key words: *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus pyogenes*, medium chain fatty acids, 1-monoglycerides

## Introduction

Antimicrobial activity of human colostrum and milk is widely believed to be mediated by the hydrolysis of the available triglycerides by lipase. This biological process releases fatty acids (FAs) and 1-monoglycerides (MGs), which put together, constitute a first line of defense of the breast fed infants against invading pathogens (Isaacs, 2005). Amongst these milk components, the medium chain FAs (C-8 to C-14) and their MGs are generally most active against viruses and Group B streptococcus, which are transmitted through mouth and nose. Such compounds are therefore applied as additives to milk formulas in order to prevent or reduce certain infections in the oral and upper respiratory mucosas of infants (Isaacs *et al.*, 1995). In addition, it was shown that some medium chain FAs and MAGs suppress the antibiotic resistance genes and provoke a relatively low frequency of spontaneous development of resistance in bacteria (Petshow *et al.*, 1996; Ruzin and Novick, 1998). This fact along with finding that these compounds are non toxic in small concentrations makes them promising as additives or alternatives to antibiotics for treatment of different diseases (Bergsson *et al.*, 2001; Nair *et al.*, 2005; Rouse *et al.*, 2005).

Lauric acid (C-12) has the greatest antibacterial activity of all medium chain aliphatic fatty acids. This effect is magnified when the acid is esterified to glycerol resulting in monolaurin, the best antibacterial MG with medium chain. Monolaurin is active primarily against Gram-positive pathogenic and spoilage bacteria. However, its spectrum of activity can be broadened when combined with other substances. For example, when combined with a cation chelator such as EDTA or citrate, monolaurin becomes active against Gram-negative bacteria. Furthermore, synergistic antimicrobial interactions between monolaurin and a number of other food ingredients such as phosphates, antioxidants, and acidulants can be observed (Marshall, 1998). Because of its longstanding safety record, monolaurin, alone or combined with antibiotics, might prove useful in the prevention and treatment of severe bacterial infections, especially

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those that are difficult to treat and/or are antibiotic resistant (Preuss *et al.*, 2005).

In this study, the antibacterial activity of all the medium chain FAs, namely caprylic (C-8), capric (C-10), lauric (C-12) and myristic (C-14) acids and their correspondent MGs (monocaprylin, monocaprin, monolaurin and monomyristin) was initially examined towards various Gram-positive bacteria belonged to *Staphylococcus*, *Corynebacterium*, *Bacillus*, *Listeria* and *Streptococcus*. The investigation was further focused on study of simple synergistic relationships of the most active compound monolaurin with selected compounds showing good antibacterial activity.

## Experimental

### Materials and Methods

**Materials.** The medium chain fatty acids caprylic, capric, lauric and myristic acids and all the chemicals necessary for the synthesis of their 1-monoglycerides were purchased in high purity from Aldrich. The test cultures: *Staphylococcus aureus* Rosenbach 209, *S. aureus* ATCC 33862 USA, *S. aureus* 146 MR, *S. epidermidis* 1093, *Corinebacterium diphteriae* 39179, *Bacillus cereus* 157, *Listeria monocytogenes* C12 and the *Streptococcus pyogenes* 15346 were obtained from the collections of the Stefan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences and the Institute for State Control of Drugs, Sofia, Bulgaria.

**Synthesis of MGs.** MGs were synthesized in two steps (Fig. 1) as described elsewhere (Batovska *et al.*, 2004). Briefly, we first obtained their acetonides from reaction of racemic 2,2-dimethyl-1,3-dioxolane-4-methanol and acid chlorides. The resulting acetonides were conveniently deprotected with strongly acidic ion-exchange resin.

**Agar-well diffusion method.** Antibacterial activity was checked by agar-well diffusion method with bacteria grown on meat-peptone agar (Spooner and Sykes, 1979). Two hundred microliter suspension of the bacteria ($10^5$ cells/ml) was plated on the agar layer in Petri dishes (10 cm in diameter). Five wells per dish were prepared, each 10 mm in diameter. One hundred microliter of each sample, dissolved in 96% EtOH (5000 μg/ml) was added to appropriate well. For pre-diffusion the Petri dishes were placed at 4°C for 2 h. The antibacterial activity was estimated by the diameter of inhibitory zones in the agar layer after incubation at 37°C for 48 h as the experiments were carried out in triplicate. Compounds giving an inhibitory zone with a diameter at least 16 mm were considered active. Control experiments were carried out with the pure solvent.

**Method of serial dilutions.** The minimal inhibitory concentration (MIC) of the individual compounds and their mixtures was measured by the Broth Tube Dilution Method as described in online laboratory manual (2000). The MIC was determined by serial dilution of each sample to 0.0–2000 μg/ml in test tubes using Mueller-Hinton broth. Each test tube was inoculated with bacterial suspension containing $10^5$ cells/ml and incubated at 35°C overnight. The highest dilution that visibly showed no growth compared to drug-free broth inoculated with microbial suspension was considered the MIC. For more precise detection, tubes that showed no visible growth were streaked on fresh meat-peptone agar plates, incubated at 35°C for 24 h, and checked for growth.

## Results and Discussion

In this study, the medium chain FAs, namely caprylic (C-8), capric (C-10), lauric (C-12) and myristic (C-14) acids and their correspondent MGs (monocaprylin, monocaprin, monolaurin and monomyristin) were evaluated for their antibacterial activity towards various Gram-positive strains. The diameters of the zones of inhibition ($d_{inh}$s) and the minimal inhibition concentrations (MICs) of the FAs and MGs are presented in Tables I and II, respectively.

It was shown that the MGs were more active than the FAs. Exception of this rule was only observed when *L. monocytogenes* was used as a test microorganism. This strain was more sensitive towards the FAs with lauric acid being the most active compound (Table I).

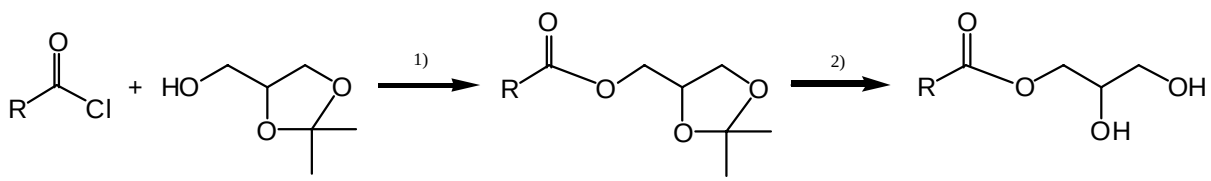


Fig. 1. Synthesis of the medium chain monoglycerides.
1) Pyridin, 4-dimethylaminopyridine, stirring at room temperature for 24 h; 2) Amberlyst 15 (wet), refluxing for 2 h

Table I
Results from the antibacterial study of the medium chain FAs

| Compounds / Bacteria | Caprylic acid | | Capric acid | | Lauric acid | | Myristic acid | |
|---|---|---|---|---|---|---|---|---|
| | $d_{inh}$, mm | MIC, µg/ml | $d_{inh}$, mm | MIC, µg/ml | $d_{inh}$, mm | MIC, µg/ml | $d_{inh}$, mm | MIC, µg/ml |
| *S. aureus* [a] | 19.3±1.2 | >500 | 19.3±1.2 | 250 | 18.0±0 | 125 | 0 | >500 |
| *S. aureus* [b] | 14.7±1.2 | >500 | 21.3±1.2 | 500 | 14.0±0 | >500 | 0 | >500 |
| *S. aureus* [c] | 15.3±1.2 | >500 | 18±0 | 500 | 14.7±1.2 | >500 | 0 | >500 |
| *S. epidermidis* | 14.7±1.2 | >500 | 23.3±1.2 | 500 | 0 | >500 | 0 | >500 |
| *C. diphteriae* | 0 | >500 | 28±0 | 500 | 16.0±0 | 62.5 | 0 | >500 |
| *B. cereus* | 16±0 | >500 | 14±0 | >500 | 12.0±0 | >500 | 0 | >500 |
| *L. monocytogenes* | 17.3±1.2 | 250 | 19.3±1.2 | 250 | 33.3±1.2 | 31.25 | 21.3±1.2 | 125 |
| *S. pyogenes* | 14±0 | >500 | 20.7±1.2 | 250 | 25.3±1.2 | 125 | 0 | >500 |

[a] *S. aureus* 209, [b] *S. aureus* 146 MR, [c] *S. aureus* ATCC 33862 USA.

Table II
Results from the antibacterial study of the medium chain MGs

| Compounds / Bacteria | Monocaprylin | | Monocaprin | | Monolaurin | | Monomyristin | |
|---|---|---|---|---|---|---|---|---|
| | $d_{inh}$, mm | MIC, µg/ml | $d_{inh}$, mm | MIC, µg/ml | $d_{inh}$, mm | MIC, µg/ml | $d_{inh}$, mm | MIC, µg/ml |
| *S. aureus* [a] | 12±0 | >500 | 21.3±1.2 | 62.5 | 21±0 | 31.25 | 22.7±1.2 | 62.5 |
| *S. aureus* [b] | 0 | >500 | 22±0 | 250 | 26±0 | 15.6 | 0 | >500 |
| *S. aureus* [c] | 0 | >500 | 27.3±1.2 | 125 | 30±0 | 7.8 | 28.7±1.2 | 500 |
| *S. epidermidis* | 12±0 | >500 | 22±0 | 125 | 28±0 | 31.25 | 36.7±1.2 | 125 |
| *C. diphteriae* | 0 | >500 | 18.7±1.2 | 125 | 21.3±1.2 | 62.5 | 17.3±1.2 | 500 |
| *B. cereus* | 12±0 | >500 | 14±0 | >500 | 22±0 | 125 | 0 | >500 |
| *L.mono.* | 0 | >500 | 35.3±1.2 | 125 | 23.3±1.2 | 62.5 | 34±0 | 250 |
| *S. pyogenes* | 14±0 | >500 | 29.3±1.2 | 62.5 | 22.3±1.2 | 31.25 | 0 | >500 |

[a] *S. aureus* 209, [b] *S. aureus* 146 MR, [c] *S. aureus* ATCC 33862 USA. L.mono. = L. monocytogenes

Monolaurin, having an aliphatic chain with 12 carbons, was the most active of all tested compounds (Table II). It inhibited strongly the growth of the staphylococcal and the streptococcal strains. This was the only compound active towards the methycilline resistant strain of *S. aureus*. The other MGs, monocaprin and monomyristin, distinguished from monolaurin by 2 carbons were sensibly less active while monocaprylin with 8 carbons in its aliphatic chain did not show any activity.

It is known that lauric acid exhibit antimicrobial activities, which intensify when the fatty acid is esterified with glycerol to give monolaurin. A hypothesis scrutinizes the glycerol moiety of monolaurin as a hydrophilic carrier that transfers lauric acid through the bacterial cell membrane where it exhibits its antibacterial activity (Ruzin and Novick, 2000). If this is the case, we can expect that monolaurin will display greater antibacterial activity than lauric acid. Moreover, elimination of the hydrophillicity of the glycerol moiety by protection of its hydroxyl groups would lead to more inactive compound. In this relation, we were interested to compare the antibacterial effects of lauric acid, monolaurin and the acetonide of the monolaurin having two blocked hydroxyl groups (Table III). The unbound lauric acid inhibited *S. pyogenes* with MIC of 125 µg/ml. This activity increased 4 times when the acid was bound to glycerol. Unexpectedly, blocking of the two hydroxyl groups of glycerol moiety in monolaurin was in favor of the antibacterial activity and the acetonide was 32-fold more active than the free lauric acid and 8-fold more active than monolaurin. The notable activity of the acetonide shows that this compound has some specific effect on the cells of *S. pyogenes*. Furthermore, the acetonide of monolaurin was totally inactive towards other bacteria, such as *L. monocytogenes* and *C. diphteriae* (Table III). This might be because the protected hydroxyl groups of the glycerol moiety disturbed penetration of the whole molecule through the cell membranes of these bacteria.

Table III
The MICs (µg/ml) of lauric acid, monolaurin and the acetonide of monolaurin towards selected bacteria

| Bacteria | Lauric acid | Monolaurin | Acetonide of monolaurin |
|---|---|---|---|
| *S. pyogenes* | 125 | 31.25 | 3.9 |
| *C. diphteriae* | 62.5 | 62.5 | >1000 |
| *L. monocytogenes* | 31.25 | 62.5 | 1000 |

Having in mind that the antibacterial activity of monolaurin can be synergistically influenced by other active compounds we examined some two-component mixtures of this MG and the compounds having the closest antibacterial activities. Thus, we selected monocaprin and monomyristin as the next most active compounds after monolaurin against *S. aureus* 209 and mixed them separately with monolaurin in different ratios. The results from the antibacterial study of these mixtures towards *S. aureus* 209 showed that monomyristin had synergistic effect only when it was in the same quantity as monolaurin (ratio 1:1) or in shortage (ratio 1:2) (Fig. 2). Monocaprin does not interact synergistically with monolaurin (Fig. 2). The same was found for lauric acid when *C. diphteriae* and *L. monocytogenes* were used as test microorganisms (Fig. 3, Fig. 4). Interesting synergistic relationships were found for monolaurin and monocaprin as well as for monolaurin and the poorly active lauric acid when their mixtures were studied against the streptococcal strain *S. pyogenes* (Fig. 5). In these cases, it was observed that the mixtures in which one of the components was 2-fold more predominant than the other one were much more active than the pure components taken individually. Moreover, the presence of the components in ratio 1:1 was disadvantageous. Presence of synergistic relationships between the active monolaurin and lauric acid show that combinations of monolaurin with compounds having weak antibacterial activity also deserve attention. In this respect, not only two-component mixtures should be examined, but also complex mixtures containing more than 3 components.

In conclusions, the medium chain MGs were more active than the medium chain FAs with monolaurin displaying the greatest antibacterial activity towards various Gram-positive strains. The following interesting effects were observed when *S. pyogenes* was used

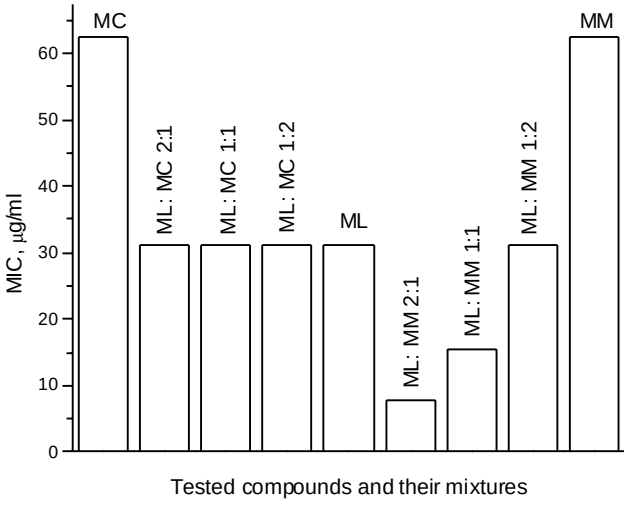


Fig. 2. The MICs of monolaurin (ML) mixtures with monocaprin (MC) and monomyristin (MM) towards *S. aureus* 209

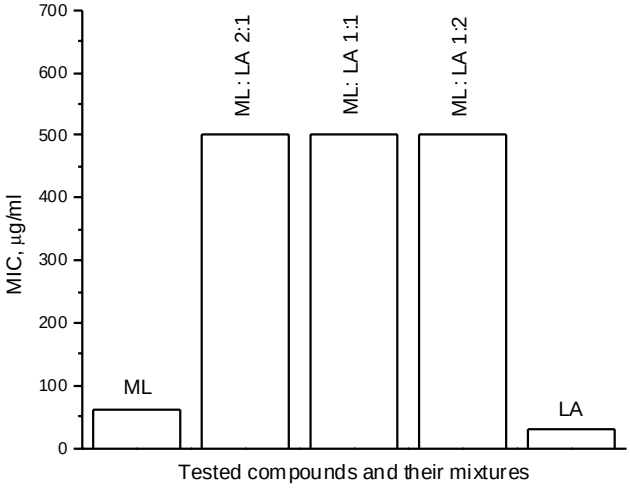


Fig. 3. The MICs of monolaurin (ML) mixtures with lauric acid (LA) against *C. diphteriae*

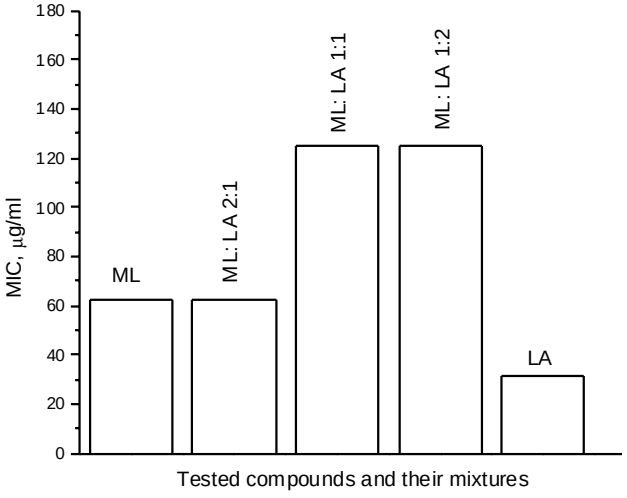


Fig. 4. The MICs of monolaurin (ML) mixtures with lauric acid (LA) against *L. monocytogenes*

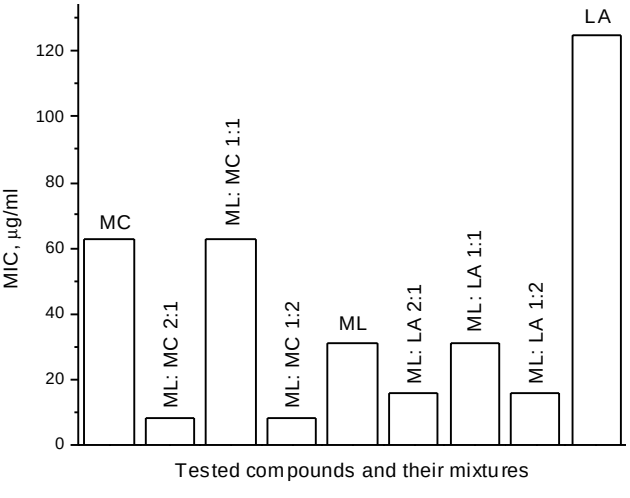


Fig. 5. The MICs of monolaurin (ML) mixtures with monocaprin (MC) and lauric acid (LA) against *S. pyogenes*

as test microorganism: 1) Blocking of the hydroxyl groups of the glycerol moiety of monolaurin led to a compound with remarkable antibacterial activity; 2) Synergistic relationships were observed between monolaurin and monocaprin as well as between monolaurin and the poorly active lauric acid when their two component mixtures were examined.

Synergistic relationships were also found between monolaurin and monomyristin towards *S. aureus* 209 when monomyristin was in the same quantity as monolaurin or in shortage.

Based on the observed effects potent antibacterial compounds and lipid mixtures could be developed.

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## Literature

**Batovska D., S. Tsubota, Y. Kato, Y. Asano and M. Ubukata.** 2004. Lipase-mediated desymmetrization of glycerol with aromatic and aliphatic anhydrides. *Tetrahedron Asymm.* 15: 3551–3559.

**Bergsson G., J. Arnfinnsson, O. Steingrimsson and H. Thormar.** 2001. *In vitro* killing of *Candida albicans* by fatty acids and monoglycerides. *Antimicrob. Agents Chemother.* 45: 3209–3212.

**Isaacs C.E.** 2005. Human milk inactivates pathogens individually, additively, and synergistically. *J. Nutr.* 135: 1286–1288.

**Issacs C., R. Litov and H. Thormar.** 1995. Antimicrobal activity of lipids added to human milk, infant formula, and bovine milk. *J. Nutr. Biochem.* 6: 362–366.

**Marshall D.L.** 1998. Antimicrobial properties of the multifunctional ingredient monolaurin. Abstracts of *International Conference of Food System Functionality*. Atlanta, GA. USA. p. 31.

**Nair M., J. Joy, P. Vasudevan, L. Hinckley and T. Hoagland.** 2005. Antibacterial effect of caprylic acid and monocaprylin on major bacterial mastitis pathogens. *J. Dairy Sci.* 88: 3488–3495.

**Online laboratory manual.** 2000. http://www.life.umd.edu/classroom/bsci424/LabMaterialsMethods/BrothTubeMIC.htm.

**Petshow B., R. Batema and L. Ford.** 1996. Susceptibility of *Helicobacter pylori* to bactericidal properties of medium-chain monoglycerides and free fatty acids. *Antimicrob. Agents Chemother.* 40: 302–306.

**Preuss H., B. Echard, M. Enig, I. Brook and T. Elliott.** 2005. Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria. *Mol. Cell Biochem.* 272: 29–34.

**Rouse M., M. Rotger, K. Piper, J. Steckelberg, M. Scholz, J. Andrews and R. Patel.** 2005. *In vitro* and *in vivo* evaluations of the activities of lauric acid monoester formulations against *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 49: 3187–3191.

**Ruzin A. and R. Novick.** 1998. Glycerol monolaurate inhibits induction of vancomycin resistance in *Enterococcus faecalis*. *J. Bacteriol.* 180: 182–185.

**Ruzin A. and R. Novick.** 2000. Equivalence of lauric acid and glycerol monolaurate as inhibitors of signal transduction in *Staphylococcus aureus*. *J. Bacteriol.* 182: 2668–2671.

**Spooner F. and G. Sykes.** 1979. Laboratory assessment of antibacterial activity, pp. 216–217. In: Norris J.R. and D.W. Ribbons (eds). *Methods in Microbiology 7B*. Academic Press, London.