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SHORT COMMUNICATION

Evaluation of Antibacterial Activity of Synthetic Aliphatic and Aromatic Monoacylglycerols

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Abstract

The antibacterial activity of synthetic aliphatic and aromatic monoacylglycerols (MAGs) was studied against two human pathogens: *Staphylococcus aureus* and *Esherichia coli*. The active compounds inhibited selectively *S. aureus*. The most active compounds amongst them were those with medium size aliphatic chain and aromatic MAGs with electron withdrawing substituents at the aryl ring. The introduction of one or two-carbon spacer between the aryl ring and the carboxylic function did not influence antibacterial effectiveness.

Key words: *Esherichia coli*, *Staphylococcus aureus*, aliphatic and aromatic monoacylglycerols

Monoacylglycerols (MAGs) are major intermediates in fat metabolism and could be found in most plant and animal tissues. They are important lipid ingredients of breast milk and propolis where serve as nutrients and antimicrobial agents constituting a defense system against microbial infections. Certain naturally occurring aliphatic MAGs have been reported to efficiently inhibit bacteria, enveloped viruses, yeasts and fungi (Bergsson *et al.*, 2001; Thormar *et al.*, 1987; Kabara, 1978). The wide-spectrum antimicrobial properties and particularly the antibacterial activities of MAGs have been thoroughly attributed to their amphiphatic nature. As nonionic surfactants MAGs are believed to easily penetrate the bacterial cells by diffusion through the peptidoglycan cell wall. Most likely they exert their antibacterial effects at the plasma membrane, but the exact mechanisms of action are still unclear. A number of studies have also revealed that some 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 MAGs are non-toxic in small concentrations makes them promising as adjuncts or alternatives to antibiotics for treatment of different diseases (Nair *et al*., 2005; Rouse *et al*., 2005).

In this study we undertook an effort to expand the range of MAGs usable for therapeutic needs *via* synthesis of various aliphatic and aromatic MAGs and evaluation of their antimicrobial effects against two human pathogens, namely *S. aureus* and *E. coli*. Our strategy was built on the fact that the antibacterial activity of MAGs is very often commensurable with that of the acids incorporated in their molecules. We therefore introduced carboxylic acids with known antibacterial properties, most of them widespread in nature.

The MAGs were synthesized in two steps following previously described by us procedures (Batovska *et al*., 2004; Batovska *et al*., 2005). Briefly, we first obtained acetonides from a reaction of racemic 2,2-dimethyl-1,3-dioxolane-4-methanol and acid anhydrides or by the Mitsunobu protocol (Batovska *et al*., 2005). The resulting acetonides were conveniently deprotected with the strongly acidic resin Amberlyst 15 (wet). Two novel compounds, derivatives of mycophenolic acid, were also synthesized (Figure 1).

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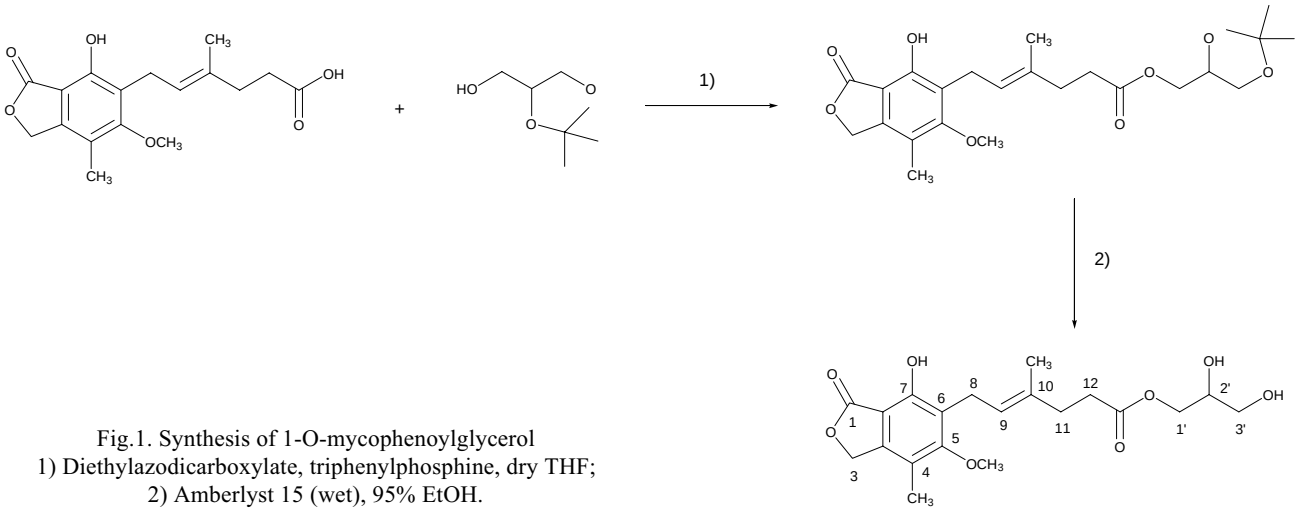


Fig.1. Synthesis of 1-O-mycophenoylglycerol
1) Diethylazodicarboxylate, triphenylphosphine, dry THF;
2) Amberlyst 15 (wet), 95% EtOH.

(2,2-Dimethyl-1,3-dioxolane-4-yl)methyl-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate, colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.67 (s, 1H, O-H), 5.23-5.25 (m, 3H, C-3-H_2, C-9-H), 4.25 (dd, J_1 = 5.4 Hz, J_2 = 10.8 Hz, 1H, C-2'-H), 4.10 (dd, J_1 = 5.4 Hz, J_2 = 10.8 Hz, 1H, C-1'-H), 4.00-4.06 (m, 2H, C-1'-H, C-3'-H), 3.75 (s, 3H, O-CH_3), 3.69 (dd, J_1 = 5.4 Hz, J_2 = 8.1 Hz, 1H, C-3'-H), 3.38 (d, J = 8.1 Hz, 2H, C-8-H_2), 2.41-2.47 (m, 2H, C-11-H_2), 2.27-2.33 (m, 2H, C-12-H_2), 2.17 (s, 3H, Ar-CH_3), 1.79 (s, 3H, CH_3), 1.41 (s, 3H, acetonide-CH_3), 1.35 (s, 3H, acetonide-CH_3); ^{13}C NMR (270 MHz, $CDCl_3$) δ 172.8, 172.6, 163.4, 153.3, 143.8, 133.8, 122.6, 121.9, 116.5, 109.6, 73.4, 69.9, 66.2, 64.5, 60.9, 34.4, 32.8, 26.6, 25.3, 22.6, 16.1, 11.6; EI-HRMS *m/z* 434.1949 (M^+ $C_{23}H_{30}O_8$ requires 434.1941).

2,3-Dihydroxypropyl-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate (**24**). Colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.25-5.22 (m, 1H, C-9-H), 5.20 (s, 2H, C-3-H_2), 4.17-4.13 (m, 1H, C-1'-H), 4.07 (dd, J_1 = 5.8 Hz, J_2 = 11.7 Hz, 1H, C-1'-H), 3.92-3.84 (m, 1H, C-2'-H), 3.76 (s, 3H, O-CH_3), 3.66 (dd, J_1 = 3.9 Hz, J_2 = 11.5 Hz, 1H, C-3'-H), 3.56 (dd, J_1 = 5.8 Hz, J_2 = 11.4 Hz, 1H, C-3'-H), 3.38 (d, J=6.9 Hz, 2H, C-8-H_2), 2.49-2.44 (m, 2H, C-11-H_2), 2.34-2.29 (m, 2H, C-12-H_2), 2.15 (s, 3H, Ar-CH_3), 1.81 (s, 3H, CH_3); ^{13}C NMR (270 MHz, $CDCl_3$) δ 173.6, 172.8, 163.4, 153.5, 144.1, 133.9, 122.8, 122.1, 116.7, 106.4, 70.2, 70.1, 65.2, 63.3, 61.1, 34.6, 32.9, 22.7, 16.3, 11.7; EI-HRMS *m/z* 394.1626 (M^+ $C_{20}H_{26}O_8$ requires 394.1628).

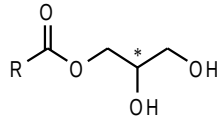
The strains *S. aureus* Rosenbach 209 and *E. coli* Castellani and Chalmers WF^+ were obtained from the collection of the Stefan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences and the Institute for State Control of Drugs, Sofia, Bulgaria, respectively.

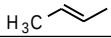
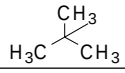
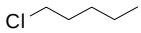
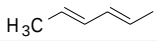
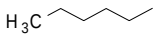
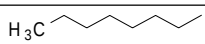
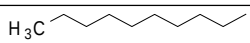
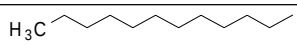
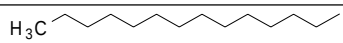
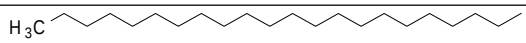
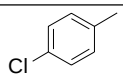
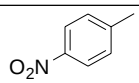
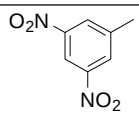
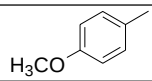
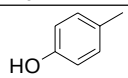
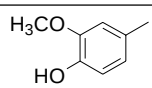
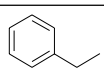
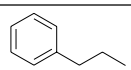
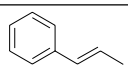
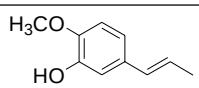
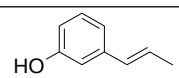
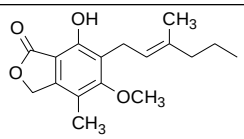
Antibacterial activity was checked by agar-well diffusion method with *S. aureus* and *E. coli* grown on meat-peptone agar (Spooner and Sykes, 1979). Two hundred microliter suspension of the bacteria (10^5 cells/ml) was plated on the agar layer in Petri dishes (10 cm in diameter). Five wells per dish were prepared, each 10 mm in diameter. One hundred microliters of each sample, dissolved in 96% EtOH (5000 μg/ml) was added to appropriate well. For prediffusion 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.

The minimal inhibitory concentration (MIC) of the MAGs was measured by the Broth Tube Dilution Method as described in online laboratory manual (Web site, 2000). The MIC was determined by serial dilution of each chalcone 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.

The aliphatic MAGs had acid moieties with various lengths (Table I). A threshold of hydrophobicity is known to have determined the medium-chain MAGs (C-8 to C-14) as the most active homologues towards Group B streptococci (Issacs *et al.*, 1995). In our study the medium-chain aliphatic esters, compounds **7**, **8** and **9** were the best antibacterials against *S. aureus*. In order to determine the optimal hydrophobicity and

Table I
Data for the anti-staphylococcal activity, log P and MR of the aliphatic and aromatic MAGs



Com-pound	R	d_{inh} (mm)	MIC (μg/ml)	Log P	MR
1		0	500	0.3	40.0
2		0	500	0.3	43.4
3		0	500	–0.1	48.5
4		14 ± 1	500	0.8	50.3
5		0	500	0.3	48.1
6		12 ± 0	500	1.1	57.3
7		21 ± 1	62.5	1.9	66.5
8		n.d.	31.25	2.6	75.7
9		23 ± 1	62.5	3.4	84.9
10		0	500	6.6	121.7
11		0	500	0.3	50.9
12		16 ± 0	500	0.8	50.3
13		12 ± 0	500	1.3	55.1
14		19 ± 1	250	0.7	57.7
15		32 ± 0	125	0.7	65.0
16		0	500	0.5	56.8
17		0	500	0.5	52.3
18		0	500	0.2	58.8
19		15 ± 1	500	0.3	54.4
20		0	500	0.7	59.0
21		0	500	1.7	60.1
22		15 ± 1	500	1.4	62.1
23		16 ± 1	500	1.2	68.5
24		17 ± 1	250	1.6	102.3

size for the active molecules we calculated 2 descriptors – the logarithm of the partition coefficient between *n*-octanol and water (log P) and molar refractivity (MR). The partition coefficient is a measure of the hydrophobicity of the molecules while MR is related to the volume of the molecules and to the London dispersive forces that act in the drug-receptor interaction (Padrón *et al.*, 2002). According to the results the active compounds had log P = 1.9 ÷ 3.4 and MR = 66.5 ÷ 84.9. This may be the optimal size of the aliphatic MAGs allowing them to penetrate the plasma membrane of the bacterial cells where they probably unfold their antibacterial potential.

1-*O*-(Lauroyl)glycerol (C-12), **8**, was the most active aliphatic MAG. This compound has recently been examined to be utilized in wound dressings or tampons (Vetter and Shlievert, 2005).

None of the aliphatic MAGs was active towards *E. coli* at concentrations under 5000 ppm. This is in accordance with the literature data showing that these substances are noticeably more efficient against Gram-positive than Gram-negative species (Kabara, 1978).

Although many aromatic acids may bring about antibacterial activity, such activity of MAGs with aromatic acid components, have not been studied so far. Benzoic acid has good antimicrobial features, but because of its low solubility in water is often applied as salt. We supposed that its coupling with the hydrophilic molecule of glycerol could solve the solubility problem and lead to better expression of its antibacterial potential. However, the bacteria were tolerant to the resulting 1-*O*-(benzoyl)glycerol – **12**. *S. aureus* was rather influenced by the introduction of substituents in the aryl ring with a preference for the electron withdrawing groups (Table I). Thus, the nitro groups-containing compounds **14** and **15** were the most active aromatic MAGs. Presence of electron withdrawing substituents probably facilitates the hydrolysis of the aromatic MAGs to glycerol and the corresponding carboxylis acids as the released acids act as antibacterials. Both compounds **14** and **15** had the same values of log P, which was in the middle of the hydrophobicity range for the aromatic MAGs. Compound 15 inhibited *S. aureus* causing an inhibitory zone with a diameter of 32 ± 0 mm (MIC 125 μg/ml), which is greater than those by the antibiotic kanamycin (d_{inh} 30 ± 0 mm, MIC<31.25 μg/ml). Compounds **13**, **16** and **17** having chloro, methoxy and hydroxyl groups at *p*-position in their aryl rings were all inactive. No relationship was found between the MIC results for the aromatic MAGs and their MR values.

We were also interested in whether a one or two-carbon aliphatic spacer introduced between the aryl ring and the carboxylic function of the aromatic MAGs will influence their antibacterial effectiveness. For this reason we replaced the benzoic acid fragment with that of some phenylalkanoic acids. Some of their MAGs (compounds **21**, **22** and **23**) are found in many plants and poplar type propolis (Batovska *et al.*, 2005). While the antibacterial activity of propolis has been widely demonstrated (Kujumgiev *et al.*, 1999) the antibacterial properties of its phenylpropanoid MAGs has not been studied so far. None of the glycerol monoesters of phenylalkanoic acids showed significant antibacterial activity towards *S. aureus* (Table I) and any activity against *E. coli*. The presence of one-carbon spacer in the molecule of MAG (**19**) retained the antibacterial activity in the same order like this of 1-*O*-(benzoyl)glycerol (**12**). However, with elongation of the spacer with one more carbon atom (compounds **20** and **21**) the activity disappeared as the presence of double bond in the spacer was not of significance. Introduction of a longer spacer between the aryl ring and the carboxylic function (1-*O*-(mycophenoyl)glycerol, **24,** resulted in a better anti-staphylococcal activity (MIC, 250 μg/ml). However, this may be due to the entire moiety of mycophenolic acid, which is a natural compound with bacteriostatic activity against *S. aureus* (Abraham, 1945).

In conclusion, several compounds with antibacterial activity towards *S. aureu*s were found amongst a series of synthesized by us MAGs. Amongst them the medium-chain aliphatic MAGs and the aromatic MAGs with electron withdrawing substituents at the aryl ring, were the most active compounds. The introduction of one or two-carbon spacer between the aryl ring and the carboxylic function did not influence the antibacterial effectiveness. Altogether, these findings could be used for developing antibacterial agents based on the MAG skeleton.

Acknowledgements

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