Acylglycosides as Future Food Preservatives

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Abstract: In this work monoesters of sucrose and D-glucose with fatty acids (both even and odd) were prepared as pure substances using the direct selective esterification of free sugar with bulky acylating agent. This compounds were examined for their antibacterial activity (against Gram-positive bacteria) and antifungal activity.

Keywords: acylglucose; acylglycosides; acylsucrose; antifungal activity; antimicrobial activity

INTRODUCTION

It has been known for a long time that free fatty acids show certain antimicrobial activity and therefore their use as food preservatives has been considered. Microorganisms in food emulsions grow strictly in water phase and thus it is necessary to follow the partition of applied preservatives between lipid and water phase. The partition coefficient of suitable fatty acids depens on chain length as well as on the water phase pH value. That is the reason for our research of fatty acid derivatives with antimicrobial properties, especially acylglycosides.

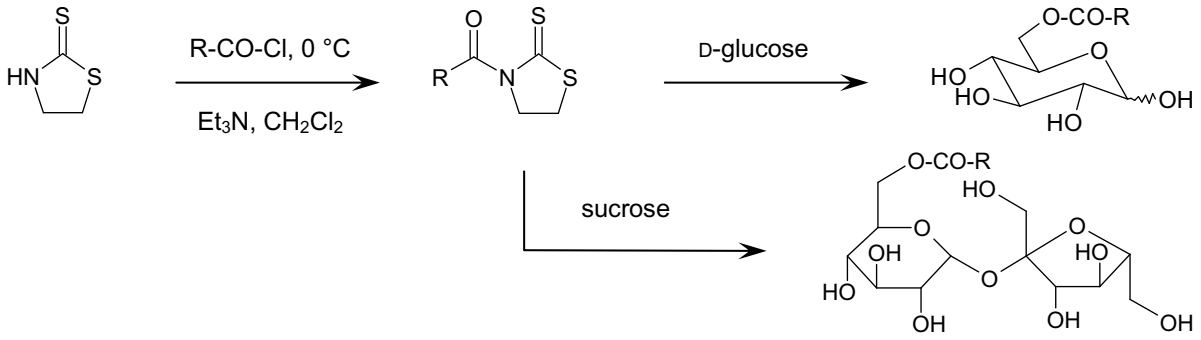
There are three types of acylglycosides, which are we focused on, namely acylglycerols, acylsucroses and acylglucoses. Recently we have been dealing with 6-*O*-esters of sucrose and D-glucose. The aim of this study is to prepare these substances as pure individuals, examine their physical-chemical properties and investigate their antimicrobial activity against selected microorganisms.

EXPERIMENTAL

Generally, there are two basic ways to obtain 6-*O*-acylglycosides [1]. The first one is using classical organic synthesis. Whether methods using protection of saccharide [2, 3], then substitution [4] followed by subsequent deprotection [5] or methods using special kind of acylating agent [1, 6], which bulkines allows esterification just of primary hydroxyl group. The other way of the synthesis is using hydrolytical enzymes – lipases, under anhydrous conditions, where the enzyme controls the stereoselectivity [7]. In this project the non enzymatic method with bulky reagent was used.

In the first step the acylating agent, 3-acylthiazolidine-2-thione, was prepared [8], from the corresponding fatty acid chloride and thiazolidine-2-thione in the presence of triethylamine and dichloromethane as a solvent. Then this acylating agent was used for selective acylation of free saccharide [1, 6] which was carried out under various conditions. Pyridine or *N*,*N*-dimethylformamide (DMF) as the solvent and sodium hydride combined with *N*,*N*--dimethylaminopyridine or NaH combined with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), just DBU or triethylamine as the catalyst were investigated. Reaction conditions were chosen according to the best results from trial experiments: DMF/NaH+DBU for 6-*O*-acylsucroses and DMF/DBU for 6-*O*-acyl--D-glucoses (see scheme). Products were isolated by column chromatography on silica gel, ethyl acetate and methanol formed the mobile phase, gradient elution 100:0 to 86:14, v:v. Crystalization from acetone was used for product purification.

TLC analyses were performed on Merck Kieselgel 60 F 254 plates. Plates were developed in the mixture



ethyl acetate:methanol:water mixture, 86:10:4, v:v:v, and visualized by spraying with $Ce(SO_4)_2$ solution in sulfuric acid and subsequet heating.

HLPC analyses were carried out using of LPP Separon SIX NH2 column (length of 150 mm, inner diameter of 3 mm, particle size of 5 μm) by isocratic elution with mixture acetonitrile:water, 95:5, v:v, flow rate programme 00–15 min 0.3 ml/min, 15–25 min 0.5 ml/min, 25–120 min 0.8 ml/min. Detection was performed by evaporative light scattering detector at 55°C.

In order to determine the antimicrobial activity these three methods were applied:

(*i*) Cultivation in liquid medium – used for the determination of acylsucroses efficiency against G^+ bacteria. Growth broth (Nutrient Broth, OXOID) supplemented with the tested compound in intended amount was inoculated with culture of *Bacillus subtilis* DMF 2006 and cultivated for 24 h at 30°C. Culture was sampled every three hours for total number of colony forming units (CFU; plate method, according to ČSN ISO 4833). At the end of cultivation the inhibiton ratio was calculated from results obtained; the inhibition ratio corresponds to the quocient of difference of CFU in the test sample and the control sample and of CFU in the control sample.

(*ii*) Gelatine cassettes – used for the determination of the antifungal activity of acylsucroses. Sterile mixture of broth with maltose extract and gelatine was fortified under aseptic conditions with the solution of tested compound and inoculated with mould spore suspension (*Aspergillus niger* DMF 0501). Medium was after that immediately injected into transparent casette and let to solidify. Every day during one week cultivation the colony count was determinated and their diameters were measured. The inhibition index calculated represents the number of not germinated spores compared to the control experiment.

(*iii*) Micro-titration plates – were used for the determination of the acylglucoses antibacterial efficiency. Broth with the solution of tested compoud was pipetted into each position of the micro-titration plate folowed by the inoculation of *Bacillus cereus* DMF 2001 culture. Plates were cultivated aerobicaly at 30°C for 10 days and the growth was measured daily by multispectrofotometer. The gowth curves were processed by integration.

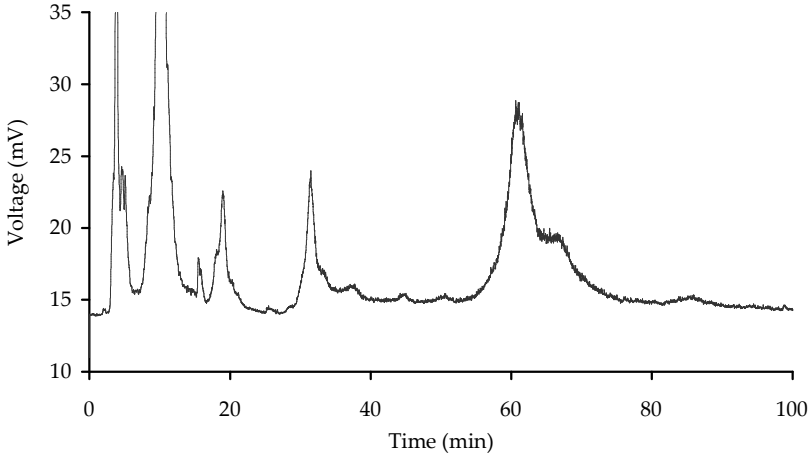


Figure 1. HPLC chromatogram of lauroylsucrose reaction mixture extract

RESULTS AND DISCUSSION

As described above these esters derived from following saccharides and fatty acids we prepared:
Sucrose – C 10:0, C 11:0, C 12:0, C 13:0, C 14:0, C 5:0, C 16:0;
D-Glucose – C 10:0, C 11:0, C 12:0, C 13:0, C 14:0.

Prepared D-glucose esters were absolutely identified by NMR (proton, COSY, APT and HMQC experiments) and IR (KBr-tablet technique). It was found out that all 6-*O*-acylsucroses, which were also synthetized by Baczko *et al*. [6] and declared to be the only one reaction product, are actually a mixture of several compounds. Elementary analysis results are in a very good agreement with values calculated theoretically for sucrose monoesters, hence we can express reasonable belief that the mixture consists of positional isomers of sucrose monoesters, in contradiction with literary resources.

The composition of reaction mixture and the disunity of reaction product can be easily disclosed by ordinary TLC analysis. In order to quantify the mixture composition the HPLC analytical method was developed. Characteristic chromatogram of lauroylsucrose reaction mixture extract is shown in Figure 1. This extract contains already no sucrose. Before reaching of 7 min there are acylating agent residues and waste products eluted, between 7 min and 25 min there are low polar by-products eluted (sucrose polyesters, degradation products) and finally from 28 min there are sucrose laurate isomers eluted – in this peak group at least eight peaks can be counted.

Due to an insignificant difference in polarity and vigorous adsorption forces the separation of acylsucrose isomers is difficult not only on aminopropylated silica gel HPLC column but also in the particular case on preparative silica gel column. In the light of these facts we were forced to use only a blend of several compounds from monoesters group for next investigation.

Bacterial and fungal strains that are commonly present in foods as contaminants were chosen for the antibacterial activity examination.

Results of the antibacterial activity of acylsucroses are shown in Figure 2. It is obvious that the addition

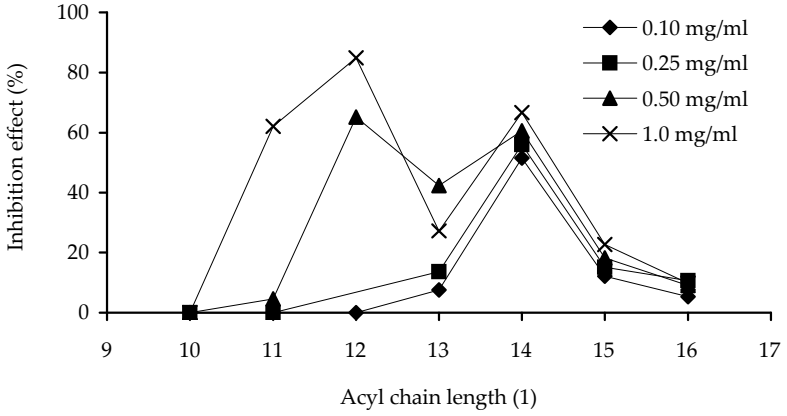


Figure 2. Antibacterial activity of acylsucroses against *Bacillus subtilis* DMF 2006

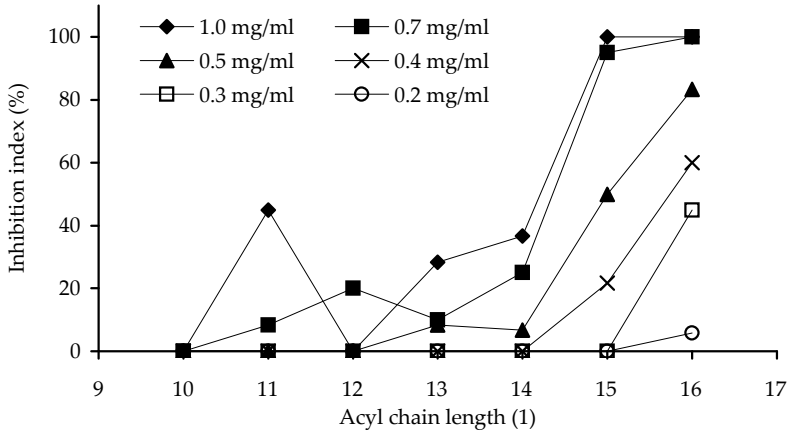


Figure 3. Antifungal activity of acylsucroses against *Aspergillus niger* DMF 0501

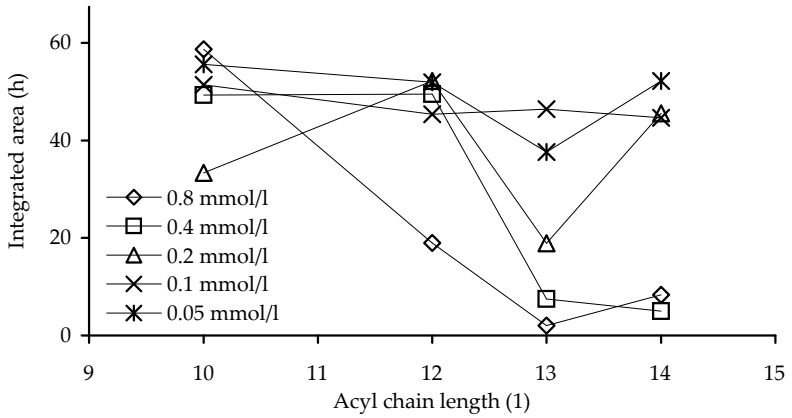


Figure 4. Antibacterial activity of acyl-D-glucoses against *Bacillus cereus* DMF 2001

of lauroylsucrose in concentration of 1 mg/ml resulted in the highest inhibitory effect agains *Bacillus subtilis* (91%). Satisfactory results were also obtained with undecanoyl- and tridecanoylsucrose, approx. 70% of inhibition at 1 mg/ml. For the antifungal activity of acylsucroses against *Aspergillus niger* (Figure 3), a sharp increase in the inhibitory effect with increasing acyl chain length was observed. Our expectations of higher antifungal activity of odd number fatty acids were not verified.

Experiments with acyl-D-glucoses showed high antibacterial efficiency of tridecanoyl and myristoyl derivatives against *Bacillus cereus*, where integrated areas under growth curves were minimal out of all (concentrations 0.4 and 0.8 mmol/ml) – Figure 4.

CONCLUSIONS

Acylation method used in this study was successful for the preparation of 6-*O*-acyl-D-glucose, nevertheless this method applied on sucrose leads to the mixture of monoester isomers.

Synthetized compounds were examinated for their antimicrobial activity. The highest antibacterial activity showed sucrose laurate and D-glucose tridecanoate or myristate, the highest antifungal activity showed sucrose palmitate. D-Glucose esteres have not been tested for their antifungal activity yet.

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