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CHEMOENZYMATIC SYNTHESIS AND CHARACTERIZATION OF A NEW TYPE OF CARBOHYDRATE-BASED CATIONIC ANTIMICROBIAL AGENTS.

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Abstract: A simple chemoenzymatic synthesis providing 6-*O*-monoesters of 3-(trimethylammonio)propyl D-glucopyranoside in high yields is described. These new compounds are very effective cationic surfactants exhibiting both antibacterial and antifungal activity and have a good compatibility with anionic surfactants.

Introduction

One of the most important classes of cationic surfactants is the quaternary ammonium cationic salts (QACs) which are widely used as antimicrobial agents for topical disinfection and as surfactants within specialized areas such as fabric softeners and hair conditioners.[1] Unfortunately, most QACs available today share some common disadvantages limiting their use. First of all, they show a poor compatibility with other materials, as exposure to *e.g.* anionic surfactants and soaps inhibits their action by forming insoluble complexes.[2] Furthermore, many of the presently available compounds show a poor biodegradation under practical sewage plant conditions.[3,4]

The incorporation of an additional noncharged hydrophilic group into a cationic surfactant has been shown to be a way of increasing the compatibility of the surfactant with other ingredients.[2] In this context, the incorporation of a carbohydrate moiety appears to be very attractive. The hydrophilic groups are more concentrated than in a polyethoxylate unit. Furthermore, if the hydrophobic part of the surfactant is attached *via* an ester bond to the carbohydrate, the resulting compound would be expected to be readily biodegradable. Recent work has shown that the incorporation of an ester functionality in a cationic surfactant can considerably accelerate the biodegradation[4] and carbohydrate esters exhibit, in general, a very rapid biodegradation.[5]

Selective *O*-acylation of the primary OH group of a suitable functionalised glucoside could be carried out by lipase-catalysis.[6] The subsequent transformation of the functionalised group to a quaternary ammonium salt would offer an attractive route to novel cationic surfactants. We herein report the characterization of a new class of carbohydrate-based quaternary ammonium salt surfactants prepared based on this synthetic strategy.

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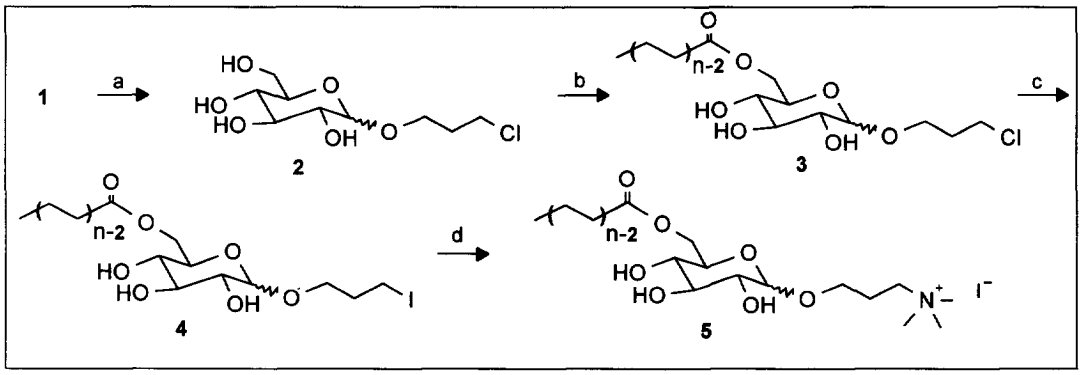
Synthesis

The glucoside **2,** obtained as a 7:3 mixture of the α- and β-anomer, was prepared in a yield of 84 % from α-D-glucose (**1**) using 15 equivalents 3-chloro-propan-1-ol and an acidic catalyst. Immobilized lipase derived from *Candida antarctica* was employed in the esterification procedure.[6] Esterification of glucoside **2** with straight-chain fatty acids containing from 10 to 18 carbon atoms furnished the 6-*O*-monoesters **3** in yields of 85-90%. Direct attempts to alkylate triethylamine with **3** failed, even after a reaction time of 48 hours employing a large excess of the amine. Therefore, the chloride **3** was transformed to the more reactive iodide **4** by a Finkelstein reaction.[7] This step proceeded in quantitative yield following treatment of **3** with 1.2 equiv. of NaI in butanone. The iodide **4** reacted readily by a Mentschutkin reaction[8] with 8 equiv. trimethylamine to afford the quaternary ammonium salt **5** in quantitative yield.[9]



Scheme 1. Reagents and conditions: (a) 3-chloro-propan-1-ol/H^+ resin/80 °C/48 hr/SiO_2 chromatography, (b) 1.8 equiv. $CH_3(CH_2)_nCOOH$ (n= 8, 10, 12, 16)/10 % (w/w) immobilized *C. antarctica* lipase/75 °C/0.01 bar/24 hr/SiO_2 chromatography, (c) 1.2 equiv. NaI/butanone/reflux/24 hr, (d) 8 equiv $(CH_3)_3N$/butanone/r.t./48 hr

Surface-active properties

As indicated in Table 1, the products **5a-e** were all found to have excellent surface-active properties. However, the chain-length of the fatty acyl chain in the 6-*O*-position appeared to have a pronounced effect. It was observed that the dodecanoyl and tetradecanoyl esters (**5b** and **5c**, respectively) exhibited the greatest ability to lower surface tension as well as having the lowest CMC values.

Antimicrobial activity

The compounds were also found to inhibit growth[10] of three selected bacterial and fungal test strains (Table 1). Again, a pronounced effect due to the acyl chain length was observed. The anti-bacterial effect was most pronounced in the case of **5b** which inhibited growth of *Micrococcus luteus, and Vibrio alginolyticus* at

Table 1. Surface active properties and antimicrobial activity of compounds **5**.

Compound	n	CMC (10^{-4} mol/l)	γ_{min} (mN/m)	Minimal inhibitory concentration[10] (MIC, ppm)		
				M. luteus	*V. alginolyticus*	*F. oxysporum*
5a	10	6.9	33.8	2000	2000	-
5b	12	0.46	29.6	10	500	500
5c	14	0.57	27.3	50	2000	-
5d	16	1.2	33.5	1000	2000	-
5e	18	1.5	39.7	-	-	-

levels of 50 and 500 ppm, respectively. Compound **5c** was found to have an almost similar effect while the other compounds worked at the highest dosage level only (2000 ppm). Only **5b** was found to inhibit growth of the fungal test strain *Fusarium oxysporum* at levels higher than 500 ppm. The quaternary ammonium functionality of the compounds was found to be essential for the antimicrobial effect as control experiments performed with esters of ethyl D-glucopyranoside (6) showed very limited activity. Thus, only ethyl 6-*O*-dodecanoyl D-glucopyranoside exhibited some effect but towards *M. luteus* only and at levels higher than 500 ppm. The anti-microbial potency of the new cationic compounds is, however, considerably lower when compared to one of the most frequently used quaternary ammonium salt disinfectants, benzalkonium chloride (a mixture of benzyldimethyldodecylammonium chloride and benzyldimethyltetradecylammonium chloride), as this compound was found to inhibit both bacterial test strains at a level of only 10 ppm and the fungal test strain at 100 ppm. Nevertheless, considering the poor compatibility of benzalkonium chloride with anionic surfactants (as outlined below), the anti-fungal effect of **5b** has, in particular, a very interesting potential.

Compatibility with anionic detergents

When compared to benzalkonium chloride the new surfactants were found to have a pronounced enhanced compatibility with anionic detergents. Thus, no precipitation occurred even in a solution containing both 1 % SDS (sodium dodecyl sulfate) and 1% **5b**. In contrast, instant precipitation took place when adding benzalkonium chloride in levels higher than 125 ppm to solutions containing 500 or 1000 ppm SDS. No precipitation took place in similar experiments adding **5b** at levels up to 1000 ppm. Both types of detergents exerted the same compatibility with sodium decanoate which formed instant precipitates with benzalkonium chloride but not with **5b** even at concentrations of 1%.

Conclusion

In summary, we have developed a highly simple chemoenzymatic synthesis of a new class of carbohydrate-ester based cationic surfactants. The products are effective surface active agents and in particular

the dodecanoyl ester **5b** which also exhibits antimicrobial activity towards both a gram positive, a gram negative and a fungal test strain. The antimicrobial effect is somewhat weaker than that of benzalkonium chloride, one of the most frequently used cationic surfactants for topical disinfection. However, **5b** exhibits a very high degree of compatibility with different anionic detergents thereby providing a much more robust antimicrobial system. Furthermore, as the carbohydrate-ester based products are expected to be readily biodegradable, these properties should encourage further optimization and studies towards commercialization.

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9 Compound **5c**; 3-(trimethylammonio)propyl 6-*O*-dodecanoyl D-glucopyranoside iodide (n=10): colorless syrup; ^{1}H NMR (300 MHz, D_2O): δ 0.82 (broad t, 3H), 1.24 (m, 16H), 1.56 (m, 2H), 2.12 (m, 2H), 2.35 (m, 2H), 3.14 (s, 9H, $-N^+(C\underline{H}_3)_3$), 3.25-3.9 (8H, m), 4.28 (m, 2H), 4.44 (0.3H, d, J=7.5 Hz, H-1β), 4.83 (0.7H, d, J=3.7 Hz, H-1α).

10 Anti-microbial activity: The bacterial strains *Vibrio alginolyticus* (gram negative) and *Micrococcus luteus* (gram positive) were grown in TY media for 16 h at 30 °C (final cell count higher than 10^7/ml). A sample (1.0 ml) was then added test substance at levels of 0, 10, 50, 100, 500, 1000, and 2000 ppm. The samples were allowed to incubate for 30 minutes before plating 10 µl samples on TY agar plates. Growth or no-growth was established by visual inspection of the plates after 16 h incubation at 30 °C. To measure anti-fungal activity, a culture of *Fusarium oxysporum* was used. Wells were made in agar plates containing the fungal spores. 15 µl samples of solutions of the test substances in concentrations of 0, 100, 500, 2500, 7500 and 15000 ppm were added to each well. After incubation for 48 h at 26 °C clearing zones were visually observed as a measure of growth inhibition.

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