Preparation and Properties of a New Type of Carbohydrate-Based Cationic Surfactant

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ABSTRACT: A new type of cationic surfactant, 6-*O*-monoesters of 3-(trimethylammonio)propyl D-glucopyranoside, was prepared in high yield by a simple chemoenzymatic synthesis. Surface-active properties of the compounds were found to be highly dependent on the fatty acyl chain length in the 6-*O*-position with the dodecanoyl and tetradecanoyl esters exhibiting the highest ability to lower surface tension as well as having the lowest critical micelle concentration values. Furthermore, the dodecanoyl ester had excellent foaming properties. The new surfactants also showed antimicrobial activity. Thus, the most potent compound, the dodecanoyl ester, was able to inhibit growth of both bacterial (Gram-positive as well as Gram-negative) and fungal test strains. The antimicrobial effect was somewhat weaker compared to benzalkonium chloride, one of the most frequently used cationics for topical disinfection. However, compared to benzalkonium chloride, the new cationics exhibit a highly improved compatibility with anionic surfactants, as no precipitation took place even in highly concentrated solutions thereby providing a much more robust antimicrobial system. Finally, the new surfactants are expected to be readily biodegradable because they are carbohydrate ester-based.

JSD 1, 37–40 (1998).

KEY WORDS: Antimicrobial activity, carbohydrate esters, cationic surfactants, chemo-enzymatic synthesis, surface-active properties.

An important class of cationic surfactants is the quaternary ammonium salt which is used within specialized areas such as fabric softeners, hair conditioners, and antimicrobial agents (1,2). Unfortunately, most quaternary ammonium cationics available today share some common disadvantages limiting their use. First of all, they show a poor compatibility with other materials, because exposure to anionic surfactants and soaps inhibits their action by forming insoluble complexes (3). Furthermore, many available compounds show poor biodegradation under practical sewage plant conditions (4,5).

Incorporation of an additional noncharged hydrophilic group into a cationic surfactant has been shown to provide one way of increasing the compatibility of the surfactant with other ingredients (3). In this context, the incorporation of a carbohydrate moiety appears very attractive because the hydrophilic groups are very concentrated when compared to a polyethoxylate unit, for example. Furthermore, if the hydrophobic part of the surfactant is attached by an ester bond to the carbohydrate, the resulting compound is expected to be readily biodegradable. Giving validity to this expectation, it has recently been shown that incorporation of ester functionality in a cationic surfactant accelerates the biodegradation considerably (5) and glucoside esters exhibit, in general, a very rapid biodegradation (6). Selective acylation in the 6-*O*-position of simple glucosides can be achieved by a very simple lipase-catalyzed process using the molten fatty acid as solvent (7). Provided that a functional group which could be transformed into a quaternary ammonium functionality was incorporated into the aglucone moiety of the glucoside, the resulting ester would be an attractive intermediate in the synthesis of a cationic surfactant. We herein report the functional characterization of a new type of carbohydrate-based quaternary ammonium salt surfactant synthesized according to the above strategy.

EXPERIMENTAL PROCEDURES

Materials. Unless otherwise specified, all chemicals and media were obtained from Merck (Darmstadt, Germany). Immobilized *Candida antarctica* B-lipase catalyst was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark) as Novozyme 435.

Analytical methods. Nuclear magnetic resonance (NMR) experiments were performed employing a Bruker ACP 300 NMR spectrometer (Bruker Instruments, Inc., Karlsruhe, Germany) using D_2O as solvent and trimethylsilane as internal standard. Operating frequency was 300 MHz. Thin-layer chromatography (TLC) analysis was performed using SiO_2 plates (Kieselgel 60, cat. 5554, Merck, Darmstadt, Germany) using toluene/ethyl acetate/methanol 8:6:3 (vol/vol/vol) as mobile phase. After drying, detection was performed by spraying with H_2SO_4 and heating to 100°C on a heating plate. High-performance liquid chromatography (HPLC) analyses were performed by using a SiO_2-NH_2 column with 96% EtOH as eluent and by using a refractive index detector as previously described (7).

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Synthesis of 3-chloropropyl D*-glucopyranoside* (**2**). The α-D-glucose (**1**) (20 g, 111 mmol) and acidic ion exchange resin (Amberlyst® A-15, H^+-form, 6 g) were suspended by stirring in 3-chloro-1-propanol (100 mL, 1.18 mol) at 80°C. Stirring was continued until TLC indicated full conversion. Purification by chromatography on SiO_2 using a gradient of heptane/ethyl acetate/methanol gave a colorless viscous syrup in 84% yield (23.9 g). The product (**2**) was found to be a 7:3 mixture of the α- and β-anomers using ^1H NMR spectroscopy as indicated by the signals at δ 4.41 (0.3H, *d, J* = 8Hz, H-1β) and 4.87 (0.7H, *d, J* = 4Hz, H-1α), respectively.

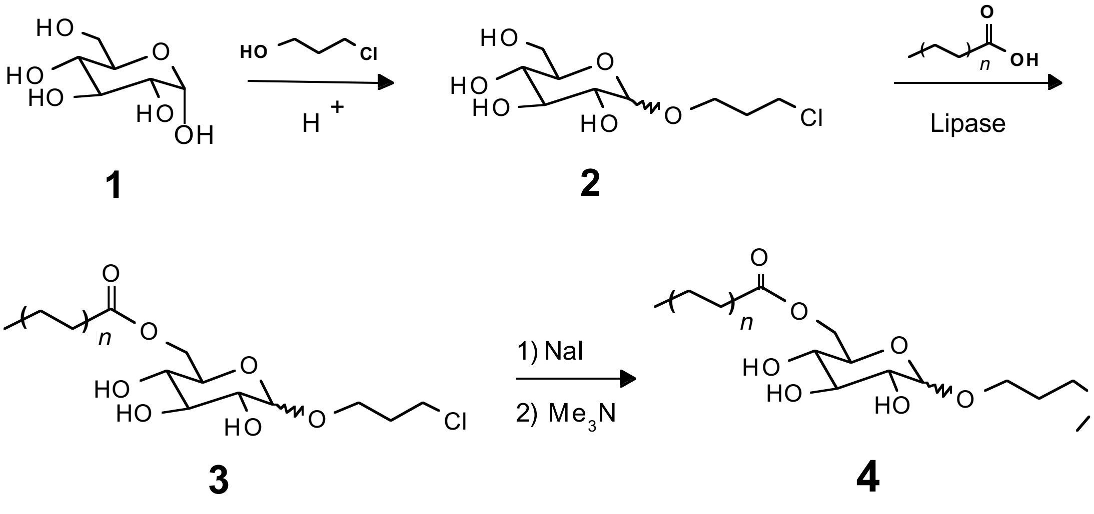
*Synthesis of 3-chloropropyl 6-*O*-alkanoyl-*D*-glucopyranoside* (**3**); *general procedure.* Compound **2** (15.6 g, 61 mmol) was dissolved in melted fatty acid (e.g., dodecanoic acid, 40 g, 0.2 mol) at 75°C. Immobilized *C. antarctica* B-lipase (5 g) was added, and the mixture was stirred at reduced pressure (0.01 bar) until HPLC indicated more than 90% conversion (typically 16–24 h). The reaction mixture was diluted with acetone (100 mL), filtered, and concentrated. The product (**3**) was purified by chromatography on SiO_2 as above, and the identity was confirmed by ^1H NMR.

*Synthesis of 3-(*N,N,N*-trimethylammonio)propyl 6-*O*-alkanoyl-*D*-glucopyranoside* (**4**); *general procedure.* Compound **3** (5.5 mmol, e.g., 3-chloropropyl 6-*O*-dodecanoyl-D-glucopyranoside, 2.4 g) and sodium iodide (0.85 g, 5.7 mmol) was dissolved in 2-butanone (40 mL), and the solution was boiled for 24 h. The precipitated sodium chloride was removed by filtration. Trimethylamine (4 mL, 40 mmol) was cooled to 0°C and then added to the solution with stirring until TLC indicated full conversion. The solvent was removed *in vacuo* giving the product **4** as a colorless syrup in quantitative yield. ^1H NMR (300 MHz, D_2O); 3-(trimethylammonio)propyl 6-*O*-dodecanoyl D-glucopyranoside iodide: δ 0.82 (*br t*, 3H), 1.24 (*m*, 16H), 1.56 (*m*, 2H), 2.12 (*m*, 2H), 2.35 (*m*, 2H), 3.14 (*s*, 9H, $-N^+(CH_3)_3$), 3.25–3.9 (8H, *m*), 4.28 (*m*, 2H), 4.44 (0.3H, *d, J* = 7.5Hz, H-1β), 4.83 (0.7H, *d, J* = 3.7Hz, H-1α).

Surface-active properties. The surface tension of the surfactant solutions was measured at 20°C using a Sigma 70 tensiometer (KSV Instruments, Helsinki, Finland). The critical micelle concentration (CMC) was determined from the break point of each surface tension vs. concentration (log scale) curve. The ability to lower surface tension (γ_{min}) is based on the surface tension at the CMC. Foaming properties were measured using the standard Ross-Miles Test (ASTM-D-1173-53; Ref. 8) at ambient temperature using a 1 g/L solution of the test substance in distilled water.

Antimicrobial activity. The bacterial strains *Vibrio alginolyticus* (Gram-negative) and *Micrococcus luteus* (Gram-positive) were grown in TY media (Difco, Detroit, MI) for 16 h at 30°C (final cell count higher than 10^7/mL). To 1.0-mL culture aliquots 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 min before plating 10-µL samples on TY agar plates (Difco). Growth or no-growth was established by visual inspection of the plates after 16 h incubation at 30°C. To measure antifungal activity, a culture of *Fusarium oxysporum* was used. Wells were made in agar plates (art. no. 5270 Merck) containing the fungal spores. Samples (15 µL) of solutions of the test substances in concentrations of 0, 100, 500, 2500, 7500, and 15,000 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.

Compatibility with anionic surfactants. All tests were performed using 200-µL solutions made in a 0.1 M sodium phosphate buffer pH 7 in 96-well PolySorp™ micro-titer plates (Nunc, Roskilde, Denmark). The level of the test substances was adjusted to 0, 15, 30, 60, 125, 250, 500, or 1000 ppm and the level of sodium dodecyl sulfate (SDS) was adjusted to 500 or 1000 ppm. Alternatively, the level of both the test substance and SDS or sodium decanoate was adjusted to 1%. Following incubation for 5 min at ambient temperature and mixing, precipitation was measured



SCHEME 1

by reading the absorption at 650 nm on a Kinetic Microplate Reader (Molecular Devices, Menlo Park, CA).

RESULTS AND DISCUSSION

Synthesis. 6-*O*-esters of 3-(*N*,*N*,*N*-trimethylammonio)propyl D-glucopyranoside (**4**) were synthesized according to the route outlined in Scheme 1. 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-chloropropan-1-ol and an acidic catalyst. Lipase-catalyzed esterification with straight-chain fatty acids containing from 10 to 18 carbon atoms using an immobilized lipase derived from *C. antarctica* (6) as catalyst proceeded smoothly, affording the 6-*O*-monoesters **3** in yields of 85–90%. As direct attempts to alkylate trimethylamine with **3** failed, even after a reaction time of 48 h employing a large excess of the amine, the chloride **3** was transformed by a Finkelstein reaction (9) to the more reactive iodide which reacted readily by a Mentschutkin reaction (10) to afford the quaternary ammonium salt **4** in quantitative yield.

*Surface-active properties***.** As indicated in Table 1, the products **4a–e** were all found to have excellent surface-active properties. However, a pronounced effect of the fatty acid chain length in the 6-*O*-position is observed with the dodecanoyl and tetradecanoyl esters (**4b** and **4c**, respectively), exhibiting the highest ability to lower surface tension as well as having the lowest CMC values. The foaming properties of the most effective surfactant **4b** were evaluated using the standard Ross-Miles test. When compared to SDS, **4b** showed equivalent good foaming ability indicated by almost identical initial foam heights. However, **4b** produced a much more stable foam in that the volume after 5 min was reduced by less than 10% while the foam of the SDS solution was reduced by more than 80%.

Antimicrobial activity. The new surfactants were found to inhibit growth of bacterial and fungal test strains (Table 2). Again, a pronounced effect of the acyl chain length was observed. The antibacterial effect was most pronounced in the case of **4b** which inhibited growth of *M. luteus*, and *V. alginolyticus* at levels of 50 and 500 ppm, respectively. Compound **4c** was found to have an almost similar effect while **4a** and **4d** gave effect at the highest dosage level only (2000 ppm), and **4e** gave no effect even at this level. Only **4b** was found to inhibit growth of the fungal test strain *F. oxysporum* at levels higher than 500 ppm. Antimicrobial potency of the new compounds is, however, considerably lower compared to one of the most frequently used quaternary ammonium salt disinfectants, benzalkonium chloride (a mixture of benzyldimethyldodecylammonium chloride and benzyldimethyltetradecylammonium chloride). 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. Even so, taking the poor compatibility of benzalkonium chloride with anionic surfactants into consideration (as outlined below), the antifungal effect of **4b** has a particularly interesting potential.

TABLE 1
Surface-Active Properties of Compounds 4[a]

Compound	n	6-*O*-Acyl side chain	CMC (10^{-4} mol/L)	$\gamma_{\min}$ (mN/m)
4a	8	Decanoyl	6.9	33.8
4b	10	Dodecanoyl	0.46	29.6
4c	12	Tetradecanoyl	0.57	27.3
4d	14	Hexadecanoyl	1.2	33.5
4e	16	Octadecanoyl	1.5	39.7

[a] CMC, critical micelle concentration.

TABLE 2
Antimicrobial Activity of Compounds 4

Compound	Minimal inhibitory concentration (ppm) *Micrococcus luteus*	*Vibrio alginolyticus*	*Fusarium oxysporum*
4a	2000	2000	—
4b	10	500	500
4c	50	2000	—
4d	1000	2000	—
4e	—	—	—

Compatibility with anionic surfactants. When compared to benzalkonium chloride, the new surfactants were found to have a pronounced and enhanced compatibility with anionic detergents. No precipitation occurred even in a solution containing both 1% SDS and 1% **4b**. 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 **4b** 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 **4b** even at concentrations of 1%.

Even though the antimicrobial effect of the new cationic surfactants, as outlined above, is somewhat weaker compared to benzalkonium chloride, the dramatically improved compatibility with anionic surfactants makes it possible to utilize the effect in much more complex matrices, thereby making the antimicrobial system much more robust. Furthermore, as the carbohydrate ester-based products are expected to be readily biodegradable, these properties should encourage further optimization and studies toward commercialization.

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[Received March 10, 1997; accepted September 23, 1997]

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