New alquil amide type cationic surfactants from arginine

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The synthesis, stability, surface activity and antimicrobial properties of a new family of cationic surfactants (the long chain arginylalkylamide dihydrochloride salts) derived from the condensation of the amino acid arginine and a long chain alkylamine are described. The surface active parameters reported are c.m.c. (critical micellar concentration), pC_{20} (negative log of the surfactant molar concentration required to reduce the surface tension of the solvent by 20 mN m^{-1}), $\gamma_{c.m.c.}$ (the surface tension at the c.m.c.), Γ_{max} (the maximum surface excess concentration) and A_{min} (the minimum area per surfactant molecule at the interface). These data and those obtained from the evaluation of the antimicrobial properties are compared with the data corresponding to another family of cationic surfactants reported earlier by our group: the long chain N^{α}-acylarginine methyl ester salts.

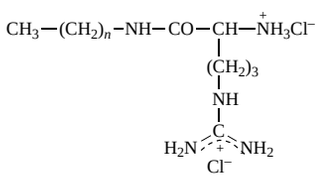
Moreover, the synthesis of analogues possessing a reactive group capable of bonding to wool or cotton fibres is described: the long chain N^{α}-dichlorotriazinylarginylalkylamide monohydrochloride salts. We expect these compounds to bond to the textile substrate by the formation of a covalent bond. Confirmation of this is, however, necessary.

Introduction

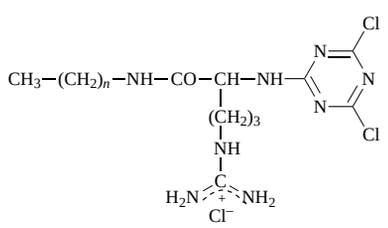
Natural proteinaceous fibres such as wool are susceptible to biological degradation by bacteria and to attack by insects such as moths. In order to increase the lifetime of woollen items, the wool needs to be protected from such agencies by antimicrobial and mothproofing finishing treatments. However, traditional antimicrobial and mothproofing finishing systems like quaternary ammonium surfactants[1] have recently become less desirable from an environmental viewpoint and the use of systems based on, *e.g.* chlorinated phenols, regardless of their effectiveness, is severely limited by environmental legislation. Given the significance of this field, the search for new biocompatible alternatives constitutes an important challenge to wool researchers.[2,3]

It is well known that amino acid derivatives with alkyl chains from fatty acids[4] or fatty amines[5,6] of varying chain length (C_{12}–C_{16}) produce biocompatible lipoaminoacids with an interesting surface active behaviour. Our group has, since 1985, undertaken research and development of cationic surfactants of the long chain N^{α}-acyl arginine alkyl ester type with excellent antimicrobial and non-toxic properties. It has been demonstrated that the antimicrobial activity is directly associated with the presence of a critical alkyl chain length of C_{12} carbon atoms and the cationic charge of the protonated guanidino group of the arginine available for interaction with the cell membrane in the molecule.[7–10]

In this paper, the synthesis, surface activity and antimicrobial properties of a new family of cationic surfactants of the type long chain arginylalkylamide dihydrochloride salts, [H–Arg-NH–C$_n$]·2HCl with n = 10, 12, 14 and 16 are described. These data will be compared with those of the long chain N^{α}-acylarginine methyl ester salts reported earlier.[7] The surface active parameters studied include c.m.c. (critical micellar concentration), pC_{20} (negative log of the surfactant molar concentration required to reduce the surface tension of the solvent by 20 mN m^{-1}), $\gamma_{c.m.c.}$ (the surface tension at the c.m.c.), Γ_{max} (the maximum surface excess concentration at the air/aqueous solution interface) and A_{min} (the minimum area per surfactant molecule at the air/aqueous solution interface). Moreover, the synthesis of their analogues, possessing a reactive group capable of bonding to wool or cotton fibres and thus endowing them with permanent properties is also described: the long chain N^{α}-dichlorotriazinyl arginylalkylamide monohydrochloride salts, [DCT–ArgNH–C$_n$]·HCl with n = 12 and 14.

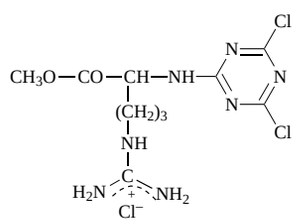


n = 9, 11, 13, 15



n = 11 and 13

It is expected that if a reactive electrophilic grouping such as the dichlorotriazine DCT is incorporated into the arginylalkylamide cationic surfactant, the resulting compound will bond to a textile substrate such as wool or cotton by way of a covalent bond in a manner analogous to that of a reactive dye.[11–14] The analogue N^{α}-dichlorotriazinylarginine methyl ester monohydrochloride salt [H–DCT–ArgOMe]·HCl (without alkylic



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chain) was also prepared in order to use it as a model to study the stability of the triazine reactive group. Data on this parameter are also reported.

The new class of compounds, whose synthesis is described in this work, contains a fatty amine of C_{10}–C_{16} chain length condensed to the α-carboxyl group of the arginine dihydrochloride and optionally the 4,6-dichlorotriazin-2-yl grouping (–DCT) attached to the α-amino group of the arginylalkylamide homologue. Arginine was combined with fatty amines in order to leave the α-amino group free to react with the triazinyl reactive compound. All these compounds were designed as new antimicrobial alternative surfactants of a presumably low toxicity for application as possible antibacterial agents to materials such as wool or cotton. Treatments of these amphiphiles with wool fibres are in progress and the results will be reported in the near future.

Experimental

Synthesis

N^{α}-*tert*-Butoxycarbonyl arginine monohydrochloride [Boc–Arg]·HCl (extra pure grade) was supplied by Novabiochem; triethylamine (TEA), dimethylformamide (DMF) and trifluoroacetic acid (TFA) (all of synthesis grade) were supplied by Merck; [benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate] reagent (BOP) was supplied by Neosystem Laboratoire; 1-decylamine, 1-dodecylamine, 1-tetradecylamine and 1-hexadecylamine (99%) were supplied by Fluka; 2,4,6-trichloro-1,3,5-triazine grouping [TCT] was supplied by Aldrich. All the other solvents, acids, alkalis and electrolytes used were general purpose grade.

TLC analysis: the following TLC systems were used throughout the experiments performed in this work to monitor the progress of the reaction. Solvent: chloroform–methanol (1:1). Substrate: aluminium backed silica gel sheets (Merck). The plates were developed using the following techniques. (*a*) Ninhydrin: this detects the presence of primary amino groups; (*b*) chlorine–toluidine: this detects any compounds containing nitrogen after chlorine bleaching and spraying with toluidine; (*c*) sakaguchi: (α-naphthol-bromine): this detects the presence of the arginyl guanidino group.

The purity of intermediate and final compounds was checked by HPLC, capillary electrophoresis (CE) and elemental analysis.

HPLC conditions: a Merck–Hitachi Model L-6200 liquid chromatograph was used with an injection valve fitted with a 20 μl loop. The detection system consisted of a UV–VIS detector, 210 nm wavelength Merck–Hitachi Model (L-4250). Chromatography was accomplished by using a Lichrospher 100 RP-18 (5 μm) in LichroCart 125-4 column (Merck). Flow rate: 1 ml min^{-1}. The mobile phase was a solvent gradient 50–100% B in 25 min, A = 0.1% trifluoroacetic acid (TFA) in water, B = 0.21% TFA in acetonitrile (ACN).

CE conditions: the separations were performed with an Applied Biosystems Model 270-A (USA) apparatus. A single column was used with 72 cm × 50 μm id, uncoated fused silica from Composite Metal Services (Works, UK). The separation unit was equipped with a UV–VIS detector operating at a 210 nm wavelength. Separation was performed at 5 kV applied voltage; aqueous buffer 0.05 M sodium dihydrogenphosphate dihydrate, pH = 4.5, 50% ACN as organic modifier, 0.5 s injection time.[15]

Melting points were determined with a FP81 measuring cell of the FP90 Mettler System. Optical rotations were measured with a 141 Perkin-Elmer spectropolarimeter (Norwalk, CT).

The structures of the intermediate and final products were checked by ^{1}H and ^{13}C NMR analyses, which were recorded with a Gemini 300 MHz spectrometer. The IR spectra were recorded on an IRFT Nicolet 510 spectrophotometer.

The UV spectra were recorded on a UV–VIS recording spectrophotometer 265FW Shimadzu.

General procedure for the preparation of the long chain arginylalkylamide dihydrochloride salts [H–ArgNH–C_n]·2HCl

Synthesis of long chain N^{α}-*tert*-butoxycarbonylarginylalkylamide monohydrochloride salts: [Boc–ArgNH–C_n]·HCl. Boc–Arg (10.0 g, 30 mmol) was dissolved in DMF (130 ml) at 25 °C. To this solution triethylamine (8.4 ml, 30 mmol) was added. After 30 min, the corresponding 1-alkylamine (30 mmol) was added, and finally BOP (13.28 g; 30 mmol). The reaction mixture was left stirring overnight at 25 °C, after which time TLC analysis was performed. The presence of a new arginyl derivative product in the reaction mixture was demonstrated.

The reaction mixture containing a precipitate was filtered under vacuum and TLC analysis showed that the solid collected was triethylamine hydrochloride. The DMF was evaporated from the filtrate to yield an oil. Diethyl ether was added and left in a freezer overnight, which resulted in the formation of a precipitate that was isolated by filtration. The solid was washed successively with diethyl ether, acetone, ethyl acetate and diethyl ether. The residue was dissolved in methanol–hexane (1:9) and washed with H_2O. The organic layer was evaporated to dryness under vacuum, dissolved in ethyl acetate and evaporated again to yield a white solid. Although routine HPLC analysis of these compounds was not possible because of their strong hydrophobic adsorption to the solid phase, [Boc–ArgNH–C_n]·HCl yielded one peak by CE.‡ N^{α}-*tert*-Butoxycarbonylarginyldecylamide monohydrochloride, [Boc–ArgNH–C_{10}]·HCl, *M* 450.064, yield 40%, R_f ($CHCl_3$–MeOH 50:50) 0.64; mp 133.6 °C (Found: C, 56.03; H, 9.96; N, 15.51. Calc. for $C_{21}H_{44}O_3N_5Cl\cdot\frac{1}{2}H_2O$: C, 56.04; H, 9.85; N, 15.56%); $[a]^D$ −6.60 (2% MeOH); CE t_M = 47.11 min. N^{α}-*tert*-Butoxycarbonylarginyldodecylamide monohydrochloride, [Boc–ArgNH–C_{12}]·HCl, *M* 478.118, yield 76%, R_f ($CHCl_3$–MeOH 50:50) 0.67; mp 140.7 °C (Found: C, 56.81; H, 10.03; N, 14.48. Calc. for $C_{23}H_{48}O_3N_5Cl\cdot\frac{1}{2}H_2O$: C, 56.66; H, 10.36; N, 14.31%); $[a]^D$ −5.48 (2% MeOH); CE t_M = 48.81 min. N^{α}-*tert*-Butoxycarbonylarginyltetradecylamide monohydrochloride, [Boc–ArgNH–C_{14}]·HCl, *M* 506.172, yield 52%, R_f ($CHCl_3$–MeOH 50:50) 0.69, mp 167.2 °C (Found: C, 58.90; H, 10.43; N, 13.71. Calc. for $C_{25}H_{52}O_3N_5Cl\cdot\frac{1}{2}H_2O$: C, 59.32; H, 10.35; N, 13.84%); $[a]^D$ −10.75 (2% MeOH); CE t_M = 50.34 min. N^{α}-*tert*-Butoxycarbonylarginylhexadecylamide monohydrochloride, [Boc–ArgNH–C_{16}]·HCl, *M* 534.226, yield 65.5%, R_f ($CHCl_3$–MeOH 50:50) 0.60, mp 159.4 °C (Found: C, 62.26; H, 11.23; N, 11.27. Calc. for $C_{27}H_{56}O_3N_5Cl\cdot\frac{1}{2}H_2O$: C, 60.70; H, 10.57; N, 13.11%); $[a]^D$ −6.1 (2% MeOH); CE t_M = 52.17 min.

Spectral characteristics of long chain N^{α}-tert-*butoxycarbonylarginylalkylamide monohydrochloride salts,* [Boc–ArgNH–C_n]·HCl.—ν(KBr)/cm^{-1}—3280 (*NH*), 3159 (*NH* amide), 2927 (CH_2), 1686 (*CO*–N amide), 1510 (*CO*–N amide Boc-group); δ_H(300 MHz, $C_2D_6O_5$) 0.80 (t, 3H, CH_3), 1.18 (m, 18H, CH_2), 1.33 (s, 9H, CH_3 Boc), 1.4–1.7 (m, 3H, CH_2), 2.99 (m, 5H, CH_2–NH), 3.80 (m, 1H, CH), 6.69 (m, 1H, NH Boc), 7.10 (m, 4H, NH_2 guanidine group), 7.73 (m, 2H, NH arginine); δ_C(300 MHz, $C_2D_6O_5$) 12.94 (CH_3), 21.11–30.30 (CH_2), 27.79–27.99 (CH_3, Boc), 52.88 (CH), 76.98 (C Boc group), 154.22 (*CO* Boc group), 156.04 (*C*=*N* guanidine group), 170.62 (*CO* amide).

Synthesis of long chain arginylalkylamide dihydrochloride salts [H–ArgNH–C_n]·2HCl. Boc–ArgNH–C_n·HCl (4.9 mmol) was dissolved in trifluoroacetic acid (TFA) (12.2 ml), at 4–5 °C. The mixture was left stirring for 30 min at 4–5 °C, after which time diethyl ether was added. The remaining TFA was elimin-

‡ CE specific conditions: aqueous buffer 0.05 M sodium dihydrogenphosphate dihydrate, pH = 4.5, 75% THF, 1 s injection time, 15 kV applied voltage.

ated by passing N_2 through the mixture. Then, methanol–HCl (0.5 M) was added and stirred for five minutes. The remaining HCl and the methanol were eliminated by evaporation and the resulting oil-paste was dissolved in water and dried in the freeze dryer yielding a pure solid by analytical HPLC and CE. Arginyldecylamide dihydrochloride [H–ArgNH–C_{10}]·2HCl, *M* 386.408, R_f ($CHCl_3$–MeOH 50 : 50) 0.33 (Found: C, 47.43; H, 9.79; N, 17.28. Calc. for $C_{16}H_{37}ON_5Cl_2·H_2O$: C, 47.52; H, 9.72; N, 17.32%); $[\alpha]^D$ 22.3 (2% MeOH); HPLC t_R = 1.75 min, CE t_M = 35.79 min. Arginyldodecylamide dihydrochloride [H–ArgNH–C_{12}]·2HCl, *M* 414.456, R_f ($CHCl_3$–MeOH 50 : 50) 0.36, mp 110–115 °C (Found: C, 48.13; H, 10.14; N, 15.50. Calc. for $C_{18}H_{41}ON_5Cl_2·H_2O$: C, 47.99; H, 10.06; N, 15.54%); $[\alpha]^D$ 15.3 (2% MeOH), HPLC t_R = 21.28 min,§ CE t_M = 36.66 min. Arginyltetradecylamide dihydrochloride [H–ArgNH–C_{14}]·2HCl, *M* 442.510, R_f ($CHCl_3$–MeOH 50 : 50) 0.37, mp 132 °C (Found: C, 52.22; H, 10.70; N, 15.16. Calc. for $C_{20}H_{45}ON_5Cl_2·H_2O$: C, 52.16; H, 10.29; N, 15.21%); $[\alpha]^D$ 16.68 (2% MeOH), HPLC t_R = 5.80 min, CE t_M = 37.53 min. Arginylhexadecylamide dihydrochloride [H–ArgNH–C_{16}]·2HCl, *M* 470.564, R_f ($CHCl_3$–MeOH 50 : 50) 0.47, mp 155–160 °C (Found: C, 57.78; H, 11.51; N, 11.93. Calc. for $C_{22}H_{49}ON_5Cl_2$: C, 56.15; H, 10.50; N, 14.88%); [*α*] 10.47 (2% MeOH), HPLC t_R = 11.66 min, CE t_M = 38.55 min.

Spectral characteristics of long chain arginylalkylamide dihydrochloride salts [H–ArgNH–C_n]·2HCl.—*ν*(KBr)/cm^{-1} 3355 (*NH*), 3178 (*NH* amide), 2917 (CH_2), 1663 (*CO*–N amide); δ_H(300 MHz, $C_2D_6O_5$) 0.83 (t, 3H, CH_3), 1.22 (m, 14H, CH_2), 1.3–1.9 (m, 6H, CH_2), 3–3.2 (m, 4H, CH_2–NH), 3.8 (m, 1H, CH), 7.34 (m, 4H, NH_2 guanidine group), 8.00 (m, 1H, NH arginine), 8.35 (m, 2H, NH_2), 8.75 (m, 1H, NH amide); δ_C(300 MHz, $C_2D_6O_5$) 13.97 (CH_3), 22.11–31.32 (CH_2), 51.56 (CH), 157.12 (*C*=*N* guanidine group), 168.07 (*CO* amide).

General procedure for the preparation of N^{α}-dichlorotriazinyl-arginylalkylamide monohydrochloride salts [DCT–ArgNH– C_n]·HCl

[H–ArgNH–C_n]·2HCl (*n* = 10, 12) (2.6 mmol) was dispersed in DMF (7 ml). Then, triethylamine was added (equimolar) and the mixture was left stirring overnight at 25 °C. The reaction mixture was filtered to remove the triethylamine hydrochloride and then DMF was evaporated from the filtrate containing the arginylalkylamide monohydrochloride [H–ArgNH–C_n]·HCl.

[H–ArgNH–C_n]·HCl (2.6 mmol) was dissolved in water–acetone (1 : 1) (20 ml), and was added dropwise to a solution containing TCT in 21 ml of acetone (equimolar + 10%) at 4–5 °C. Sodium carbonate (0.5 equimolar) was added in order to hold the pH at about 7 throughout the reaction. Then, the reaction was left stirring at 4–5 °C for one hour, after which time the presence of the new product was demonstrated by HPLC analysis.

The acetone was evaporated from the reaction mixture, which was dried in the freeze dryer yielding a white solid. The pure product was obtained from this solid by successive extraction with acetone. N^{α}-Dichlorotriazinylarginyldodecylamide monohydrochloride [DCT–ArgNH–C_{12}]·HCl, *M* 525.953, R_f ($CHCl_3$–MeOH 50 : 50) 0.59, mp 137 °C (Found: C, 45.14; H, 7.46; N, 20.81; Cl, 17.48. Calc. for $C_{21}H_{39}ON_8Cl_3·2H_2O$: C, 44.84; H, 7.65; N, 19.93; Cl, 18.93%); HPLC t_R = 12.97 min. N^{α}-Dichlorotriazinylarginyltetradecylamide monohydrochloride [DCT–ArgNH–C_{14}]·HCl, *M* 554.006, R_f ($CHCl_3$–MeOH 50 : 50) 0.59, mp 145 °C (Found: C, 44.68; H, 7.33; N, 17.62; Cl, 16.23. Calc. for $C_{23}H_{43}ON_8Cl_3·3H_2O$: C, 45.39; H, 8.06; N, 18.42; Cl, 17.49%); HPLC t_R = 15.84 min.

Spectral characteristics of long chain N^{α}*-dichlorotriazinyl-arginylalkylamide monohydrochloride salts* [DCT–ArgNH–C_n]·HCl.—*ν*(KBr)/cm^{-1} 3413 (*NH*), 3294 (*NH* amide), 2923 (CH_2), 1655–1700 (*CO* amide) and (*C*=*N* DCT group); δ_H(300 MHz, [$^2H_4$]methanol) 0.894 (t, 3H, CH_3), 1.28 (m, 18H, CH_2), 1.4–2.0 (m, 6H, CH_2), 3.23 (m, 4H, CH_2–NH), 4.45 (m, 1H, CH); δ_H(300 MHz, [$^2H_6$]acetone) 0.86 (t, 3H, CH_3), 1.26 (m, 18H, CH_2), 1.4–1.9 (m, 6H, CH_2), 3.2–3.4 (m, 4H, CH_2–NH), 4.60 (m, 1H, CH), 7.08 (m, 4H, NH_2 guanidine group), 8.14 (m, 2H, NH); δ_C(300 MHz, [$^2H_6$]acetone) 14.32 (CH_3), 23.27–46.81 (CH_2), 55.78 (CH), 158.40 (*C*=*N* guanidine group), 166.83 (*CO* amide), 170.32, 170.99, 171.65 (*C*=*N* DCT group).

Procedure for the preparation of N^{α}-dichlorotriazinylarginine methyl ester monohydrochloride salt [DCT–ArgOMe]·HCl

Arginine methyl ester dihydrochloride (H–ArgOMe) (1 g, 3.83 mmol) was dispersed in DMF (3.2 ml) at 25 °C. Then, triethylamine (0.53 ml, 3.83 mmol) was added and the reaction mixture was left stirring overnight at 25 °C.

The reaction mixture was then filtered and TLC analysis showed that the solid collected was triethylamine hydrochloride. Then, the DMF was evaporated from the filtrate which contains the arginine methyl ester monohydrochloride salt to yield an oil. This oil was dissolved in water–acetone (1 : 1) and was added dropwise to a solution containing TCT (0.73 g, 3.96 mmol) in acetone. The pH was held at about 7–8 with sodium carbonate throughout the reaction. The reaction mixture was left stirring at 4–5 °C for one hour. Some more acetone was added to the reaction mixture which was left in the freezer overnight. This resulted in the precipitation of sodium carbonate and sodium chloride. The reaction mixture was filtered and the acetone was evaporated to obtain an aqueous medium in which the residual TCT precipitated. This precipitate was isolated by filtration. Finally, the filtrate was dried in the freeze dryer yielding a solid. *M* 371.04, yield 59.3%, R_f ($CHCl_3$–MeOH 50 : 50) 0.52 (Found: C, 32.66; H, 4.64; N, 25.13; Cl, 27.07. Calc. for $C_{10}H_{16}O_2N_7Cl_3$: C, 32.34; H, 4.35; N, 26.42; Cl, 28.27%); $[\alpha]^D$ −25.77 (2% MeOH); HPLC t_R = 18.60 min;¶ CE t_M = 10.6 min.‖

Spectral characteristics of N^{α}*-dichlorotriazinylarginine methyl ester monohydrochloride salt* [DCT–ArgOMe]·HCl.—*ν*(KBr)/cm^{-1} 1700.8 (*C*=*O* ester), 1649.9 (*C*=*N* DCT group); δ_H(300 MHz, DMSO) 1.55 (m, 2H, CH_2), 1.81 (m, 2H, CH_2), 3.12 (m, 2H, CH_2–NH), 4.42 (m, 1H, CH), 7.24 (m, 4H, NH_2 guanidine group), 7.87 (m, 1H, NH arginine), 9.54 (d, 1H, CH–*NH*–DCT); δ_C(300 MHz, D_2O) 25.941, 28.36, 35.99 [(CH_2)$_3$–CH], 52.55 (O–CH_3), 54.92 (CH), 158.35 (*C*=*N* guanidine group), 166.70 (*C*=*O* methyl ester), 170.19, 170.61, 171.84 (*C*=*N* DCT group).

Stability

The stability of [DCT–ArgOMe]·HCl and [DCT–ArgNH– C_n]·HCl as a function of pH and temperature was evaluated by UV spectrometry at 232 nm and ^{13}C NMR spectroscopy. Given that [DCT–ArgNH–C_n]·HCl compounds are not soluble in water even at very low concentrations, these compounds were suspended in an aqueous solution at the appropriate pH and temperature.

Surface active properties

The surface tension measurements at equilibrium (γ) were determined with a tensiometer (Krüss K-12) with a Wilhelmy plate. All solutions, at different surfactant concentrations, were prepared with deionized water and allowed to equilibrate for 2 h at 25 °C in appropriate cells. Effectiveness of adsorption (pC_{20}), critical micellar concentration (c.m.c.), maximum

§ The mobile phase was a solvent gradient 20–100% B in 40 min, A = 0.1% TFA, 70 μl l^{-1} TFA in water, B = 0.085% TEA, 70 μl l^{-1} TEA in ACN.

¶ The mobile phase was a solvent gradient 5–70% B in 32 min, A = 0.1% TFA in water, B = 0.085% TFA, in ACN–H_2O 80 : 20.

‖ Aqueous buffer 0.05 M sodium dihydrogenphosphate dihydrate, pH = 4.5, 25 kV applied voltage, 5 s injection time.

surface excess concentration (Γ_{max}) and area per molecule (A_{min}) were calculated from the γ/log C curves at equilibrium.[16]

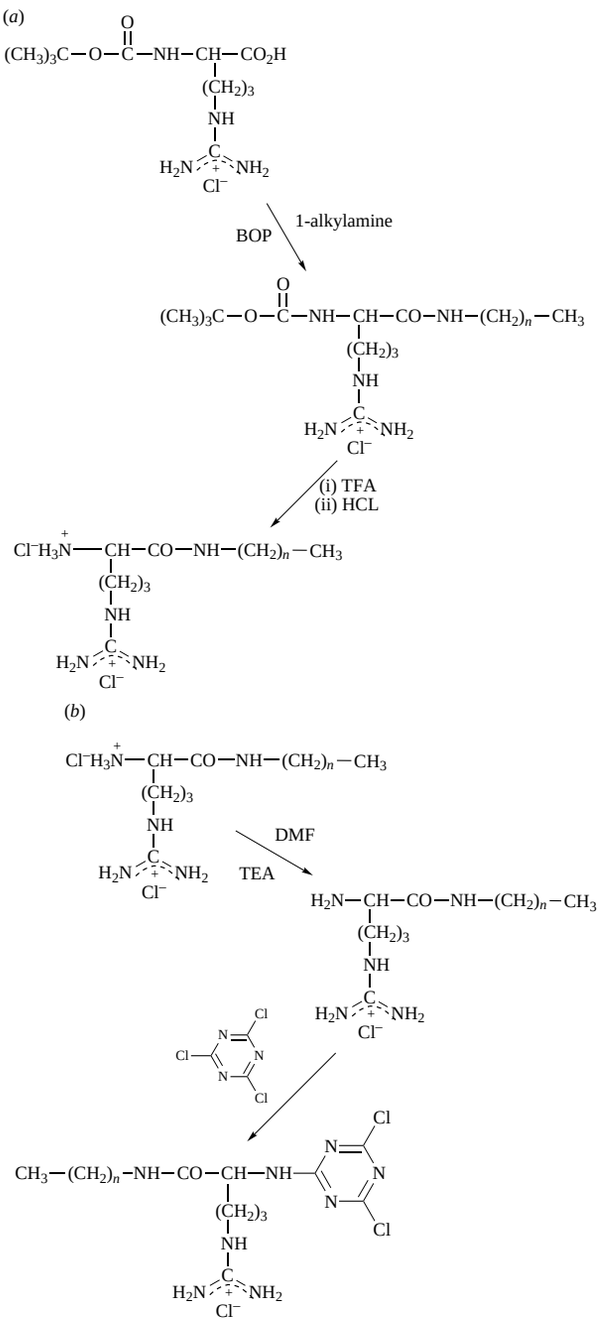
Antimicrobial properties

The minimum inhibitory concentration values (m.i.c.) were determined by using a 'microtriters' system (disposable multiple-well plates. Reg: 25850-96, Corning Lab. Sci. Co., USA) consisting of a plate containing a series of cups classified in files and columns. Each cup is filled with the same concentration of microorganism in Müller Hilton broth and a variable concentration of surfactant. The growth of each microorganism is visually controlled at 24 h of incubation at 37 °C.

Results and discussion

Synthesis

The synthetic pathways for the preparation of [H–ArgNH–C_n]·2HCl (n = 10, 12, 14 and 16) as dihydrochloride salts and [DCT–ArgNH–C_n]·HCl (n = 12, 14) as monohydrochloride salts are outlined in Scheme 1(*a*) and (*b*), respectively.



Scheme 1

In this strategy the heart of the synthesis involves the use of (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluoroposphate) BOP reagent in the presence of an activating base to condense the α-carboxyl group of the Boc–Arg to the primary amino group of the 1-alkylamine (n = 10, 12, 14 and 16) to yield a long chain N^{α}-*tert*-butoxycarbonylarginylalkylamide monohydrochloride [Boc–ArgNH–C_n]·HCl. The employment of BOP to condense a primary alkylamine to the carboxylic group of the arginine obviates the need for converting the essential non-electrophilic carboxylic group into the highly electrophilic acyl chloride using reagents such as thionyl chloride and phosphorus(V) chloride. The coupling reaction was achieved rapidly by using Et_3N as a base without the protection of the guanidino group of arginine.

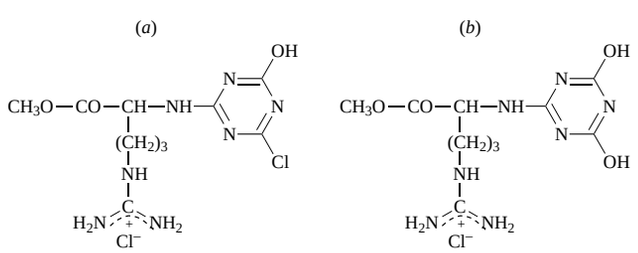
The subsequent deprotection procedure yielded quantitatively the required H–ArgNH–C_n compounds which were not further purified. White hygroscopic solids, such as dihydrochloride salts with optical activity were thus prepared as final products. The effectiveness of the reaction was demonstrated by HPLC and CE analysis of the final compounds. HPLC analysis yielded one peak for each of the H–ArgNH–C_n·2HCl compounds, whose t_R decreased when the chain length decreased. CE analyses also yielded one peak for each of the compounds, whose t_M decreased when the chain length decreased. The purity of these compounds was about 99.5% on the basis of elemental analysis, HPLC and CE.

Reactive DCT–ArgNH–C_n derivatives were prepared using the arginylalkylamide salts as starting materials. The synthetic mechanism for DCT–ArgNH–C_n·HCl (n = 12 and 14) compounds consisted of nucleophilic attack of the α-amino group of the H–ArgNH–C_n·HCl on one of the C-atoms in the TCT molecule. The purity of these compounds was about 93% on the basis of elemental analysis, HPLC and CE.

The preparation of the H–DCT–ArgOMe·HCl model resembled that of DCT–ArgNH–C_n·HCl. The synthetic conditions yielded the required product with a purity of 99.7%, showing a single peak by HPLC and CE analyses. Satisfactory elemental analyses were obtained from these materials giving FABMS and NMR spectra which were consistent with the desired compounds.

Stability

Whereas the H–ArgNH–C_n·2HCl compounds were very stable in solid state or in aqueous solution over a wide range of pH (3.0–9.0) even up to a temperature of 50 °C, the compounds containing a –DCT group showed a short lifetime. H–DCT–ArgOMe·HCl, stored dessicated at 0 °C showed purity for only 3–4 months; in alkaline aqueous solution at pH > 7 and at room temperature it decomposed as a result of hydrolysis in a few hours. The reaction with hydroxide ions in the aqueous medium results in the displacement of one of the chloride atoms, producing a less reactive N^{α}-monochloromonohydroxy compound or a non-reactive N^{α}-dihydroxy compound. This



reaction occurs despite the fact that the DCT–ArgNH–C_n·HCl compounds are not soluble in water. They are present in aqueous solution as a solid suspension. Even in this form, the greater electronegativity of the chlorine atoms in the molecule makes them labile and readily susceptible to stepwise nucleophilic displacement by an hydroxide ion. Dye-stuffs incorporating TCT groups show a similar stability behaviour.[17,18]

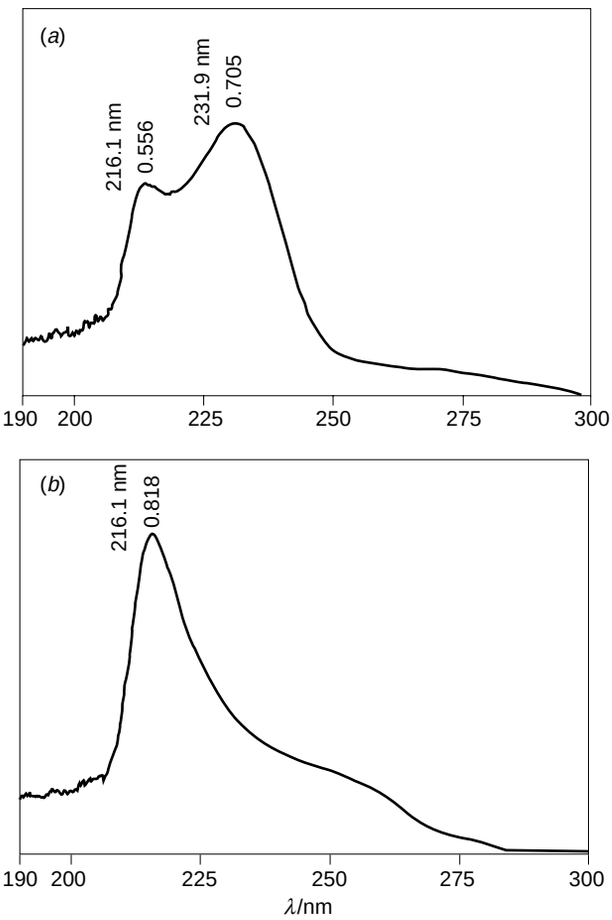


Fig. 1 UV spectra for (*a*) the model H–DCT–ArgOMe·HCl and (*b*) its hydrolysed form

Table 1 Solubility of H–ArgNH–C_n·2HCl compounds and DCT–ArgNH–C_n·HCl compounds in 100 ml of water at 25 °C. The results are indicated in % (w/v).

Compound	% (w/v)
H–ArgNH–C_{10}·2HCl	34.37
H–ArgNH–C_{12}·2HCl	32.22
H–ArgNH–C_{14}·2HCl	29.82
H–ArgNH–C_{16}·2HCl	1.53
DCT–ArgNH–C_{12}·HCl	<0.1
DCT–ArgNH–C_{14}·HCl	<0.1

The presence of these compounds in a satisfactory N^{α}-dihydrochloride form could be demonstrated by their UV spectra, which show a characteristic maximum peak at 232 nm changing the maximum toward the short wavelength region of UV light, about 210 nm. The UV spectrum for the model analogue H–DCT–ArgOMe·HCl and the one for its hydrolysed form are shown in Fig. 1.

The ^{13}C NMR analysis of the N^{α}-dichlorotriazine compounds exhibits characteristic signals of the three carbon atoms in triazine bonding to chlorine atoms (C–Cl) at δ 170–174, whereas, when hydrolysed, the resulting compound containing C–OH groups shows a characteristic signal at about δ 150. It is noticeable that the alkylamide compounds H–ArgNH–C_n·2HCl (in which no ester bonds exist) are more stable at alkaline pH than the earlier described long chain N^{α}-acylarginine alkyl ester surfactants.

Surface active properties

The solubilities in 100 ml of water at 25 °C were determined for all H–ArgNH–C_n and DCT–ArgNH–C_n hydrochloride salts. The results are indicated in Table 1. An isotropic liquid phase was observed for the arginylalkylamide salts. Their solubilities are influenced by the alkyl chain length, in particular, for the homologue of 16 carbon atoms. The solubility properties of arginylalkylamide salts are higher than those of the long chain N^{α}-acylarginine alkyl esters reported earlier as a consequence of possessing two ionic groups per molecule (δ-guanidine and α-amino groups); for example, the water solubility was 25% (w/v) for N^{α}-dodecylarginine methyl ester (LAM) and 10% (w/v) for N^{α}-tetradecylarginine methyl ester (TAM).[19] The introduction of a group such as dichlorotriazine into the H–ArgNH–C_n molecule implies a dramatic loss of water solubility. These reactive compounds were not soluble in water even at very low concentrations. To ascertain whether or not these compounds could be applied in a water solution, the water solubility of mixtures DCT–ArgNH–C_{12} and H–ArgNH–C_{12} up to 50% (mol/mol) were determined in the range of temperatures 25–40 °C. All the solutions were water soluble at 25 °C except the most concentrated mixture (50/50), which was soluble at 40 °C. This solution maintained its solubility when the temperature decreased up to 25 °C. Given the solubility properties of these compounds, the surface active properties were only evaluated for the H–ArgNH–C_n dihydrochloride salts.

One of the main characteristics of surfactants is their tendency to adsorb at interfaces in an oriented fashion as a consequence of their amphipathic structure, consequently reducing the surface tension of the solvent. This adsorption is important in determining the amount of surfactant adsorbed per unit area of the saturated interface or saturation absorption Γ_{max}, which is a measure of the change undergone by the interface because of the surfactant. This depends on the structural groupings in the surfactant molecule and its orientation at the interfaces. The effectiveness of adsorption is related to the interfacial area occupied by the surfactant molecule A_{min}; the smaller the effective cross-sectional area of the surfactant at interface the greater its effectiveness of adsorption.[16] Micelle formation is an alternative mechanism to adsorption for removing hydrophobic groups from contact with water in which a number of interfacial phenomena such as solubilization, surface or interfacial tension reduction are involved. For surfactants in aqueous solution the adsorption and micellization processes are both related to the hydrophobic–hydrophilic balance of the molecule and they intensify with the increase in the hydrophobic character of the surfactant, since this distorts the structure of the water and therefore increases the free energy of the system.[16] Fig. 2 shows the surface tension (γ) *vs.* log surfactant concentration plots for the four homologues in water solutions. c.m.c. values were determined from the surface tension γ/log C curves at 25 °C. These were taken as the concentrations at the point of intersection of the two linear portions of γ/log C plots. Maximum surface excess concentration values or saturated adsorption (Γ_{max}), in mol cm^{-2} and the minimum area per molecule (A_{min}) in the air/water interface were calculated using the Gibbs adsorption equation (1), where $(\partial\gamma/\partial \log C)_T$ is the slope of the γ/log C plot

$$\Gamma_{max} = \frac{-1}{2.303nRT}\left(\frac{\partial\gamma}{\partial \log C}\right)_T \quad (1)$$

$$A_{min} = (N_A\Gamma)^{-1} \times 10^{16} \quad (2)$$

at a constant absolute temperature, T, $R = 8.314$ J mol^{-1} K^{-1}, n is the number of species whose concentration at interface alters with changes in the surfactant concentration in the solution. For our cationic surfactants $n = 3$ (the dicationic amphiphile residue and two chlorides) and N is the number of Avogadro.

Table 2 summarizes all these parameters together with the $\gamma_{c.m.c.}$ and pC_{20} which measure the effectiveness and the efficiency of adsorption of the surfactants respectively.

The c.m.c. values of H–ArgNH–C_n compounds show a fall with the rise in the number of methylene groups in the alkyl chain, as would be expected from the increase in the hydrophobic character of the molecule. In agreement with the rel-

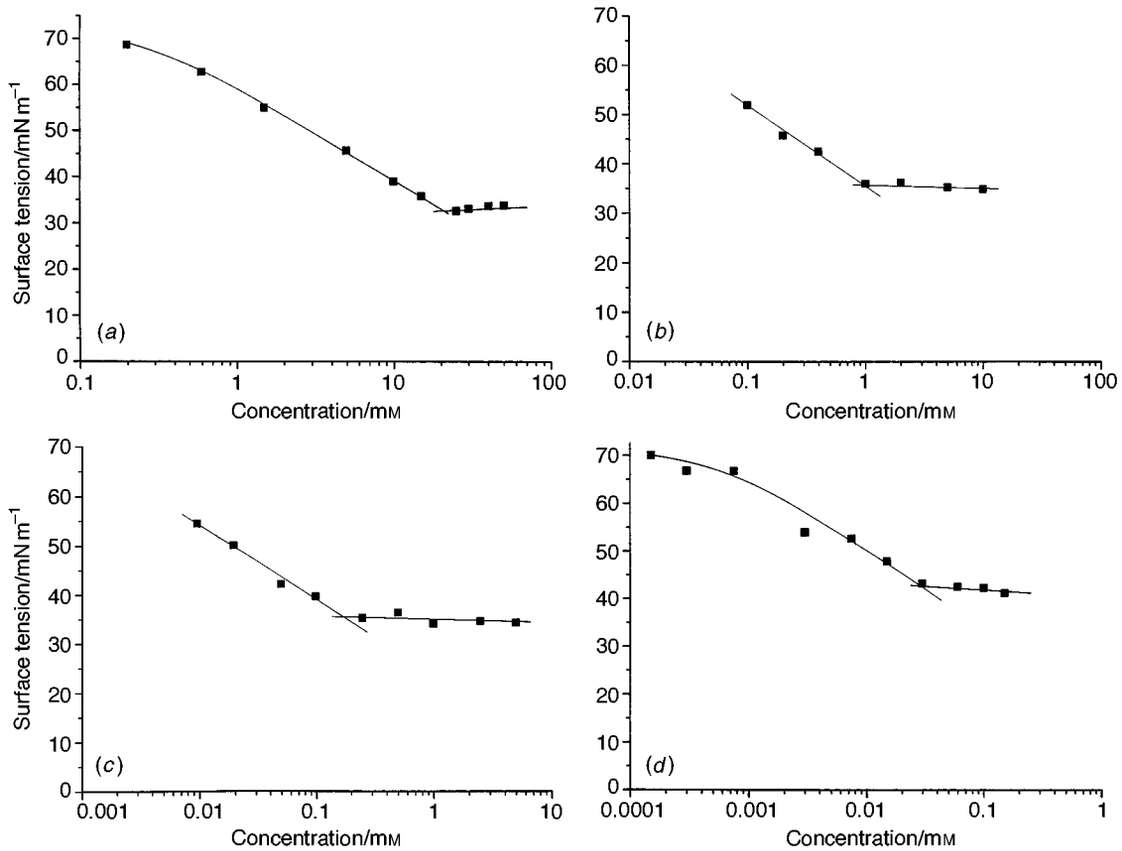


Fig. 2 Surface tension *vs.* log concentration at 25 °C for (*a*) H–ArgNH–C_{10}, (*b*) H–ArgNH–C_{12}, (*c*) H–ArgNH–C_{14} and (*d*) H–ArgNH–C_{16}

Table 2 Surface active parameters for H–ArgNH–C_n·2HCl compounds at 25 °C

Compound	$\gamma_{c.m.c.}/10^{-3}$ N m^{-1}	pC_{20}	c.m.c./10^{-3} mol dm^{-3}	$\Gamma_{max}/10^{14}$ mol m^{-2}	A_{min} [a]/10^2 nm^2 molecule^{-1}
H–ArgNH–C_{10}	34	2.60	20	1.17	142 (95)
H–ArgNH–C_{12}	35	4.00	1.0	0.99	167 (111)
H–ArgNH–C_{14}	35	4.80	0.16	0.98	169 (113)
H–ArgNH–C_{16}	43	5.09	0.038	0.92	180 (120)

[a] Values in parentheses correspond to $n = 2$.

ationship observed for the long chain N^{α}-acylarginine methyl ester salts,[20] the variation of log c.m.c. of H–ArgNH–C_n with the number of carbon atoms (10, 12, 14 and 16) of the alkyl chain is linear, with a slope significantly higher than that for the N^{α}-acylarginine derivatives, thereby suggesting that the hydrophobic contribution to micellation of the present surfactants is higher (Fig. 3). As in a conventional series of homologues with a different alkyl chain length, the efficiency of adsorption, pC_{20}, increases as the number of carbon atoms grows. The larger the pC_{20} value, the more efficiently the surfactant is adsorbed at the interface and the more efficiently it reduces surface tension.

For surfactants with a single hydrophilic group, the area occupied by a surfactant molecule at the surface appears to be determined by the area occupied by the hydrated hydrophilic group, rather than by the hydrophobic group. For the H–ArgNH–C_n compounds, the minimum area per molecule at the aqueous solution/air interface, A_{min} (obtained from the values of the saturated adsorption Γ_{max}), assuming $n = 3$ in the Gibbs equation, is very large (A_{min} in the range 142–180 × 10^2 nm^2 molecule^{-1}) if this is compared with the A_{min} values of the N^{α}-acylarginine methyl ester derivatives: A_{min} (LAM) = 70.1 × 10^2 nm^2 molecule^{-1}; A_{min} (TAM) = 67.2 × 10^2 nm^2 molecule^{-1}. This fact seems to indicate that given the size and nature of their hydrophilic head groups, the molecules of arginylalkylamide surfactants are less packed at the interface than those of N^{α}-acylarginine methyl ester derivatives. The hydrophilic portion of the new molecules contains two cationic groups per molecule (instead of one in the case of N^{α}-acylarginine methyl ester compounds) which tend to spread out on the interface and increase the repulsion inter–intramolecular forces yielding higher A_{min} values. However, since A_{min} values of H–ArgNH–C_n compounds when $n = 3$ are higher than expected, we are not sure whether $n = 3$ or 2 for these surfactants (values in parentheses for $n = 2$ are more reasonable). The constant n depends on the number of species constituting the surfactant which are adsorbed at the interface. It has been argued that for other surfactants of the type bisQuats with two quaternary ammonium groups per molecule, one counterion is associated with one ionic head group and, therefore, the value $n = 2$ should be used.[21] A_{min} values of H–ArgNH–C_n salts are consistent with the values of $\gamma_{c.m.c.}$. The higher the value of A_{min}, the higher the $\gamma_{c.m.c.}$ values. They exceed those of the N^{α}-acylarginine methyl ester derivatives because of the number of molecules adsorbed at the surface and do not change significantly with the alkyl chain length, except for the H–ArgNH–C_{16} derivative whose A_{min} value is 180 × 10^2 mm^2 molecule^{-1}. For this homologue the value of $\gamma_{c.m.c.}$ is 43 mN m^{-1}. It has been postulated that when the number of carbon atoms in a straight-chain hydrophobic group exceeds 16 there is a significant decrease in the effectiveness of adsorption (the maximum change in γ), which has been attributed to the coiling of the long chain with a subsequent increase in the cross-sectional area of the molecule at the interface.[22]

Table 3 Minimum inhibitory concentration (m.i.c.) (μg ml^{-1}) of H–ArgNH–C_{12}·2H_2O, H–ArgNH–C_{14}·2H_2O, LAM and TAM

Microorganism	m.i.c./μg ml^{-1} H–ArgNH–C_{12}·2HCl	H–ArgNH–C_{14}·2HCl	LAM	TAM
Gram negative				
1 *Alcaligenes faecalis* ATCC 8750	32	4	128	—
2 *Bordetella bronchiseptica* ATCC 4617	>128	128	16	—
3 *Citrobacter freundii* ATCC 11606	>128	128	>128	209
4 *Enterobacter aerogenes* ATCC 10938	128	128	128	—
5 *Salmonella typhimurium* ATCC 14028	128	64	32	—
6 *Streptococcus faecalis* ATCC 1054	32	64	8	—
7 *Escherichia coli* ATCC 27325	>128	>128	128	104
8 *Klebsiella pneumoniae* ATCC 13882	32	32	32	209
9 *Pseudomonas aeruginosa* ATCC 9721	>128	128	128	—
10 *Arthrobacter oxydans* ATCC 8010	128	64	8	—
Gram positive				
11 *Bacillus cereus var. mycoides* ATCC 1178	>128	64	64	—
12 *Bacillus subtilis* ATCC 6633	128	32	26	52
13 *Staphylococcus aureus* ATCC 25178	>128	1	4	52
14 *Staphylococcus epidermis* ATCC 155-1	>128	128	6	13
15 *Micrococcus luteus* ATCC 9341	128	64	8	52
16 *Candida albicans* 10231	32	64	32	—

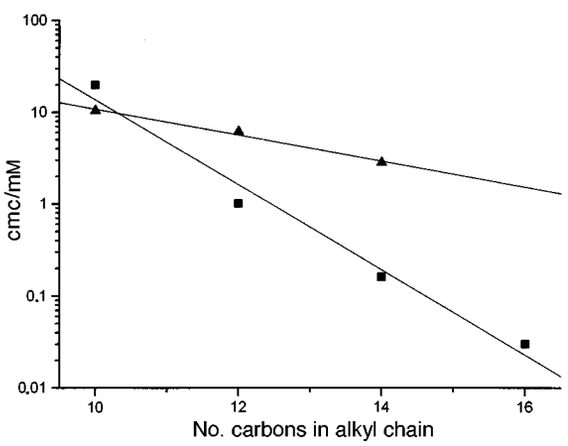


Fig. 3 Effect of length of the hydrophobic group on the c.m.c., (■) arginylalkylamide salts, (▲) *N*-acylarginine methyl ester salts

Antimicrobial properties

The evaluation of antimicrobial activity was carried out by determination of the m.i.c. parameter (Table 3) (the lowest concentration of surfactant at which the microorganisms tested do not show visible growth). Given the water insolubility of DCT–ArgNH–C_n compounds, the antimicrobial study was only restricted to the H–ArgNH–C_n surfactants. Comparing data for 16 selected microorganisms demonstrated that H–ArgNH–C_n (n = 12 or 14) salts exhibited a broad range of antimicrobial activity comparable to that of the analogue LAM. As expected, the Gram negative bacteria were more resistant than the Gram positive bacteria, which makes them suitable for the subsequent biodegradability process of these surfactants. The alkyl chains exert a slight influence on the antimicrobial activity as in a conventional series of surfactants with different alkyl chains. Whereas LAM was more active than TAM in the series of N^{α}-acylarginine methyl esters salts, in the present compounds, H–ArgNH–C_{14} seems to be more active than the homologue of 12 carbon atoms H–ArgNH–C_{12}. There is a displacement of the maximum of activity to a higher alkyl chain because of the increased hydrophilic character of the H–ArgNH–C_n head groups.

From these results we conclude that H–ArgNH–C_n·2HCl salts are a new family of amino acid based surfactants with a surface active performance which is slightly lower than that of long chain N^{α}-acylarginine methyl ester derivatives (owing to the differences in the hydrophilic head groups) but with a higher stability at alkaline pH values. They exhibit antimicrobial activity which depends on the alkyl chain length of the hydrophobic part. These arginylalkylamide antimicrobial surfactants can be regarded as a possible alternative to conventional quaternary ammonium surfactants and as a good starting material for preparing DCT–ArgNH–C_n reactive compounds for covalent bonding to keratine or cotton substrates.

These compounds were prepared with a view to developing a new system of cationic surfactants with antimicrobial activity for textile applications. Treatments of DCT–ArgNH–C_n compounds solubilized in H–ArgNH–C_n solutions with wool fibres and the testing of microbial resistance of fibres are currently in progress. Synergistic and toxic studies of these mixtures are also being carried out.

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