Lipopeptidic Surfactants: I. Neutral N-Lauroyl-l-Arginine Dipeptides from Pure Amino Acids

M.R. Infante, J. Molinero, P. Bosch, M.R. Julià and P. Erra
Instituto de Tecnologia Quimica y Textil (C.S.I.C.), Jorge Girona Salgado, 18-26, 08034 Barcelona, Spain.

Neutral N^{α}-lauroyl-dipeptides such as N^{α}-lauroyl arginylglycine and N^{α}-lauroyl-arginylphenylalanine as methyl ester (cationic surfactants) and free acid (amphoteric surfactants) derivatives have been prepared by synthetic methods. Fundamental surfactant properties and antimicrobial activity have been evaluated.

The properties of these compounds have been compared to the properties of the monomer derivatives N^{α}-lauroyl-arginine methyl ester (cationic) and N^{α}-lauroyl-arginine (amphoteric). These new molecules are very soluble in water, good surfactants, and exhibit a good antimicrobial activity, independently of their ionic character.

INTRODUCTION

The synthesis of antimicrobial surfactants derived from pure basic amino acids have been studied by our research team for the last few years. This kind of surfactant constitutes an interesting class of biocompatible amphiphilic molecules attributed mainly to their chemical structure (1,2).

In a recent paper (and also to obtain protein-based amphoteric surfactants with antimicrobial properties) we described the synthesis and properties of N^{α}-lauroyl-l-arginine dipeptides from collagen. We found that they were soluble in water, they performed surfactant properties and their antimicrobial character was slightly positive (3).

From these results we planned to make a systematic study which is partially presented in this paper. The whole study deals with the preparation of N^{α}-lauroyl-arginine dipeptides from L-arginine and pure amino acis, instead of the amino acid mixture from collagen, and the study of the influence of terminal amino acid as well as the influence of the ionic character on their properties.

The work that we present in this paper is concerned with the synthesis of neutral N^{α}-lauroyl-dipeptides such as N^{α}-lauroyl-arginylglycine and N^{α}-lauroyl-arginylphenylalanine as methyl esters (cationic amphiphiles) and free acid (amphoteric amphiphiles) derivatives; the determination of several surfactant properties; and the determination of antimicrobial activity of four prepared compounds.

A special emphasis has been placed on the amphoteric compounds, which have been compared with the water-insoluble monomer derivative N^{α}-lauroyl-arginine (LA) described previously (4).

EXPERIMENTAL

Materials. Two cationic surfactants, N^{α}-lauroyl arginylglycine methyl ester (LA-GOM) and N^{α}-lauroyl-arginylphenylalanine methyl ester (LA-POM), and two amphoteric surfactants, N^{α}-lauroyl-arginylglycine (LA-GOH) and N^{α}-lauroyl-arginylphenylalanine (LA-POH), were all synthesized in our labortory according to the method described by Molinero *et al.* (3), following Scheme 1, which can be seen in Figure 1.

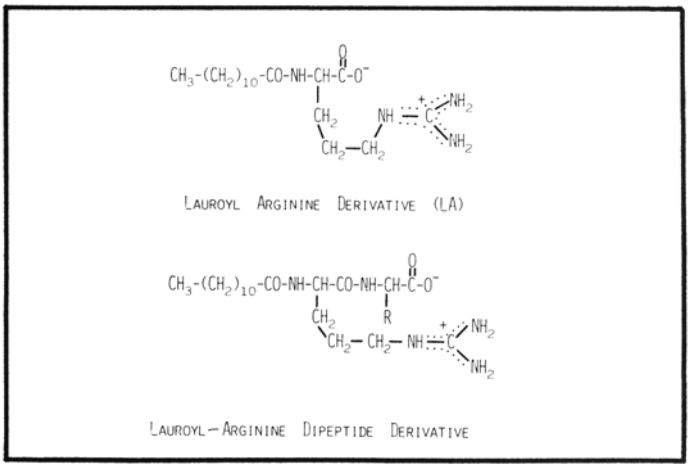

FIG. 1. Schematic method of synthesis (3).

Since the purity of final compounds was an important requisite for this study, the unprotected peptide derivatives were finally purified by column chromatography on Silica Gel 60 (230-400 mesh, Merck), using different compositions of methanol/chloroform mixture as solvents. The products were obtained with a purity of 99.5%. They were used without further crystallization.

Solubility. Water solubility up to 25 wt% surfactant concentration was checked by weighing all components (surfactant and water) in ampoules, which were homogenized by stirring and placed under thermostatted conditions. The pH was previously adjusted by adding an appropriate amount of dilute HCl or NaOH solutions. Undissolved material was separated, if necessary, by centrifugation.

Surface tension. A Du Noüy ring tensiometer (Lauda) with a platinum-indium ring was used for surface tension measurements (γ). Solutions were allowed to equilibrate at 25°C until stabilization of surface tension readings. Equilibration times ranged from 15–30 min.

Critical micelle concentration. From the γ-log C curves the c.m.c., saturation absorption τ and area/molecule were determined. The dependence of c.m.c. on pH was not studied.

Foaming ability. The foam was produced by the following method: 10 ml of a 0.1 wt% solution of each compound was placed at room temperature in a glass column (50 cm $\times$ 2 cm) supplied with a porous plate and a constant flow of wet air bubbled from the bottom of the column in order to generate foam. The relative efficiency of the surfactant was determined by measuring the foam height (mm) vs time (second) generated into the column until 150 seconds for each solution. The foam stability was not measured.

Antimicrobial activity. The minimum inhibitory concentration (MIC) of each compound was determined by a

TABLE 1

Characteristic Properties of the Synthesized Products

Compound	MW	Elemental analysis (%) Calc.	Found	Rf	m.p.[2] (°C)	[α] 25 D (c = 1% MeOH)	Yield[3] (%)	U.V.[1] max (nm)
LA-GOM	463.5	C: 54.37 H: 9.06 N: 15.10	54.12 8.99 15.09	0.64[a] 0.43[b]	47–48	– 1.5	70	209
LA-POM	553.5	C: 58.79 H: 8.75 N: 12.25	58.85 8.85 12.21	0.53[b]	35–39	– 1.68	90	207
LA-GOH	449.5	C: 51.34 H: 8.98 N: 14.97	50.74 9.10 13.84	0.54[b]	55–60	– 2.65	—	209
LA-POH	539.5	C: 58.12 H: 8.61 N: 12.56	57.45 8.74 12.86	0.63[b]	27–31	– 1.60	90	209

[a]Butanol/acetic acid/water (5:2:5).

[b]Chloroform/methanol (7:3).

1: methanol; 2: hygroscopic; 3: without column purification.

TABLE 2

Water Solubility at Room Temperature

	pH:4.0 0.5–25 wt%	pH:7.0 0.5–25 wt%	pH:9.0 0.5–25 wt%
LA-GOM	S	S	S
LA-POM	S	S	S
LA-GOH	S	S	S
LA-POH	S	S	S
LA	I	I	I

S = Soluble.
I = Insoluble.

dilution test in Müeller-Hinton agar medium, against Gram positive and Gram negative bacteria and one yeast.

The dilution test was performed to determine the minimal concentration of an antimicrobial agent required to inhibit microbial growth. Serial dilutions of the antimicrobial agent were inoculated with the organism and incubated at 37°C for 24 hr (3).

RESULTS AND DISCUSSION

Table 1 indicates the characteristics of the synthesized compounds.

The structural formulas of all compounds were confirmed by elemental analyses, amino acid analyses, H'-NMR and Fast Atom Bombardment Mass Spectrometry.

All compounds (both the cationic and the amphoteric ones) displayed good solubility in distilled water at room temperature up to 25% w/w at pH below 9.0 (Table 2). This was in contrast with the amphoteric monomer derivative lauroyl arginine (LA), which was not soluble in water. Gel-like formation was observed for LA-POM and LA-POH surfactants at concentrations higher than 0.15% wt and below 20°C. The condensation of a second amino acid to N^{α}-lauroyl arginine has significantly enhanced the water solubility of derivatives, mainly in the case of amphoteric ones.

Since the structure of N^{α}-lauroyl-arginine is able to form intramolecular guanidine-carboxylate interactions it could be inferred that the insolubility of N^{α}-lauroyl-aginine would be promoted by this effect by the formation of a nonionic hydrophobic structure as shown in Figure 2. This kind of non-covalent hydrogen-bonding interaction should not take place if the guanidine and carboxylate groups were kept away each other, such as in the case of lauroyl-arginine dipeptides and, consequently, the water solubility would increase (5). Conformational studies by X-Ray diffraction are in progress in order to confirm this hypothesis.

Table 3 shows the surface tension values at c.m.c. in water solution at 25°C and pH 4.0, as well as the c.m.c.

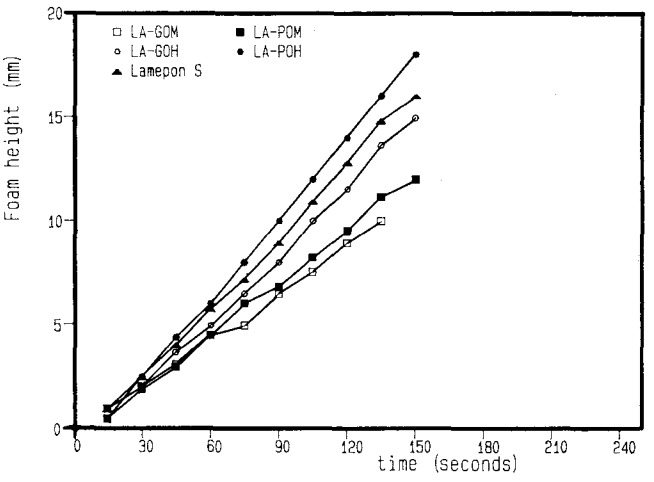

FIG. 2. Chemical structure of the LA and any LA-dipeptide derivative.

TABLE 3

Surface Properties at Room Temperature

Compounds	Surface Tension ($mN.m^{-1}$)	c.m.c. Surf. Tens. Method ($mole.l^{-1}$)	$\pi(mole.m^{-1})$	AM ($\AA^2$)
LA-GOM	37.6	6.5×10^{-3}	2.32×10^{-6}	71
LA-POM	34.6	1.5×10^{-3}	1.86×10^{-6}	88
LA-GOH	30.0	1.5×10^{-3}	2.27×10^{-6}	72
LA-POH	30.0	0.25×10^{-3}	2.08×10^{-6}	79

values, saturation adsorption τ, and area/molecule Am of LA-GOM, LA-POM, LA-GOH and LA-POH. The saturation adsorption τ at air/water interface was calculated using the Gibbs adsorption equation. The area/molecule was calculated from τ for each surfacant.

In general, these results show that surface tension γ, c.m.c. and area per molecule Am obtained with the amphoteric derivatives are lower compared to their corresponding cationic homologues with the same length of the hydrophobic group. Although this is probably the result of several combined effects the decreasing of the electrostatic repulsion forces because of the effect of the zwiterionic nature of the amphoteric molecules probably has a major effect to improve these surfactant properties (6).

On the other hand, the hydrophobic nature of the terminal amino acid does not produce a substantial change of the surface tension, nor in the adsorption values, if LA-GOH and LA-POH are compared. However, the substitution of a hydrogen atom by a phenyl group when the molecule is cationic gives rise to higher adsorption and lower surface tension values. It is very difficult to explain all of these results, but in any case, the nature of the adsorbed films (e.g., the interaction between adsorbed surfactant molecules and the packing of the molecules within the film because of dimension of the molecule, etc.) should be different when cationic molecules or amphoteric molecules are considered.

Critical micelle concentrations are lower in both LA-POM and LA-POH if they are compared with LA-GOM and LA-POM, respectively. Among the factors known to affect the c.m.c. in aqueous solution, the introduction of a phenyl group could increase the hydrophobic component in the molecule, consequently decreasing its c.m.c. value.

Figure 3 shows the foam height vs time for all synthesized compounds using the method described in the experimental section. Each line was the average of five determinations. The advantage of this method was the use of 10 mg of surfactant for each measurement. For comparative purposes, the same experiment was carried out for a commercial potassium salt of a polypeptide coco fatty acid condensate (MW:400) from Stepan Chem. Co. which is considered to be a good foam agent.

As can be observed from the results in Figure 3, there was not an important foaming difference between all surfactants synthesized and the commercial surfactant when the foam height of the commercial surfactant and LA-GOH derivative was determined according to the Ross-Miles method. The foam height value obtained for both compounds was similar and, consequently, it can be concluded that these kinds of compounds are good foaming agents, comparable to a commercial fatty acid polypeptide condensate.

The antimicrobial activity of all synthesized compounds was established by estimating their corresponding Minimum Inhibitory Concentration values (in $\mu g/ml$) at pH 7.0, which are given in Table 4.

These values were compared to the N^{α}-lauroyl-arginine methyl ester (LAM, cationic monomer derivative) and to

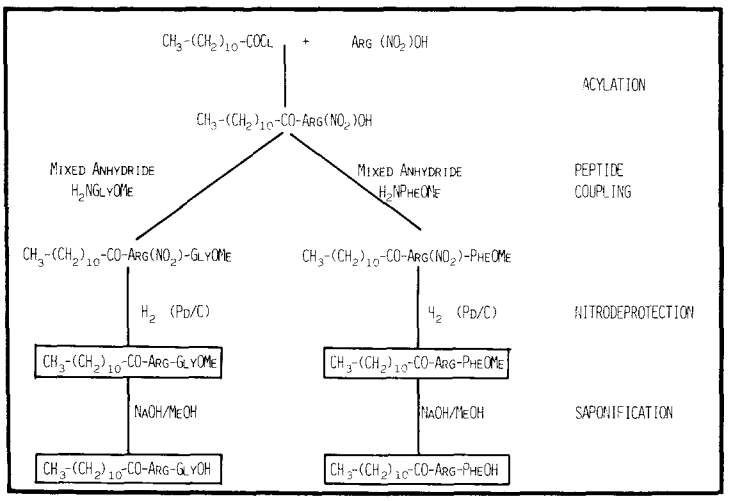

FIG. 3. Foam height (mm) vs time (seconds) generated into the column by bubbling a flow of wet air.

TABLE 4

Minimum Inhibitory Concentration (μg/ml)

	Compounds	LAM	LAa[b]	LA-GOM	LA-GOH	LA-POM	LA-POH
Gram Positive	Candida albicans CCM[a]	32	256	32	32	16	4
	Staphylococcus epidermidis ATCC 12228	8	64	16	16	4	4
	Streptococcus faecalis ATCC 10541	16	256	64	64	32	4
	Corynebacterium agropyri CCM	8	64	8	8	4	2
	Bacillus subtilis ATCC 6623	8	128	16	16	16	4
	Bacillus pumilus CCM	—	—	—	16	—	4
	Micrococcus luteus ATCC 10240	16	64	32	16	8	2
	Micrococcus aurantiacus ATCC 11731	16	128	16	16	4	2
	Alcaligenes faecalis ATCC 8750	256	—	256	128	256	32
Gram Negative	Escherichia coli ATCC 10536	16	>256	32	16	16	4
	Klebsiella pneumoniae ATCC 13883	256	256	>256	>128	256	>128
	Citrobacter freundii ATCC 22636	256	>256	>256	>128	256	>128
	Serratia marcescens ATCC 13880	256	>256	>256	>128	256	>128
	Pseudomonas aeruginosa ATCC 10145	128	>256	128	>128	256	>128
	Salmonella typhimurium ATCC 14028	32	—	64	>128	128	128
	Bordetella bronchiseptica ATCC 10580	16	—	64	>128	32	32

[a]From Cátedra de Microbiología, Universidad de Barcelona.

[b]N-Lauroyl-L-arginine dipeptides mixture from Collagen (3).

the N^{α}-lauroyl-arginine (LA, amphoteric monomer derivative).

In earlier communications we believed that the biological activity depended on the cationic character of the long chain N^{α}-Acyl amino acid derivatives (4,8). Only the monomer derivatives with protected α-carboxyl groups were antimicrobial. Thus, while LAM and other cationic surfactant homologues were good antimicrobial agents, LA and another amphoteric homologues did not show any activity, giving MIC values exceeding 256 μg/ml against all bacteria tested. But when a second neutral amino acid has been incorporated into the monomer derivative lauroyl–arginine, the results show that the condition of α-carboxyl protection is not necessary. MIC values of amphiphile dipeptides with protected carboxyl group, that is to say cationic surfactants, were very similar to those of the amphoteric ones which α-carboxyl group was free. All of them were substantially active.

The antimicrobial activity of these pure amphoteric dipeptides derivatives is higher than those of amphoteric dipeptides derivatives mixture from collagen (see LAa values from Table 4).

It seems likely that the antimicrobial activity is due to N^{α}-lauroyl–arginine residue, which is the common structural factor in all the amphiphile under study. In our opinion, the amphoteric dipeptides derivatives could yield antimicrobial activity because of the absence of intramolecular ionic interactions leaving the guanidine group free increasing their water solubility and performing as a surfactant. This strong basic group could interact with the polyanionic components of the cell surface due to their cationic condition. Consequently, a weakness of the cell wall would occur, allowing the surfactant to pass through the membrane and prevent in this way the microbial growth.

From the result of this work we can conclude that the condensation of a second amino acid into the monomer derivative LA has totally transformed their properties. Thus these new molecules are very soluble in water, good

surfactants and, the most important in our opinion, exhibit a good antimicrobial activity independently of their ionic character.

Further work is in progress in order to understand the influence of acid or basic terminal α-amino acids as well as the influence of pH on the physico-chemical properties and, consequently, to be able to assess the area of application of these materials.

ACKNOWLEDGMENTS

The authors thank CICYT for financial support, and Cátedra Microbiológica, Facultad de Farmacia (U.B.) for MIC determinations, and also A. Vilchez and I. Carrera for assistance in the experimental work.

REFERENCES

1. Richtler, H.J. and J. Knaut, *Proc. 2nd World Surfactant Congress 1*:3 (1988).
2. Than, P., in *Surfactants in Cosmetics*, edited by M.M. Rieger, Marcel-Dekker Inc., NY, 1985, pp.361.
3. Molinero, J., R. Juliá, P. Erra, M. Robert, and M.R. Infante, *J. Am. Oil Chem. Soc. 65*:975 (1988).
4. Infante, M.R., J.J. García Domínguez, P. Erra, M.R. Juliá, and M. Prats, *Int. J. Cosm. Sci. 6*:275 (1984).
5. Eggleston, D. and D.J. Hodgson, *Int. J. Peptide Protein Res. 25*:242 (1985).
6. Aveyard, R., in *Surfactants*, edited by Th.F. Tadros, Academic Press, Inc., 1984, pp. 153–173.
7. Takai, M., H. Hidaka, and M. Moriya, *J. Am. Oil Chem. Soc. 56*:537 (1979).
8. Infante, M.R., J. Molinero, P. Erra, M.R. Juliá, and J. García Domínquez, *Fette Seifen Anstrichmittel 87*:309 (1985).

[Received October 25, 1988; accepted June 17, 1989]
[JSD5592]