Synthesis of N-Acyl Amino Acids and Correlation of Structure with Surfactant Properties of Their Sodium Salts[1]

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The effect of structural variation in fatty acid and amino acid moieties on surfactant properties of sodium salts of N-acyl condensates of amino acids was investigated. Pure N-acyl leucines and N-lauroyl condensates of different amino acids were synthesized and neutralized. Among the N-acyl leucinates, N-lauroyl leucinate exhibited optimum properties and compared well with sodium lauryl sulfate (SLS). Among the salts of Nα-lauroyl amino acids, Nα-lauroyl lysinate was found comparable to SLS. Salts of Nα-lauroyl condensates of leucine, tryptophan, phenylalanine and proline showed good wetting ability; proline also displayed high calcium ion tolerance. Salts of N-lauroyl tyrosine and phenylalanine exhibited good foaming ability. N-Lauroyl aspartate showed inferior properties compared to SLS in spite of having an additional carboxylic group.

KEY WORDS: N-acyl leucines, N-lauroyl amino acids, structure vs surfactant properties.

Sodium salts of N-acyl condensates of amino acids are anionic surfactants with good biodegradability, skin compatibility and antibacterial activity (1-4). They find use in detergent, cosmetic and food formulations. The surfactant properties of sodium salts of some N-acyl condensates prepared from a few pure fatty acids and either pure amino acids or protein hydrolysates have been studied (4-7). However, a systematic study on the effect of structural variation in fatty acid and amino acid moieties on surfactant properties of N-acyl condensates does not appear to have been investigated. This forms the subject of the present communication. The variation examined in the structure of fatty acyl moieties in N-acyl condensates of L-leucine was in chain length (10,12, 14,16,18 carbons) and in unsaturation (18:1, 9-*cis*). The variation in amino acid moiety studied was in R- of condensate R.CH(NHOC.R_1)COONa, where R is hydrogen, isobutyl, benzyl, mercaptomethylene, hydroxymethylene, p-hydroxybenzyl, 3-methyleneindole, 4-aminobutyl or pyrrolidine ring (in proline) and R_1.CO- is lauroyl moiety.

MATERIALS AND METHODS

Materials. Capric, lauric and myristic acids were purchased from BDH Ltd. (Poole, England) and palmitic and stearic acids were from ACME Synthetic Chemicals (Bombay, India). Fatty acid methyl esters were tested by gas chromatography (GC) and found to be 99% pure. Chromatographically homogenous amino acids, namely, glycine, L-leucine, L-phenylalanine, L-proline, L-aspartic acid, L-cysteine, L-serine, L-tyrosine and L-tryptophan were purchased from Hi-Media Ltd. (Bombay, India). Silica gel (60-120 mesh) for column chromatography and silica gel G for thin-layer chromatography were obtained from ACME Synthetic Chemicals. Sodium lauryl sulfate (SLS) was purchased from Glaxo Laboratories, Bombay, India. Analytical grade reagents and chemicals (BDH Ltd.; and Indian Drug and Pharmaceutical Ltd., Hyderabad, India) were used.

Methods. Melting points of products were measured using a Ketan melting point apparatus (Shivam Scientific Instruments, Bombay, India). GC was carried out on Silar 10C column using a Hewlett Packard 5840A (Hewlett Packard, Co., Palo Alto, CA) fitted with a hydrogen flame detector and data processor. The column, injection port and detector were maintained at 195, 250 and 300°C, respectively. Flow rate of carrier gas (N_2) was 30 mL. Infrared (IR) spectra were recorded on a Perkin Elmer 683 spectrometer (Perkin Elmer, Norwalk, CT). Proton nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ on a Varian 60-FT (Varian Associates, Palo Alto, CA). Nitrogen was estimated by the Kjeldahl method as described by Cocks and van Rede (8).

Surfactant properties. Surface tension (ST) was measured using Torsion balance (White Electrical Company, Ltd., Worcestershire, England) at constant temperature. Foaming property was determined using a Ross-Miles pour foam apparatus (9). Emulsifying power was determined according to Subrahmanyam and Achaya (10). The Draves-Clarkson method was used as described in IS specification (11) for determining the wetting ability . Detergency was tested by gain in reflectance (ΔR) obtained after washing soiled cloth (supplied by U.S. Testing Company, Hoboken, N.J.; reflectance of 30.5 units) in Terg-O-tometer (U.S. Testing Company) under uniform conditions (12). The minimum amount of Ca^{11} ions (expressed as ppm of $CaCO_3$) required to make the surfactant solution attain defined turbidity was determined according to the modified Hart method (13). Critical micelle concentration (CMC) was determined by plotting equivalent conductance against concentration of a surfactant solution on a conductometer (supplied by Digisun Electronics Ltd., Bombay, India).

Preparation of fatty acid chlorides and N-acyl L-leucines. Saturated fatty acid chlorides were prepared using thionyl chloride (14), and oleoyl chloride was prepared using oxalyl chloride (15). The acid chlorides were distilled under vacuum. N-acyl leucines were prepared by the Schotten-Baumann reaction by adding acid chloride dropwise to an aqueous solution of sodium salt of leucine at pH 10 ± 0.5 (16). The product was acidulated with 30% sulfuric acid, taken up in ethyl acetate and crystallized out by addition of hexane. N-Oleoyl leucine was purifed on a silica gel column using hexane/ethyl acetate (90:10, v/v).

Preparation of N-Lauroyl amino acids. N-Lauroyl condensates of simple amino acids (e.g., glycine, leucine, phenylalanine and proline) and dilauroyl condensates of lysine and tyrosine were prepared as described earlier. In the case of dilauroyl condensates the mole ratio of lauroyl chloride to amino acid was 3:1. N-Lauroyl condensates of

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glycine, leucine, phenylalanine were purified on silica gel column using chloroform/methanol (95:5, v/v). Dilauroyl-lysine and -tyrosine were purified by washing off the unreacted fatty acid with warm hexane. N-lauroyl aspartic acid was prepared by reacting disodium aspartate in acetone/water (25:30 v/v) with lauroyl chloride at pH 12 (17). The product was crystallized from ethanol/petroleum ether.

Preparation of N-lauroyl cysteine. S-Benzyl cysteine was first prepared by protecting the SH group of cysteine (0.055 mole) with benzyl chloride (0.06 mole) in sodium hydroxide (25 mL) and ethanol (60 mL) and crystallizing the product from hot water after precipitating it from cold 2N hydrochloric acid. S-Benzyl cysteine (0.04 mole in 20 mL of 2N sodium hydroxide) was acylated with lauric acid anhydride (0.04 mole) by carboxylic-carbonic anhydride method using ethyl chloroformate (0.04 mole) in absolute tetrahydrofuran (75 mL) in the presence of triethylamine (0.04 mole) (18). The product was crystallized from cyclohexane after acidulating it with hydrochloric acid. The S-benzyl group was deprotected using Na-liquid NH_3 and absolute tetrahydrofuran as the solvent, and the crude product was crystallized from benzene.

Preparation of Nα-lauroyl lysine. Nε-Tosyl lysine was prepared by condensing tosyl chloride (0.09 mole) with Cu complex of lysine (0.06 mole) in alkaline condition (40 mL 2N NaOH) (19). The copper complex was cleaved with H_2S gas in acidic medium (100 mL 2N HCl) and then Nε-tosyl lysine was precipitated by adjusting the pH to 6 with pyridine. Nε-Tosyl-Nα-lauroyl lysine was prepared by the carboxylic-carbonic acid anhydride method as described for N-lauroyl cysteine except benzyl chloroformate was used instead of ethyl chloroformate. The crude product was purified on a silica gel column using chloroform/methanol (85:15, v/v). Deprotection of tosyl group was done with Na-liquid NH_3 method and the product was crystallized from acetonitrile to give Nα-lauroyl lysine.

Preparation of N-lauroyl tyrosine. O-Tosyl tyrosine was prepared by condensing the tosyl chloride (0.05 mole) in ether (40 mL) with the copper complex of tyrosine (0.05 mole) in alkaline medium (100 mL of 3N NaOH) (20). The copper complex of O-tosyl tyrosine was isolated as the hydrochloride in 100 mL conc. HCl after cooling the mixture for 48 hr. O-Tosyl tyrosine was then obtained by adjusting the pH of the solution to 6 with ammonium hydroxide and recrystallizing the product from hot water. O-Tosyl-N-lauroyl tyrosine was prepared by condensing O-tosyl tyrosine (0.25 mole) and lauroyl chloride (0.3 mole) via the Schotten-Baumann reaction. The tosyl group was deprotected using the Na-liquid NH_3 reaction and the product was purified on a silica gel column with chloroform/methanol (85:15, v/v).

Preparation of Nα-lauroyl tryptophan and N-lauroyl serine. The N-hydroxy succinimide ester of lauric acid was prepared by condensing lauric acid (0.05 mole) with N-hydroxy succinimide (0.05 mole) using dicyclohexyl-carbodiimide in dry ethly acetate (145 mL). Precipitated dicyclohexyl urea was filtered to get pure N-hydroxy succinimide ester which, after removal of solvent, was crystallized from ethanol. Nα-Lauroyl tryptophan was prepared by adding N-hydroxy succinimide ester to tryptophan (0.03 mole) and triethylamine (0.06 mole) in a mixture of water and acetone (50:50, v/v) (3). The reaction was carried out for 6 hr under N_2 and at the end of reaction the product was precipitated by adjusting the pH to 2 after removal of the solvent. Crude product was crystallized from chloroform-hexane to get pure Nα-lauroyl tryptophan. N-Lauroyl serine was prepared by the carboxylic-carbonic anhydride method using ethyl chloroformate as described earlier. The product was crystallized from benzene.

Preparation of sodium salts of N-acyl derivatives. N-Acyl amino acids were dissloved in 95% ethanol (10:90, w/v), warmed to 60°C and neutralized with ethanolic sodium hydroxide to the exact end point. The solutions were filtered and the products were dried under vacuum.

Preparation of aqueous solutions of sodium salts. Aqueous solutions for testing surfactant properties were prepared in double distilled water (ST 72 dynes/cm at room temperature, 27°C). For N-acyl leucinates, 0.25% solutions were prepared and for salts of N-lauroyl amino acid condensates 0.1% solutions were prepared.

RESULTS AND DISCUSSION

Synthesis of N-acyl condensates. N-acyl leucines were obtained by the acid chloride method in 70–80% yield after two crystallizations. The TLC, NMR and mass spectral analyses showed that these compounds did not contain either free amino acid or fatty acid. Lauroyl condensates of glycine, phenylalanine, proline and aspartic acid were similarly obtained in 70–80% yield. N-lauroyl serine was prepared by the carboxylic-carbonic anhydride method which gave higher yield (72%) than the acid chloride method due to non-acylation of the free-OH group in the former. The thiol group of cysteine was protected with a benzyl group and the overall yield of N-lauroyl cysteine was 40%. The ε-NH_2 group of lysine as well as the phenolic OH group of tyrosine, were protected by tosyl group by making Cu complex of α-NH_2 and COOH group, and then reacting them with tosyl chloride. Deprotection of the tosyl group by HBr-acetic acid proved unsuccessful, whereas Na-liquid NH_3 gave the required fission. Nα-lauroyl lysine was obtained in low yield (30%) after crystallization from acetonitrile. Each of these above compounds was obtained as a single spot on TLC.

TABLE 1

^{1}H NMR Data (δ ppm) of N-Lauroyl Amino Acids

Amino acid moiety	β-CH_2[a]	-NH-[a]	Characteristic peaks
Glycine	—	6.6	—
Leucine	1.2	7.3	0.9 (d, 6H, 2 ×-CH_3)
Phenylalanine	2.1	5.8	7.2 (s, 5H, aromatic)
Aspartic acid	2.9	6.9	10.0(br, 1H, -COOH)
Serine	3.9	6.9	4.0 (br, 1H, -OH)
Cysteine	3.2	7.6	1.2 (br, 1H, -SH)
Lysine	1.2	7.4	2.8 (br, 2H, -NH_2)
Tryptophan	3.2	7-8[b]	7.8 (m, 4H, indole ring)
Proline	—	—	3.6 (t, 2H, N-CH_2-) 2.2 (m, 4H, cyclic CH_2-)
Tyrosine	3.3	8.9	7.5 & 7.9 (d, A_2B_2, 4H, aromatic portons)

[a]Peaks were observed as doublets.

[b]Overlapped with aromatic protons.

Spectral properties. All the N-acyl amino acids gave the characteristic alkyl stretching at 2920 cm^{-1} and bending at 1460 cm^{-1}, secondary amide carbonyl stretching at 1670 cm^{-1} and acid carbonyl stretching at 1720 cm^{-1} in IR spectroscopy. N-lauroyl proline (cyclic amino acid with tertiary amide) showed carbonyl stretching at 1630 cm^{-1}. Nα-lauroyl lysine exhibited a band at 3400 cm^{-1} owing to the primary ϵ-NH_2 group. N-lauroyl cysteine showed the thiol group at 2500 cm^{-1}.

^1H NMR studies of all the condensates showed common peaks (δ) in N-acyl leucines as follows: 5.5-7 (d, 1H, -NH-), 5.45 (m,2H, CH=CH in unsaturated acid), 4.51 (m, 1H, -CαH), 2.22 (m merged with t, 6H, allylic protons, CO-CH_2-), 1.25 [δ, n $\times$ 2H, -$(CH_2)_n$], 0.96 (d,6H, $(CH_3)_2$-CH), 0.88 (t, 3H CH_3-CH_2). The NMR data (δ ppm) of amino acid moieties of N-lauroyl amino acids are given in Table 1.

Surfactant properties of N-acyl leucinates. The surfactant properties of N-acyl leucinates are given in Table 2. The CMC of N-acyl leucinates increased with decrease in chain length and increase in unsaturation of acyl chain. Calcium tolerance of N-palmitoyl and N-stearoyl leucinates could not be determined as the solutions were turbid even at 60°C. Out of these remaining, N-myristoyl leucinate showed better tolerance, though it is still less than that of SLS. ST lowering ability of N-acyl leucinates was found to be superior to that of SLS. N-Myristoyl, -palmitoyl and -stearoyl leucinates showed better ST-lowering than the other N-acyl condensates. The foaming stability was not good, though initial foam was satisfactory at 60°C. The N-myristoyl leucinate proved to be a better foaming agent of all the condensates. The N-acyl condensates were comparable in wetting ability to that of SLS, except for N-lauroyl and -myristoyl leucinates which gave instantaneous wetting, and N-stearoyl leucinate which was inferior to SLS. N-Oleoyl leucinate showed good wetting as compared to stearoyl leucinate showing the favorable effect of unsaturation on wetting ability. All N-acyl leucinates were superior in emulsification power to SLS. No correlationship was seen between emulsification power and acyl chain length. N-lauroyl leucinate showed the best emulsification out of all condensates. N-Stearoyl leucinate showed better emulsification than N-oleoyl leucinate. Detersive power of products increased with increase in acyl chain length up to 16 carbons. Unsaturation showed a negative effect on detergency. Lauroyl-leucinate was found to possess optimum properties which are generally superior to SLS; hence lauric acid was fixed as acyl moiety in N-acyl condensates of different amino acids.

Surfactant properties of sodium salts of N-lauroyl condensates. Lauric acid was acylated with ten amino acids having different functional groups, viz., simple and neutral (glycine, L-leucine, L-phenylalanine); acidic (L-aspartic acid); basic (L-lysine, L-tryptophan); primary hydroxyl (L-Serine); phenolic hydroxyl (L-tyrosine); pyrrolidine ring (L-proline); and thiol (L-cysteine); to study the effect of functional groups present in amino acids on surface-active properties. The surfactant properties are given in Table 3. All the condensates gave lower CMC values than SLS. The condensates of basic amino acids had lower CMCs, whereas the condensates of amino acids with either additional acidic or hydroxyl group had higher CMCs.

TABLE 2

Physicochemical and Surfactant Properties[a] of Sodium N-Acyl Leucinates

Acyl moiety	MP[b] (°C)	CMC (mmol/10 L)	Ca^{II} tolerance (ppm: $CaCo_3$)	Surface tension (dynes/cm)	Foam ht. (mm)		Wetting time (sec)	Aq. phase sepn. from emulsion (sec)		Detergency as gain in reflectance (ΔR)
					Initial	after 5 min		10 mL	20 mL	
10:0	109	20.1	37	29.0	20	Nil	4.0	191	373	1.5
12:0	107	9.2	37	30.0	125	5	<1	390	928	2.0
14:0	101	2.0	97	26.5	150	40	<1	270	625	3.0
16:0	91	0.8	—[c]	25.0	145	35	6.5	200	390	5.5
18:0	65	0.2	—[c]	26.5	95	40	44.0	285	490	4.5
18:1	Oily	2.1	66	30.0	100	5	6.5	213	455	1.0
SLS[d]	—	12.4	113	33.5	130	5	2.5	147	520	4.0

[a]Determined on 0.25% aqueous solutions at 60°C except CMC and C^{II} tolerance, which were determined at 27°C. Refer to text for test procedures.

[b]Melting point of N-acyl leucine.

[c]Solution was turbid under test conditions.

[d]Sodium lauryl sulfate.

TABLE 3

Physicochemical and Surfactant Properties[a] of Sodium Salts of N$^{\alpha}$-Lauroyl Amino Acids

Amino acid moiety	R in R–CH–COOH | NH–12:0	MP[b] (°C)	CMC (mmol/10 L)	Ca^{II} tolerance (ppm: $CaCo_3$)	Surface tension 0.05% (dynes/cm)	Surface tension 0.1% (dynes/cm)	Foam ht. Initial (mm)	Foam ht. after 5 min (mm)	Wetting time (sec)	Aq. phase sepn. from emulsion 10 mL (sec)	Aq. phase sepn. from emulsion 20 mL (sec)	Detergency as gain in reflectance (ΔR)
Glycine	Hydrogen	118	8.3	19	48.5	42.0	20	5	8	Fast sepn.		0.5
Leucine	Isobutyl	106	9.2	51	31.5	29.5	60	20	3	63	110	1.0
Phenylalanine	Benzyl	98	7.0	32	30.5	28.0	115	90	7	152	278	1.0
Aspartic acid	Carboxymethylene	113	10.8	222	56.5	49.0	20	5	124	6	15	1.5
Serine	Hydroxymethylene	107	11.8	16	42.0	39.5	25	10	156	180	210	1.0
Cysteine	Mercaptomethylene	124	10.0	177	32.5	30.5	55	35	62	148	353	2.0
Lysine	4-Aminobutyl	132	4.4	759	35.0	33.5	165	145	14	320	750	3.0
Tryptophan	3-Methyleneindole	110	5.5	13	32.5	31.5	75	30	5	288	585	2.0
Proline	Cyclic	Liq.	7.4	>1000	40.5	35.5	65	25	9	30	45	0.5
Tyrosine	p-Hydroxybenzyl	128	8.8	139	33.0	30.0	100	95	10	65	110	1.5
SLS	—	—	12.4	50	39.5	34.0	150	135	8	115	291	2.0

[a]Determined on 0.1% aqueous solutions at 27°C.

[b]Melting point of N$^{\alpha}$-lauroyl amino acid.

TABLE 4

Physicochemical and Surfactant Properties[a] of Sodium Salts of N$^{\alpha}$-Lauroyl and -Dilauroyl Amino Acids

Amino acid moiety	MP[b] (°C)	CMC (mmol/10 L)	Ca^{II} tolerance (ppm: $CaCO_3$)	Surface tension 0.05% (dynes/cm)	Surface tension 0.1% (dynes/cm)	Foam ht. Initial (mm)	Foam ht. after 5 min (mm)	Wetting time (sec)	Aq. phase sepn. from emulsion 10 mL (sec)	Aq. phase sepn. from emulsion 20 mL (sec)	Detergency as gain in reflectance (ΔR)
Lysine (monoacyl)	132.0	4.4	759	35.0	33.5	165	145	14	320	750	3.0
Lysine (diacyl)	118.5	10.1	—[c]	42.0	39.5	20	10	40	120	145	2.0
Tyrosine (monoacyl)	128.0	8.8	139	33.0	30.0	100	95	10	65	110	1.5
Tyrosine (diacyl)	decomp.	5.5	44	52.0	47.0	10	—	151	65	110	1.0

[a]Determined on 0.1% solutions at 27°C.

[b]Melting point of N$^{\alpha}$-lauroyl amino acid.

[c]Solution was turbid under test conditions.

Ca^{11} ion tolerance was very much (>1000 ppm) higher for N-lauroyl prolinate than for all the other condensates and SLS (50 ppm) depicting the stability of cyclic amino acid structure to calcium ions. Nα-lauroyl lysinate also showed significant stability of 759 ppm of Ca^{11}. The sodium salts of Nα-lauroyl glycine, -serine and tryptophan showed very low tolerance (19, 16 and 13 ppm, respectively). The N-lauroyl aspartate showed a Ca^{11} tolerance of 222, as expected due to the additional carboxylic group.

Increase in molecular weight of alkyl chain (glycine vs leucine) increased ST-lowering ability. The presence of additional carboxyl (aspartic acid) and hydroxy group (serine) retarded ST-lowering ability, unlike benzyl (phenylalanine), thiol (cysteine) and p-hydroxybenzyl (tyrosine) groups. The presence of additional -NH_2 group (lysine vs leucine) retarded the ST-lowering ability. The ST-lowering abilities (in decreasing order) of condensates of phenylalanine, leucine, tryptophan, cysteine, tyrosine and lysine were superior, of proline comparable and of serine, glycine and aspartic acid inferior to that of SLS. The salts of Nα-lauroyl lysine, -phenylalanine and -tyrosine exhibited foaming power comparable to SLS. All the other condensates were found to be poor foaming agents. The wetting abilities of all condensates were comparable to that of SLS, with the exception of salts of aspartic acid, serine and cysteine suggesting, the unfavorable effect of carboxylic, hydroxy and thiol groups, respectively.

Increase in bulkiness of R in RCH(NH-12:0)COONa enhanced emulsifying property. While comparing the same bulkiness of R, the presence of hydrophilic group (e.g., phenylalanine and tyrosine) decreased the stability of the emulsion. Basic amino acid condensate salts exhibited better emulsifying property. Separation of aqueous phase (10 mL) from emulsion took longer time for serine (-OH group) than for cysteine (-SH group) condensate salt, though the stability of emulsion was less for the former.

Detergency as judged by a gain in reflectance (ΔR) was found to be maximum in case of Nα-lauroyl lysinate. In general, additional hydrophilic groups enhanced the detersive power. N-Lauroyl prolinate showed poor detergency in spite of having the best calcium ion tolerance.

Dilauroyl condensate salts were found to be inferior in solubility and surfactant properties to monolauroyl condensate salts (Table 4). Dilauroyl tyrosine salt exhibited lower CMC and similar emulsifying power as compared to monolauroyl tyrosine salt.

In general, Nα-Lauroyl lysinate was found to be the better surfactant than the other condensates, as well as SLS. N-Lauroyl aspartate exhibited inferior surfactant properties in all aspects. Hydrophobic, cyclic and basic groups in amino acid moieties contributed to better surfactant properties. Some of these condensates may be used in different surfactant formulations to enhance a particular property. For example, the salts of N-lauroyl condensates of proline are Ca^{11} ion tolerent; tryptophan, phenylalanine, glycine and proline are good wetting agents; and lysine and tryptophan can be used as emulsion stabilizers.

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