Nonionic Amphiphilic Compounds from Aspartic and Glutamic Acids as Structural Mimics of Lecithins

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ABSTRACT: Monodisperse nonionic surfactant molecules, based on aspartic or glutamic acid, with two hydrogenated or fluorinated fatty amide chains in the hydrophobic part and one polyoxyethylene methoxy-capped chain (EOn-Me) in the hydrophilic head group have been synthesized. These compounds are structural mimics of natural lecithins. Their solubility in water or in formamide and the surface activities at 60°C have been measured and discussed in comparison with lecithin analogues that contained short chains. The compounds reported in this study showed physicochemical properties comparable with those of lecithins.

JAOCS 73, 87–96 (1996).

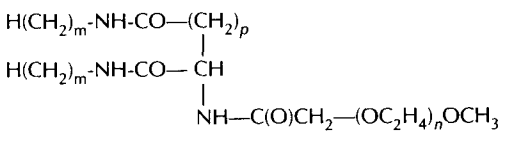
KEY WORDS: Mimics of lecithins, nonionic amphiphilic compounds, surface activity properties.

The interest in surfactant molecules has become increasingly important during the last fifteen years as a consequence of the numerous applications of these compounds in biology, biochemistry, biophysics, and chemistry (1–5). In a recent paper, we reported the preliminary results of the synthesis, physicochemical properties, and some biological effects of new monodisperse nonionic surfactants that are based on trifunctional amino acids, such as lysine (Lys, a basic amino acid), aspartic acid (Asp), and glutamic acid (Glu) (acidic amino acids), bearing two hydrophobic alkyl chains and one or two short polyoxyethylene-chains with their terminal hydroxyls protected by O-methylation (6).

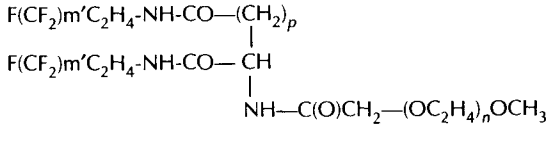
A more detailed and systematic description on the Lys analogues was reported in a previous paper, which dealt with the synthesis and physicochemical study of a large number of monodisperse hydrogenated nonionic long-chain N^{α}, N^{ε} diacyl lysine polyoxyethylene methoxycapped amide compounds that were prepared by condensation of N^{α}, N^{ε} diacyl lysine amphiphiles with polyoxyethylene amine derivatives (7). The effects of several structural parameters, such as fatty acid hydrophobic chainlength, polyoxyethylene hydrophilic chainlength, and a number of polyoxyethylene hydrophilic chains, have been studied.

The present paper is concerned with the synthesis and physicochemical properties in water of a large number of nonionic monodisperse compounds, analogues to those of Lys, but based on Glu and Asp acidic amino acids, with a structural resemblance to natural lecithins. Scheme 1 shows the structures of Asp and Glu nonionic surfactants, mimics of lecithin: $P = 1$, aspartic acid (D); and $P = 2$, glutamic acid (E). Code used for compounds (see Table 4, later) that are based on aspartic acid are H*mm*D*n* and F*mm*D*n*; those based on glutamic acid are H*mm*E*n* and F*mm*E*n*. In this paper, the effect of hydrophobic chain nature (hydrogenated or perfluorinated), hydrophobic chainlength, and type of amino acid (Asp or Glu) on the physicochemical properties are presented. The results are compared with those of Lys analogues described in a previous article (7) and with those of lecithin analogues described in the literature. Additionally, some physicochemical properties in formamide solution are also reported.

The present compounds possess two hydrophobic chains of different length and nature (hydrogenated or fluorinated), which come from the corresponding fatty amine compounds. They are condensed through amide linkages to both the α- and ω-COOH groups of the acidic amino acid. The hydrophilic group consists of one amide function between the



H*mm*D*n*



F*mm*D*n*

SCHEME 1

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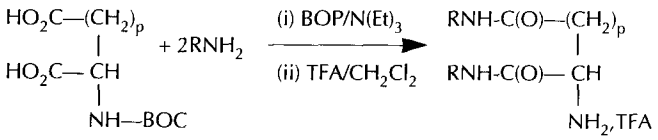
α-amino group of amino acid and a monomethyletherpolyoxyethylene carboxylic chain. As for the Lys analogues, the central pivot for the present compounds is constituted by the trifunctional acidic amino acid, which plays a structural role similar to the glycerol in lecithins.

Preparation of molecular mimics of lecithins was expected to yield surfactant compounds with similar physicochemical properties to those of lecithin analogues and, therefore, with biocompatible characteristics. Allouch's results (8) concerning biocompatibility showed that those compounds were indeed nontoxic materials at concentrations below 0.1 g dm^{-3}. Higher chemical stability of these compounds compared to lecithins was expected because the amide linkages in the molecule are more resistant to hydrolysis than those of esters (in lecithins). Compared with the Lys analogues (7), the present compounds have their amide functionality of the central pivot in a reversed position.

EXPERIMENTAL PROCEDURES

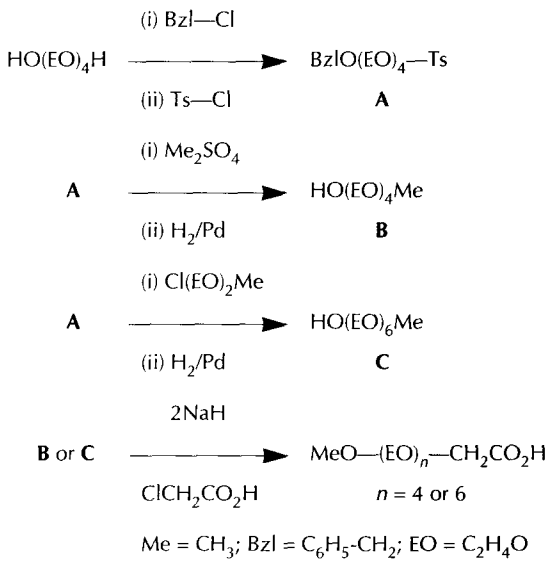
Synthesis. To understand the relationship between the chemical structure of these surfactants and their physicochemical properties correctly, pure nonionic compounds were synthesized. These compounds were prepared with monodispersed polyoxyethylene of known true chainlength. Synthesis of these compounds was based on a modular strategy (9), which consisted of the following three main steps: (i) Preparation of the hydrophobic part. Synthesis of long-chain C^{α}, C^{ω} dialkylamide Boc-Asp (or Boc-Glu) acids by the reaction of the α-amino–protected N^{α}-Boc amino acid derivative (Boc = tertbutoxycarbonyl) with hydrogenated or fluorinated fatty amines by a classical activation method with the BOP reagent [benzotriazole-1-yl-oxy-*tris*-(dimethylamino)-phosphonium hexafluorophosphate] (see Scheme 2) for synthesis of the hydrophobic part (H*mm*D0; F*mm*D0 and H*mm*E0 or F*mm*E0). (ii) Preparation of the hydrophilic part. Synthesis of monomethyletherpolyoxyethylene ethanoic acids of the type $Me\text{-}(OC_2H_4)_n\text{-}OCH_2CO_2H$ with n = 2, 3, 4, and 6, with commercial starting materials (see Scheme 3) for synthesis of the hydrophilic part [$Me\text{-}(OC_2H_4)_n\text{-}OCH_2CO_2H$). (iii) Condensation of the hydrophobic part with the hydrophilic module. The hydrophilic monomethyletherpolyoxyethylene carboxylic chains were coupled to the amino function of the hydrophobic dialkylamide amino acid derivative by the BOP activation method (see Scheme 4) for synthesis of the surfactants (H*mm*D*n*; F*mm*D*n* and H*mm*E*n* or F*mm*E*n*).

The synthetic experimental procedures and characteristics of the compounds were as follows.



p = 1, aspartic acid; p = 2, glutamic acid; $R = H(CH_2)_m$-[H*mm*D0 or H*mm*E0] or $R = F(CF_2)_mC_2H_4$-[F*mm*D0 or F*mm*E0]

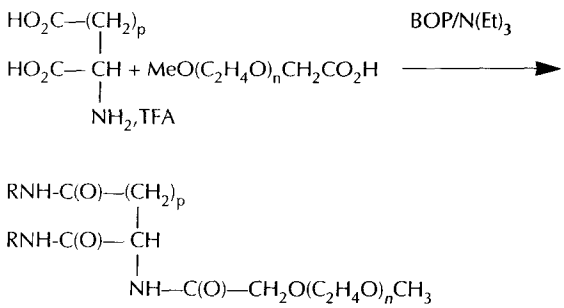
SCHEME 2



SCHEME 3

MATERIALS AND METHODS

Reagent-grade solvents (SDS, Marseille, France), BOP reagent (Propeptide, Paris) and Boc-acids (Bachem, Budendorf, Sweden) were used without further purification. Monomethyletherpolyoxyethylene ethanoic acid was prepared according to the method described by Seguer *et al.* (6). Progress of the reaction and purity of the products were evaluated on silica-gel thin-layer chromatography (TLC) plates (Kieselgel 60 F_{254}; Merck, Darmstadt, Germany) with eluents ethyl acetate (AcOEt) or chloroform/methanol 7:3 (C/M). Purification was effected by flash silica-gel column chromatography with the same eluents. Melting points were determined with an electronic apparatus (Electrothermal; Prolabo, Nogent, France) and were not corrected. Proton ^1H nuclear magnetic resonance (NMR) spectra were recorded with a Bruker (Karlsruhe, Germany) AM 400 or AM 250 spectrometer, and chemical shifts are reported in parts per million (δ in ppm) downfield from tetramethylsilane (TMS). Infrared spectra were recorded with a Perkin-Elmer (Norwalk, CT) 580D or 1600 Fourier transform infrared (FTIR) spectrometer. After several recrystallizations, elemental analysis of a few com-



$R = H(CH_2)_m$ with m = 6, 8, 10; $F(CF_2)_mC_2H_4$ with m = 6, 8; n = 2, 3, 4, 6; p = 1 for Asp(D) and 2 for Glu(E)

SCHEME 4

TABLE 1
Characteristics of Compounds H*mm*D0, H*mm*E0, F*mm*D0, and F*mm*E0 with Structural Formula

Compound	*R*	Yield (%)	m.p. (°C)	R_f(AcOEt)
H66D0	C_6H_{13}	93	140	0.75
H88D0	C_8H_{17}	90	145	0.80
H1010D0	$C_{10}H_{21}$	94	135	0.81
H88E0	C_8H_{17}	88	105	0.76
H1010E0	$C_{10}H_{21}$	97	106	0.78
F66D0	$C_6F_{13}C_2H_4$	66	165	0.75
F88D0	$C_8F_{17}C_2H_4$	64	167	0.85
F66E0	$C_6F_{13}C_2H_4$	55	125	0.75
F88E0	$C_8F_{17}C_2H_4$	60	105	0.80

TABLE 2
Results of Microanalysis of Compounds H*mm*D0 and H*mm*E0

	C		H		N	
Compound (formula)	Calc. (%)	Found (%)	Calc. (%)	Found (%)	Calc. (%)	Found (%)
H66D0 ($C_{21}H_{41}N_3O_4$)	63.12	63.47	10.34	10.84	10.52	10.73
H88D0 ($C_{25}H_{49}N_3O_4$)	65.90	65.21	10.84	11.14	9.22	9.37
H1010D0 ($C_{29}H_{57}N_3O_4$)	68.06	67.35	11.23	11.39	8.21	8.52
H88E0 ($C_{26}H_{52}N_3O_4$)	66.49	65.98	10.94	10.44	8.95	8.87
H1010E0 ($C_{30}H_{59}N_3O_4$)	68.53	68.21	11.31	11.54	7.99	7.87

pounds was off by more than 0.5%; however, they gave only single peaks by high-performance liquid chromatography (HPLC) and spectral analyses consistent with the expected structures. No impurities were detected. The solids probably crystallized with the same molecules of water. The same results were obtained for the Lys analogues (7). Surface-tension measurements were made with a Dognon Abribat or Kruss tensiometer (Prolabo) by using the Wilhelmy method.

*General procedure for preparing long-chain dialkylamides Boc-aspartic (D) or Bocglutamic (E) acids (H*mm*D0, F*mm*D0 or H*mm*E0, F*mm*E0).* In a round-bottom flask, to a solution of acetonitrile (30 mL), containing 0.4 mole (2 eq) fatty amine, 0.8 mole (4 eq) triethylamine, and 0.4 mol (2 eq) BOP reagent, was added 0.1 mole (1 eq) Boc-aspartic (or glutamic) acid. Stirring was continued at room temperature for 3 h. During reaction, precipitation of a white solid was observed. After reaction, the white solid was filtered off. The product was generally pure. Recrystallization from chloroform/diethyl ether furnished a very pure compound. Yields and characteristics of all synthesized compounds are presented in Tables 1–3.

General spectral characteristics of dialkylamides Boc-aspartic or Boc-glutamic acids. H*mm*D0: IR (KBr), ν: NM = 3,000–3,200 cm^{-1}; C = O = 1,730 cm^{-1}; C(O)N = 1,660 cm^{-1}; ^1H NMR ($CDCl_3$/TMS) ppm: 0.9 (*t*, $2CH_3$); 1.3 [*m*, (m – 2)CH_2]; 1.4 (*s*, $3CH_3$); 1.5 [*m*, $2CH_2$]; 2.7 [*m*, CH_2(Asp)]; 3.2 (*m*, $2CH_2N$); 4.4 (*m*, C × H); 6.1 (*m*, NH); 6.25 (*m*, NH); 7 (*m*, NH); H*mm*E0: I.R. (KBr), ν: NH = 3,000–3,200 cm^{-1}; C = O = 1,730 cm^{-1}; C(O)N = 1,660 cm^{-1}; ^1H NMR ($CDCl_3$TMS) ppm: 0.9 (*t*, $2CH_3$); 1.3 [*m*, (m – 2)CH_2]; 1.4 (*s*, $3CH_3$); 1.5 [*m*, $2CH_2$]; 2.3 [*m*, $2CH_2$(Glu)]; 3.2 (*m*, $2CH_2N$); 4.4 (*m*, C × H); 6.1 (*m*, NH); 6.25 (*m*, NH); 7 (*m*, NH); F*mm*D0: I.R. (KBr), ν: NH = 3,000–3,200 cm^{-1}; C = O = 1,750 cm^{-1}; C(O)N = 1,590 cm^{-1}; ^1H NMR (acetone-D6/TMS) ppm: 1.6 (*s*, $3CH_3$); 2.65 [*m*, 2RFCH_2]; 2.8 [*m*, CH_2(Asp)]; 3.7 (*m*, $2CH_2N$); 4.55 (*m*, C × H); 6.1 (*m*, NH); 6.5 (*m*, NH); 7.7 (*m*, NH); 7.8 (*m*n, NH); F*mm*E0: I.R. (KBr), ν: NH = 3,000–3,200 cm^{-1}; C = O = 1,745 cm^{-1}; C(O)N = 1,590 cm^{-1}; ^1H NMR (acetone-D6/TMS) ppm: 1.6 (*s*, $3CH_3$); 2.7 [*m*, 2RFCH_2]; 2.45 [*m*, $2CH_2$(Glu)]; 3.7 (*m*, $2CH_2N$); 4.2 (*m*, C × H); 6.4 (*m*, NH); 7.6 (*m*, NH); 7.75 (*m*, NH).

General procedure for preparing methyletherpolyoxyethylene long-chain dialkylamides of aspartic or glutamic acids by BOP method. Deprotection of synthesized dialkylamide Boc-amino acid derivatives (H*mm*D0, F*mm*D0 or H*mm*E0, F*mm*E0) was effected by a classical method with trifluoroacetic acid (41). The crude trifluoroacetate was used as purchased.

To a solution of 0.01 mole (1 eq) monomethyletherpolyoxyethylene-ethanoic acid (6), 3 equivalents of triethylamine, and 0.01 mole (1 eq) BOP-reagent in dry acetone, were added 0.01 mole trifluoroacetate of dialkylamide Asp (or Glu) acid with stirring. Stirring was continued at room temperature for 12 h. After reaction, the solvent was evaporated to dryness *in*

TABLE 3
Results of Microanalysis of Compounds F*mm*D0 and F*mm*E0

	C		H		N		F	
Compound (formula)	Calc. (%)	Found (%)	Calc. (%)	Found (%)	Calc. (%)	Found (%)	Calc. (%)	Found (%)
F66D0 ($C_{25}H_{23}F_{26}N_3O_4$)	32.51	32.64	2.51	2.50	4.55	4.67	53.49	52.88
F88D0 ($C_{29}H_{23}F_{34}N_3O_4$)	31.00	30.87	2.06	1.94	3.74	3.91	57.49	56.93
F66E0 ($C_{26}H_{25}F_{26}N_3O_4$)	33.31	33.52	2.69	2.41	4.48	4.63	52.69	51.98
F88E0 ($C_{30}H_{25}F_{34}N_3O_4$)	31.68	31.44	2.22	2.10	3.69	3.87	56.79	55.94

TABLE 4
Principal Characteristics of Perhydrogenated Surfactants Based on Aspartic or Glutamic Acids (H*mm*D*n* and H*mm*E*n*)

Compound	Molecular weight (g/mol)	m.p. (°C)	TLC[a] (R_f)	Yield (%)	HPLC[b] retention time (min)
H66D2	459.60	78	0.66	70	23.58
H88D2	515.70	102	0.62	72	32.46
H1010D2	571.8	80	0.6	58	41.86
H66D3	503.70	85	0.59	71	23.48
H88D3	559.80	93	0.52	68	32.35
H1010D3	615.90	90	0.51	60	40.24
H66D4	547.70	80	0.54	65	23.47
H88D4	587.80	95	0.52	73	32.25
H1010D4	643.90	89	0.50	68	40.10
H66D6	635.80	95	0.43	72	23.06
H88D6	691.90	97	0.45	75	30.96
H1010D6	748.1	95	0.48	71	39.80
H88E2	529.80	70	0.55	65	33.95
H1010E2	585.90	80	0.60	58	41.86

[a]Thin-layer chromatography in ethyl acetate/methanol 85:15.
[b]High-performance liquid chromatography.

TABLE 5
Principal Characteristics of Fluorinated Surfactants Based on Aspartic or Glutamic Acids (F*mm*D*n* and F*mm*E*n*)

Compound	Molecular weight (g/mol)	m.p. (°C)	TLC[a] (R_f)	Yield (%)
F66D2	983.5	94	0.67	76
F88D2	1183.5	108	0.72	80
F66D3	1027.5	70	0.57	75
F88D3	1227.5	95	0.53	78
F66D4	1071.6	78	0.54	64
F88D4	1271.6	92	0.58	74
F66D6	1159.7	85	0.44	62
F88D6	1359.7	90	0.40	90
F66E2	997.5	70	0.66	70
F88E2	1197.5	105	0.57	65
F66E3	1041.6	55	0.52	68
F88E4	1285.6	80	0.45	68

[a]Thin-layer chromatography in ethyl acetate/methanol 85:15.

vacuo. The crude material was dissolved in ether (100 mL) and washed with aqueous solution of 1N HCl (3 × 100 mL) and 1N $NaHCO_3$ (3 × 100 mL). The organic phase, dried over anhydrous $MgSO_4$, was evaporated to dryness *in vacuo*. Several recrystallizations of the solid from diethyl ether furnished a very pure compound. The yields and characteristics of all synthesized compounds are presented in Table 4 for H*mm*D*n*, H*mm*E*n*, Table 5 shows compounds for F*mm*D*n*, F*mm*E*n*, and results of microanalyses appear in Tables 6 and 7.

RESULTS AND DISCUSSION

Pure and optically active long-chain $C^{\alpha}C^{\omega}$ dialkylamide Boc-Asp (or Boc-Glu) acids with perhydrogenated or perfluoroalkylated hydrophobic chains (H*mm*D0, H*mm*E0, F*mm*D0, and F*mm*E0) were prepared in yields of about 85% by following the BOP-coupling procedure (see Tables 1–3). Removal of the terminal Boc-protected group was carried out by classical treatment with trifluoroacetic acid (6,10). The hydrophobic amino acid derivatives were isolated as white solids by simple filtration. Further recrystallization was carried out from a chloroform/ether mixture. All compounds showed a well-defined melting point as indicated in Table 1.

Monodisperse methyl polyoxyethylene hydrophilic moieties were obtained in four steps with a global yield of 50%. The synthesis of methyletherpolyoxyethylene carboxylic acids [$Me(OC_2\text{-}H_4)_n\text{-}OCH_2CO_2H$] with an n = 2, 3, 4, and 6 was achieved by the classical Williamson ether synthesis (7). The purities of the intermediate and final hydrophilic products were monitored by TLC in several solvent systems.

Final compounds of the type methyletherpolyoxyethylene long-chain $C^{\alpha}C^{\omega}$ dialkylamide Asp (H*mm*D*n*, F*mm*D*n*) or Glu (H*mm*E*n*, F*mm*E*n*) were obtained as white solids by reaction of the hydrophobic part with the hydrophilic part in the presence of the well-known BOP reagent, the bonding functional group being of the amide type. Further purification was achieved by recrystallization from ether. Purity of these compounds was determined by NMR, FTIR, HPLC, and elemental analyses, as described previously (6,7). Yields for perhydrogenated compounds were higher than 70%. Fluoroalkyl derivatives were obtained with yields comparable to those of the hydrogenated type (see Tables 4 and 5).

Physicochemical properties. Fundamental physicochemical properties in two polar solvents, water and formamide,

TABLE 6
Results of Microanalysis of Compounds H*mm*D*n* and H*mm*E*n*

Compound (formula)	C Calc. (%)	C Found (%)	H Calc. (%)	H Found (%)	N Calc. (%)	N Found (%)
H66D2 ($C_{23}H_{45}N_3O_6$)	60.10	59.55	9.86	9.92	9.14	8.93
H88D2 ($C_{27}H_{53}N_3O_6$)	62.88	62.33	10.36	10.41	8.15	8.25
H1010D2 ($C_{31}H_{61}N_3O_6$)	65.10	64.43	10.76	10.38	7.35	7.59
H66D3 ($C_{23}H_{49}N_3O_7$)	59.62	58.96	9.81	10.05	8.34	8.46
H88D3 ($C_{29}H_{57}N_3O_7$)	62.22	61.62	10.26	10.21	7.50	7.76
H1010D3 ($C_{35}H_{65}N_3O_7$)	62.35	61.66	10.64	10.23	6.82	6.92
H66D4 ($C_{27}H_{53}N_3O_8$)	59.21	58.96	9.75	9.65	7.67	7.46
H88D4 ($C_{31}H_{61}N_3O_8$)	61.66	60.98	10.18	10.05	6.96	6.46
H1010D4 ($C_{35}H_{69}N_3O_8$)	63.70	63.09	10.54	10.46	6.37	6.26
H66D6 ($C_{31}H_{61}N_3O_{10}$)	58.56	58.01	9.67	9.64	6.61	7.05
H88D6 ($C_{31}H_{69}N_3O_{10}$)	60.75	59.95	10.05	9.78	6.07	6.85
H1010D6 ($C_{39}H_{77}N_3O_{10}$)	62.62	61.09	10.38	10.14	5.62	5.55
H88E2 ($C_{28}H_{55}N_3O_6$)	63.47	62.63	10.47	10.05	7.94	7.10
H1010E2 ($C_{32}H_{63}N_3O_6$)	65.60	64.79	10.83	10.64	7.17	7.20

TABLE 7
Results of Microanalysis of Compounds F*mm*D*n* and F*mm*E*n*

Compound (formula)	C		H		N		F	
	Calc. (%)	Found (%)	Calc. (%)	Found (%)	Calc. (%)	Found (%)	Calc. (%)	Found (%)
F66D2 ($C_{27}H_{27}F_{26}N_3O_6$)	32.97	32.85	2.77	2.71	4.27	4.39	50.23	49.84
F88D2 ($C_{31}H_{27}F_{34}N_3O_6$)	31.46	31.42	2.30	2.28	3.55	3.51	54.58	54.46
F66D3 ($C_{29}H_{31}F_{26}N_3O_7$)	33.90	32.83	3.04	2.93	4.09	4.22	48.07	47.53
F88D3 ($C_{33}H_{31}F_{34}N_3O_7$)	32.29	32.37	2.55	2.31	3.42	3.63	52.61	52.58
F66D4 ($C_{31}H_{35}F_{26}N_3O_8$)	34.75	34.63	3.29	3.18	3.92	4.01	46.10	45.89
F88D4 ($C_{35}H_{35}F_{35}N_3O_8$)	33.06	32.98	2.77	2.68	3.30	3.42	50.80	50.15
F66D6 ($C_{35}H_{43}F_{26}N_3O_{10}$)	36.25	36.13	3.74	3.63	3.62	3.88	42.59	41.98
F88D6 ($C_{39}H_{43}F_{34}N_3O_{10}$)	34.45	34.01	3.19	3.65	3.09	3.80	47.50	44.82
F66E2 ($C_{28}H_{29}F_{26}N_3O_6$)	33.71	33.54	2.23	2.11	4.21	4.33	49.52	48.29
F88E2 ($C_{32}H_{29}F_{34}N_3O_6$)	32.10	32.95	2.44	2.32	3.51	3.63	53.94	53.76
F66E3 ($C_{30}H_{33}F_{26}N_3O_7$)	34.60	34.48	3.19	3.08	4.03	4.12	47.42	46.98

were determined. Solubility data, at 22 and 60°C, for all synthesized compounds in water and in formamide were summarized in Tables 8 and 9. Surface-tension values for all synthesized compounds were determined at 22°C in water and in formamide as saturated solutions after separation of excess crystals by filtration. These data are also shown in Tables 8 and 9 as γ_{sat}. It was expected that, as for the precedent Lys analogues, the present compounds bearing short alkyl chains with six units of ethylene oxide (*mm*D6, *mm*E6) would possess pronounced solubilities in these two polar solvents at room temperature (6). Only the hydrogenated substances, however, showed good solubility in formamide (between 1 and 10^{-2} mol • L^{-1}), the solubilities in water being poor at 22°C (between 10^{-3} and 10^{-5} mol • L^{-1}). Results of γ_{sat} revealed that our compounds, in particular those of fluorinated alkyl chains, were surface active compounds that were able to lower surface-tension values of polar solvents, such as water and formamide, at low concentrations. Solubilities of all hydrogenated compounds in these solvents increased at temperatures around 60°C. On the contrary, no important differences concerning solubilities were observed for the fluorinated compounds. Solubilities at 60°C were slightly higher than those at 22°C. Consequently, surface tension vs. concentration in water and in formamide solvents was studied only at 60°C for the hydrogenated compounds H*mm*D*n* and H*mm*E*n*. At this temperature, hydrogenated compounds were

TABLE 8
Limits of Solubilities of H*mm*D*n* and H*mm*E*n* in Water and in Formamide at 22 and at 60°C and Surface Tension at Saturation in Water at 22°C

Compound	Limit of solubility in water (mol • L^{-1})		Limit of solubility in formamide (mol • L^{-1})		γ_{sat} in water at 22°C
	22°C	60°C	22°C	60°C	(mNm^{-1})
H66D2	$<5 \times 10^{-3}$	$<1 \times 10^{-2}$	<1.5	<2.0	29
H66D3	$<5 \times 10^{-3}$	$<1 \times 10^{-2}$	<1.5	<2.0	28
H66D4	$<5 \times 10^{-3}$	$<1 \times 10^{-2}$	<1.5	<2.0	28
H66D6	$<5 \times 10^{-3}$	$<2 \times 10^{-2}$	<1.0	<2.0	28
H88D2	$<5 \times 10^{-4}$	$<5 \times 10^{-3}$	$<2 \times 10^{-2}$	<1.0	27
H88D3	$<5 \times 10^{-4}$	$<5 \times 10^{-3}$	$<1 \times 10^{-1}$	<1.0	27
H88D4	$<5 \times 10^{-4}$	$<5 \times 10^{-3}$	$<5 \times 10^{-2}$	<1.5	27
H88D6	$<1 \times 10^{-3}$	$<1 \times 10^{-2}$	$<5 \times 10^{-2}$	<2.0	25
H1010D2	$<1 \times 10^{-5}$	$<1 \times 10^{-3}$	$<1 \times 10^{-2}$	$<5 \times 10^{-1}$	28
H1010D3	$<1 \times 10^{-4}$	$<5 \times 10^{-3}$	$<1 \times 10^{-2}$	$<5 \times 10^{-1}$	29
H1010D4	$<1 \times 10^{-4}$	$<5 \times 10^{-3}$	$<1 \times 10^{-2}$	$<5 \times 10^{-1}$	29
H1010D6	$<1 \times 10^{-4}$	$<1 \times 10^{-3}$	$<1 \times 10^{-2}$	$<5 \times 10^{-1}$	24
H88E2	$<1 \times 10^{-3}$	$<1 \times 10^{-2}$	$<1 \times 10^{-2}$	$<5 \times 10^{-1}$	29
H1010E2	$<5 \times 10^{-4}$	$<1 \times 10^{-2}$	$<1 \times 10^{-2}$	$<5 \times 10^{-1}$	31

TABLE 9
Limits of Solubilities and Surface Tension at Saturation at 22°C of F*mm*D*n* and F*mm*E*n* in Water and in Formamide

Compound	Limit of solubility in water (mol • L^{-1})	Limit of solubility in formamide (mol • L^{-1})	γ_{sat} in water at 22°C (mNm^{-1})	γ_{sat} in formamide (mNm^{-1})
F66D2	5×10^{-5}	10^{-3}	18	19
F88D2	10^{-5}	10^{-4}	22	18
F66D3	5×10^{-5}	10^{-3}	17	19
F88D3	3×10^{-5}	5×10^{-4}	24	17
F66D4	2×10^{-4}	5×10^{-3}	16	17
F88D4	10^{-4}	5×10^{-4}	23	18
F66D6	10^{-3}	5×10^{-3}	17	19
F88D6	2×10^{-4}	10^{-3}	22	16
F66E2	5×10^{-5}	10^{-3}	17	19
F88E2	10^{-5}	10^{-4}	27	23
F66E3	5×10^{-5}	10^{-3}	16	18
F88E4	10^{-4}	5×10^{-3}	24	20

all apparently soluble (formation of an isotropic monophasic system took place), and a simultaneous decrease of surface tension of the solvent was observed when concentrations of the compounds were increased. Stabilization of surface tension was reached from a critical concentration because of surface saturation by the surface-active compound. Similar behavior was observed by Wells (11), who studied the aqueous solution surface-tension reduction as a function of the bulk concentration of short-chain diacyl lecithins analogues with 6, 7, 8, and 9 carbon atoms in the alkyl chains at different temperatures. He found that dihexanoyl lecithin (55Lec) and dioctyl lecithin (77Lec) homologues, which correspond to H66D*n* and H88D*n* (or H88E*n*) series, respectively, gave an isotropic monophasic system in water at temperatures above 40°C. Therefore, he reported the critical micelle concentration (CMC) parameter at 45°C. This agreement could be due to the chemical structural analogy between the compounds in the present study and the lecithins.

Figures 1 through 3 give the surface-tension values exhibited by the H*mm*D*n* series in aqueous solution at 60°C, expressed as a function of log concentration. Figure 4 illustrates the plot of surface tension vs. concentration of the H88D*n* homologues in formamide at 60°C. A critical concentration (CC), corresponding to the formation of aggregates in the solvent, was taken for each compound as the concentration at the point of intersection between the two linear portions of the curves. From such plots, the surface-excess concentrations were calculated with the appropriate Gibbs equation (7). The areas per molecule (A_{min}) at the surface were calculated from the excess concentration values in the usual manner.

CC data, the ability to lower surface tension above CC (γ_{CMC}), areas per molecule, and surface excess concentrations (Γ) of H*mm*D*n* and H*mm*E*n* homologues in water at 60°C are summarized in Table 10, along with those of the reference short-chain lecithin analogues described in the literature (11,12). Table 11 shows CC data and γ_{CMC} in formamide solutions at 60°C. Surface-tension values obtained in water are between 24 and 29 mNm^{-1}, within the range obtained with other hydrogenated nonionic surfactants. As usual, for the same hydrophilic group, these surface activities increase with the alkyl chain-lengths (13,14). Conversely, for the same hydrophobic alkyl chainlength, surface-tension values increase with an increase in the number of oxyethylene units. Their CC values in aqueous solution are in the range of 10^{-3} to 10^{-5} mol • L^{-1}. These values are also of the same order of magnitude as those of other classical nonionic surfactants (15). Compared with the previously reported Lys analogues with the same fatty acid alkyl chains and bearing one hydrophilic head group with the same ethyleneoxide units, CC values of hydrogenated Glu and Asp surfactants are lower than those of Lys surfactants (i.e., $CC_{55K06} = 35 \times 10^{-3}$ mol • L^{-1}; $CC_{H66D6} = 65 \times 10^{-4}$ mol • L^{-1}; $CC_{77K06} = 1.6 \times 10^{-3}$ mol • L^{-1}; $CC_{H88D6} = 4.1 \times 10^{-4}$ mol • L^{-1}), indicating that the chemical structure of the hydrophilic moiety (type of

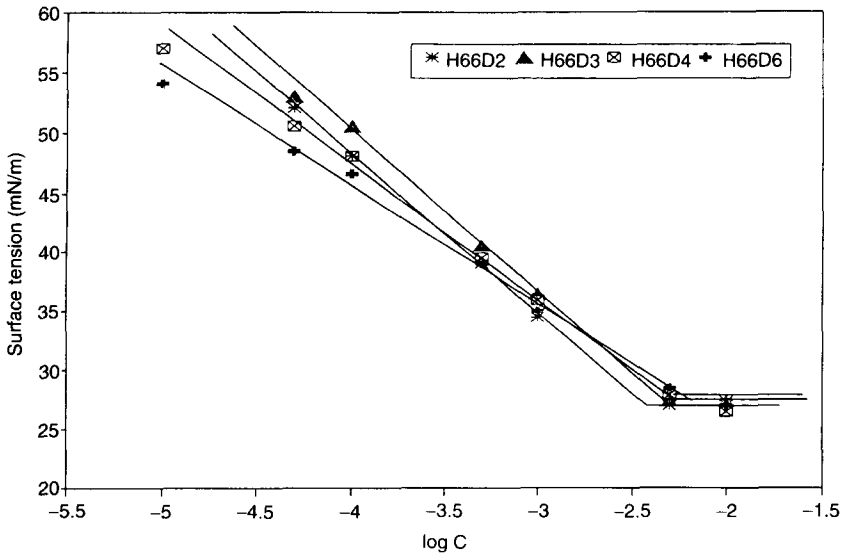


FIG. 1. Plot of surface tension/logC for binary systems water/66D*n* at 60°C.

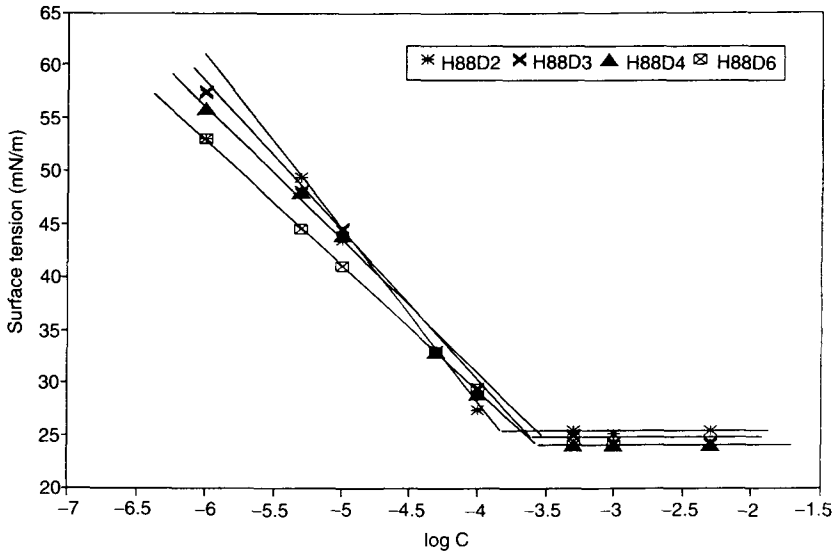


FIG. 2. Plot of surface tension/logC for binary systems water/88D*n* at 60°C.

amino acid an orientation of the amide groups in the central pivot) does influence their ability to form aggregates. For the same alkyl chainlength, CC values increase with an increase in the polyoxyethylene chainlength from 2 to 6. Data on other nonionic surfactants also indicate that increasing the size of the hydrophilic group inhibits the formation of micelles (16,17). In contrast, CC values decrease as the number of carbon atoms in the alkyl chains increase; this is due to an increase in the hydrophobicity of the molecules (18). Results in Table 10 show that the CC value of a surfactant is reduced each time that two methylene groups per alkyl chain are added, the relative change being lower when the surfactant is more hydrophobic.

When CC values of these surfactants are compared with those of the corresponding singlechain nonionic surfactants of the type C*m*EO*n* (in which the CMC decreases by a factor of 120 when there is an increase in four methylene groups in the alkyl chain) (19–21), together with the lecithin homologues (Table 10), it appears that CC values of the compounds in this study are of the same order of magnitude as those of the natural compounds (i.e., $CMC_{55Lec} = 98 \times 10^{-4}$ mol • L^{-1}; $CC_{H66Dn} = 37.5 - 63 \times 10^{-4}$ mol • L^{-1} and $CMC_{77Lec} = 1.7 \times 10^{-4}$ mol • L^{-1}; $CC_{H88Dn} = 1.46 - 4.13 \times 10^{-4}$ mol • L^{-1}).

Regarding surface-tension reduction vs. logC in formamide solution, the γ_{CMC} values were slightly higher than those in aqueous medium. The break point in the γ vs. logC curves of Figure 4 corresponds to the formation of aggregates in solution. The CC that was associated with this break point was in the range of 10^{-1} to 10^{-2} mol • L^{-1}. These values were

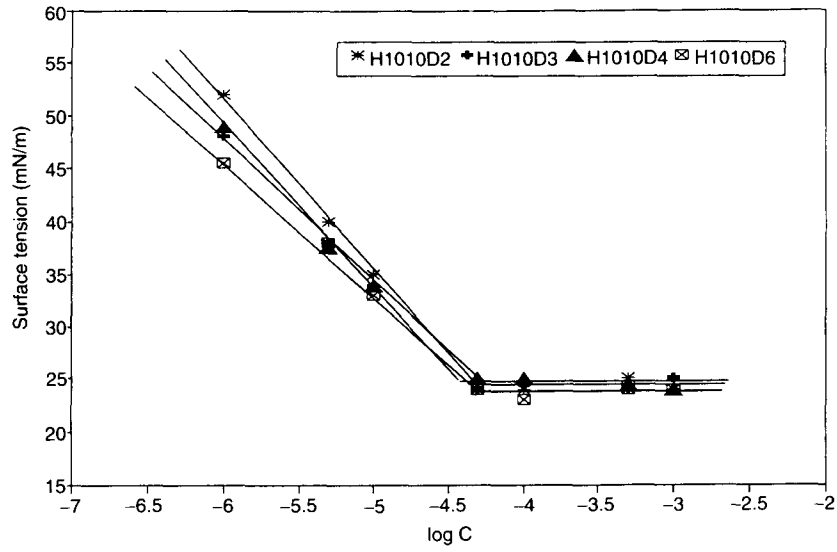


FIG. 3. Plot of surface tension/logC for binary systems water/1010D*n* at 60°C.

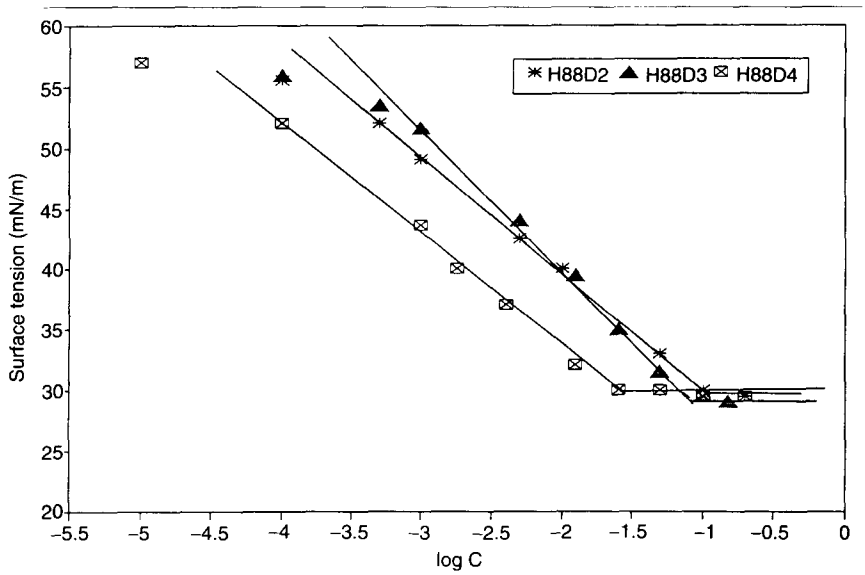


FIG. 4. Plot of surface tension/logC for binary systems formamide/88D*n* at 60°C.

much higher than the corresponding CC values in aqueous solution. These results agree with CMC values of ionic surfactants in formamide obtained by Ray (22), Couper *et al.* (23), and Lattes and coworkers (24–26).

From the γ vs. logC curves in water, the surface-excess concentrations (Γ) and the area per molecule (A_{min}) for these surfactants were determined. From these curves, the effect of both the hydrophobic group and the number of ethyleneoxide (EO) groups on the A_{min} at the surface can be determined.

TABLE 10
Surface Properties of Compounds H*mm*D*n* and H*mm*E*n* in Water at 60°C

Compound	$10^4 \times$ CC ± 0.05 (mol • L^{-1})	γ_{CC} ± 1 (mNm^{-1})	A_{min} ± 4 ($nm^2 \times 10^2$)	Γ_{max} (mols/cm^2 $\times 10^{10}$)
H66D2	37.30	27	80	2.07
H88D2	1.46	25	69	2.40
H1010D2	0.42	24	62	2.67
H66D3	54.70	27	85	1.95
H88D3	2.29	25	74	2.23
H1010D3	0.47	24	67	2.42
H66D4	59.00	27	93	1.79
H88D4	3.50	24	84	1.97
H1010D4	0.49	24	75	2.20
H66D6	65.00	27	109	1.52
H88D6	4.13	24	101	1.64
H1010D6	0.55	24	83	2.00
H88E2	5.23	28	66	2.50
H1010E2	0.81	27	64	3.81
55Lec[a]	98.00[a]	—	66 ± 1[b]	—
66Lec[b]	—	—	60 ± 1[b]	—
77Lec[a]	1.70[a]	—	63 ± 3[b]	—
88Lec[b]	—	—	85 ± 2[b]	—

[a]55Lec and 77Lec are, respectively, dihexanoyllecithin and dioctanoyllecithin. Critical micelle concentration values are measured at 45°C by Wells (Ref. 11).
[b]88Lec is the nonanoyllecithin. The A_{min} are evaluated by Tausk *et al.* (Ref. 12).

These data are shown in Table 8. These values are of the same order as those of lecithin analogues 55Lec, 77Lec, and 99Lec (12). Also, A_{min} in the current series of surfactants decreased when the alkyl chainlength was increased. Compound H66D2 produced a value of 80 $nm^2 \times 10^2$ for the minimum surface area per molecule at the water/air interface, whereas H88D2 gave a value for the area per molecule of about 69 $nm^2 \times 10^2$. The slopes of the γ vs. logC curves below the CC, as indicated by a sharp break, decreased with an increasing number of EO groups; from this, it follows that the surface concentration (Γ) diminishes progressively. Consequently, the A_{min} increases as a function of the number of EO units. Thus, for example, compound H66D2 has an A_{min} of 80 $nm^2 \times 10^2$, whereas H66D6 has an A_{min} of 109 $nm^2 \times 10^2$. As was expected, these values were higher than those of the single-chain nonionic surfactants of the type C_mEO_n (27,28). Values ranging from 21.7–24.8 $nm^2 \times 10^2$ at 25°C have been reported for the nonionic single-chain surfactants if C_mEO_n(m = 12, n = 2–6). Compared with the corresponding Lys analogues (6,7,29), the A_{min} values of the present compounds agree in certain characteristic features. They are of the same order of magnitude and change in a similar way with the molecular structure pa-

TABLE 11
Surface Properties of Compounds H*mm*D*n* and H*mm*E*n* in Formamide at 60°C

Compound	$10^2 \times CC^a$ ± 0.1 (mol • L^{-1})	γ_{CC} ± 1 (mNm^{-1})
H66D2	9.5	30
H66D3	10.2	30
H88D2	2.7	29
H88D3	8.9	29
H1010D2	1.8	28
H1010D3	2.3	28
H1010E2	2.5	2

[a]Critical concentration.

rameters of hydrophobic chainlength and EO units. According to Kuwamura and Takahashi (30,31), values of A_{min} for compounds H*mm*D*n* are the result of the effect of three structural parameters: (i) hydrophobic alkyl chainlengths; (ii) structure of the connecting amino acid, Asp or Glu; and (iii) number of EO units in the hydrophilic head group. For H*mm*D*n*, the effect of (i) and (iii) are the most important. It is noticeable, for example, that the values of A_{min} for this series decrease with an increase of methylene groups and increase for each successive EO group added. No important changes, however, were observed in A_{min} when Asp was substituted by Glu; for example, $A_{H88D2} = 69\ nm^2 \times 10^2$, while $A_{H88E2} = 66\ nm^2 \times 10^2$; $A_{H1010D2} = 62\ nm^2 \times 10^2$ and $A_{H1010E2} = 64\ nm^2 \times 10^2$.

When comparing the A_{min} of the 88Lec ($85\ nm^2 \times 10^2$) homologue, reported by Tausk *et al.* (12), with those of the corresponding H88D*n* series (with the same chainlengths), homologue H88D4 showed $A_{min} = 84\ nm^2 \times 10^2$, comparable with that of the 88Lec homologue.

The surface-tension study in water showed these compounds to be surface-active materials, in particular those with fluorinated alkyl chains. Their water solubility properties at 22°C, however, as for lecithins, was poor but increased with temperature. As expected, the solubility of these compounds decreased with increasing alkyl chainlength. No important changes were observed when Asp was substituted by Glu. Nevertheless, when compared to Lys analogues, some differences were observed. The orientation of the amide groups in the central pivot of the molecule should influence the surface activity and aggregation ability of these new molecules. In contrast to Lys analogues, the present compounds show comparable properties to those of natural compounds.

Surface properties vs. concentration plots of the fluorinated F*mm*D*n* and F*mm*E*n* compounds were not determined due to the poor solubility properties in water and in formamide. Despite this, the lowering of the surface tension of pure solvents by the addition of small amounts of these compounds (Table 9) suggests that they are potential surface-active materials with potential applications in the biological field (32–34).

To improve the solubility properties of these fluorinated compounds, synthesis of analogue compounds with poly-ols or sugar functions in the hydrophilic head group, instead of the polyoxyethylene chains, is in progress (35). Fluorinated compounds appear to be interesting surfactants for oxygenation purposes in the field of biology (34,36,37).

Further investigation in this series are currently being prepared to extend the work. As indicated in a previous paper (7), following the theories of Israelachvili (38) and Tanford (39) concerning the structure of micellar aggregates, a study of the shape and size of the aggregates will be undertaken. Moreover, from the work carried out by Katz (40), the effect of the CH_2 contribution to thermodynamic parameters of air/water adsorption and of micellization for the three series of lecithin analogues, based on Lys, Asp, and Glu amino acids, will be evaluated.

ACKNOWLEDGMENTS

We thank J.J. Delpuech and J.-C. Ravey (CNRS) for helpful discussions of this work. This study has been generously supported by the "Quimica Fina Program" (QFN 89/4011) project, a fellowship to Joan Seguer, and "Action Intégrée Picasso" N-92021 and 104B.

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[Received January 11, 1995; accepted November 4, 1995]