GRASAS Y ACEITES, 57 (3),
JULIO-SEPTIEMBRE, 319-327, 2006,
ISSN: 0017-3495

Amphoteric surfactants containing α-hydroxy ester group and an amino acid residue

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RESUMEN

Tensioactivos anfóteros conteniendo un grupo alfa hidroxi éster y un residuo de aminoácido

Se prepararon una serie de tensioactivos anfóteros conteniendo un grupo alfa hidroxi éster y un residuo de aminoácido por adición de derivados epoxy (obtenidos mediante epoxidación de metacrilato de alquilo) a diferentes tipos de aminoácidos (glicina, alanina, valina, isoleucina, fenilalanina, tirosina, serina, treonina y ácidos aspártico y antranílico). Las estructuras de los compuestos preparados se confirmaron por los espectros de infrarrojo, de masa, resonancia magnética nuclear de protones y análisis elemental. Se determinaron la tensión superficial, el punto de Kraft, el poder espumante, la concentración micelar crítica en emulsión y las estabilidades de Ca++. También se estudiaron la actividad antimicrobiana y la biodegradabilidad.

PALABRAS-CLAVE: Actividad antimicrobiana – Biodegradabilidad – Tensioactivos anfóteros.

SUMMARY

Amphoteric surfactants containing α-hydroxy ester group and an amino acid residue

A series of amphoteric surfactants containing α-hydroxy ester group and an amino acid residue were prepared with the addition of epoxy derivatives (which were prepared from epoxidation of alkyl methacrylate) to different types of amino acids (glycine, alanine, valine, isoleucine, phenylalanine, tyrosine, serine, threonine, aspartic and anthranilic acid). The structures of the prepared compounds were confirmed by infrared spectra, proton magnetic resonance spectra, Mass spectra and elementary analysis. Surface tension, Kraft point, foaming power, critical micelle concentration emulsion and Ca^{++} stabilities were determined. Antimicrobial activity and biodegradability were also screened.

KEY-WORDS: Anphoteric surfactants – Antimicrobial activity – Biodegradability.

1. INTRODUCTION

As a part of our program (Eissa., 2002; Eissa et al. 1996; Eissa. 1995; Eissa et al. 2003; Amin et al., 2004; El-Dougdoug et al., 2001) on the synthesis and characterization of different types of surface active agents, the author attempts to synthesize a novel group of amphoteric surfactants based on amino acid residue. Amino acids are not only essential components of the human body but also interesting raw materials for surfactants (Nasreddine et al., 1993). The presence of anionic and cationic moieties in the molecule of an amino acid makes it possible to prepare various kinds of surfactants by introducing a hydrophobic group into the molecule (Klrvens., 1953; Herrmann., 1963; Tokiwa et al., 1967; Bluestein et al., 1973; Andersen., 1957).

Sodium salts of long chain N-alkyl-β-alanines are well known amphoteric surfactants. Surfactants of the β-alanine type have been used in such applications as shampoos, cosmetics, emulsion paints, various products in the textile industry, corrosion inhibitors, industrial cleaning products and many others (Hikota., 1979). One of the most important features of these surfactants is that they are effective over a wide pH range, in their cationic form in an acidic solution, and in their anionic form in an alkaline solution, except at the isoelectric points. Furthermore, these surfactants are less toxic to higher animals and are not irritating to human skin (Christophe et al., 2002; Marion et al., 2002; Hironari et al., 2002; Infante et al., 2003).

The present paper deals with the preparation and functional properties of a series of amphoteric surfactants containing the α-hydroxy group and an amino acid residue. A series of these compounds was synthesized from alkyl methacrylate as raw starting material (alkyl; a, C_8H_{17}; b, $C_{12}H_{25}$; c, $C_{18}H_{37}$).

2. MATERIAL AND METHODS

The IR spectra in KBr were recorded on a Shimadzu 470 Spectrometer. The ^1H NMR were measured on Varian EM-390-90 MHz a spectrometer using TMS as internal reference and the chemical shifts are expressed as δ (ppm). The mass spectra were recorded on HP Model: MS 5988 at 70 eV. The physical and spectral data are listed in (Tables 1 and 2).

2.1. General procedure of formation of epoxy fatty ester (2a-c)

Alkyl methacrylate 1a-c (1 mmole) (Figure 1) in glacial acetic acid (40 ml) was mixed with 8 % aqueous sodium hydroxide (12 ml) followed by the addition of hydrogen peroxide (30 % 5 ml). The

Table 1.
Physical data of the synthesized compounds

Compds	R	R'	m.p °C Yield%	M. F M.wt	Analysis Calcd/Found %		
					C	H	N
2a	C_8H_{17}		65-67 (69%)	$C_{12}H_{22}O_3$ 214.31	67.26 67.32	10.35 10.41	
2b	$C_{12}H_{25}$		63-65 (75%)	$C_{16}H_{30}O_3$ 270.24	71.07 71.12	11.18 11.24	
2c	$C_{18}H_{37}$		66-68 (70%)	$C_{22}H_{42}O_3$ 354.58	74.52 74.57	11.94 11.99	
3a	C_8H_{17}	-H	120-122 (76%)	$C_{14}H_{27}NO_5$ 289.37	58.11 58.16	9.40 9.45	4.84 4.89
3b	$C_{12}H_{25}$	-H	125-127 (74%)	$C_{18}H_{35}NO_5$ 345.48	62.586 2.63	10.211 0.25	4.05 4.09
3c	$C_{18}H_{37}$	-H	123-125 (78%)	$C_{24}H_{47}NO_5$ 429.65	67.09 67.13	11.031 1.08	3.26 3.30
4a	C_8H_{17}	$-CH_3$	90-92 (77%)	$C_{15}H_{29}NO_5$ 303.40	59.38 59.43	9.63 9.68	4.62 4.67
4b	$C_{12}H_{25}$	$-CH_3$	90-92 (68%)	$C_{19}H_{37}NO_5$ 359.51	63.48 63.52	10.371 0.42	3.90 3.95
4c	$C_{18}H_{37}$	$-CH_3$	91-93 (65%)	$C_{25}H_{49}NO_5$ 443.67	67.686 7.73	11.131 1.17	3.16 3.20
5a	C_8H_{17}	$CH(CH_3)_2$	85-87 (75%)	$C_{17}H_{33}NO_5$ 331.46	61.60 61.65	10.04 10.09	4.23 4.27
5b	$C_{12}H_{25}$	$CH(CH_3)_2$	80-82 (70%)	$C_{21}H_{41}NO_5$ 387.56	65.086 5.12	10.661 0.70	3.61 3.65
5c	$C_{18}H_{37}$	$CH(CH_3)_2$	88-90 (71%)	$C_{27}H_{53}NO_5$ 471.73	68.756 8.79	11.321 1.37	2.97 3.02
6a	C_8H_{17}	$CH_3CH_2CH(CH_3)-$	86-88 (65%)	$C_{18}H_{35}NO_5$ 345.48	62.58 62.63	10.211 0.26	4.05 4.09
6b	$C_{12}H_{25}$	$CH_3CH_2CH(CH_3)-$	85-87 (68%)	$C_{22}H_{43}NO_5$ 401.59	65.806 5.85	10.791 0.83	3.49 3.53
6c	$C_{18}H_{37}$	$CH_3CH_2CH(CH_3)-$	79-81 (66%)	$C_{28}H_{55}NO_5$ 485.75	69.246 9.29	11.411 1.45	2.88 2.94
7a	C_8H_{17}	$C_6H_5CH_2$-	105-107 (74%)	$C_{21}H_{33}NO_5$ 379.24	66.46 66.50	8.76 8.81	3.96 4.00
7b	$C_{12}H_{25}$	$C_6H_5CH_2$-	106-108 (77%)	$C_{25}H_{41}NO_5$ 435.3	68.93 68.98	9.49 9.54	3.22 3.27
7c	$C_{18}H_{37}$	$C_6H_5CH_2$-	109-111 (69%)	$C_{31}H_{53}NO_5$ 519.39	71.64 71.69	10.28 10.33	2.96 3.01
8a	C_8H_{17}	*p*-$OHC_6H_5CH_2$	105-107 (65%)	$C_{21}H_{33}NO_6$ 395.23	63.78 63.83	8.41 8.48	3.54 3.60
8b	$C_{12}H_{25}$	*p*-$OHC_6H_5CH_2$	108-110 (60%)	$C_{25}H_{41}NO_6$ 451.29	66.49 66.54	9.15 9.20	3.10 3.16
8c	$C_{18}H_{37}$	*p*-$OHC_6H_5CH_2$	115-117 (74%)	$C_{31}H_{53}NO_6$ 535.39	69.50 69.55	9.97 10.02	2.61 2.66
9a	C_8H_{17}	$HOCH_2$	140-142 (61%)	$C_{15}H_{29}NO_6$ 319.39	56.41 56.44	9.15 9.19	4.39 4.43
9b	$C_{12}H_{25}$	$HOCH_2$	139-141 (60%)	$C_{19}H_{37}NO_6$ 375.26	60.77 60.82	9.93 9.98	3.73 3.77
9c	$C_{18}H_{37}$	$HOCH_2$	128-140 (66%)	$C_{25}H_{49}NO_6$ 459.36	65.32 65.37	10.74 10.78	3.05 3.09

Table 1. (cont.)
Physical data of the synthesized compounds

Compds	R	R'	m.p °C Yield%	M. F M.wt	Analysis Calcd/Found %		
					C	H	N
10a	C_8H_{17}	$CH_3CH(OH)-$	120-122 (70%)	$C_{16}H_{31}NO_6$ 333.22	57.64 57.68	9.37 9.41	4.20 4.24
10b	$C_{12}H_{25}$	$CH_3CH(OH)-$	118-120 (75%)	$C_{20}H_{39}NO_6$ 389.28	61.67 61.71	10.09 10.14	3.60 3.66
10c	$C_{18}H_{37}$	$CH_3CH(OH)-$	121-123 (74%)	$C_{26}H_{51}NO_6$ 473.37	65.93 65.98	10.85 10.88	2.96 2.99
11a	C_8H_{17}	$HOOC-CH_2$	150-152 (60%)	$C_{16}H_{29}NO_7$ 347.19	55.32 55.37	8.41 8.44	4.03 4.08
11b	$C_{12}H_{25}$	$HOOC-CH_2$	152-154 (65%)	$C_{20}H_{37}NO_7$ 403.26	59.53 59.57	9.24 9.29	3.47 3.52
11c	$C_{18}H_{37}$	$HOOC-CH_2$	150-152 (75)	$C_{26}H_{49}NO_7$ 487.35	64.03 64.08	10.13 10.17	2.87 2.90
12a	C_8H_{17}	$o\text{-}NH_2C_6H_5COOH$	130-132 (68%)	$C_{19}H_{29}NO_5$ 351.45	64.93 64.98	8.32 8.37	3.99 4.05
12b	$C_{12}H_{25}$	$o\text{-}NH_2C_6H_5COOH$	133-135 (73%)	$C_{23}H_{37}NO_5$ 407.56	67.78 67.83	9.15 9.19	3.44 3.49
12c	$C_{18}H_{37}$	$o\text{-}NH_2C_6H_5COOH$	130-132 (75)	$C_{29}H_{49}NO_5$ 491.72	70.84 70.88	10.04 10.09	2.85 2.89

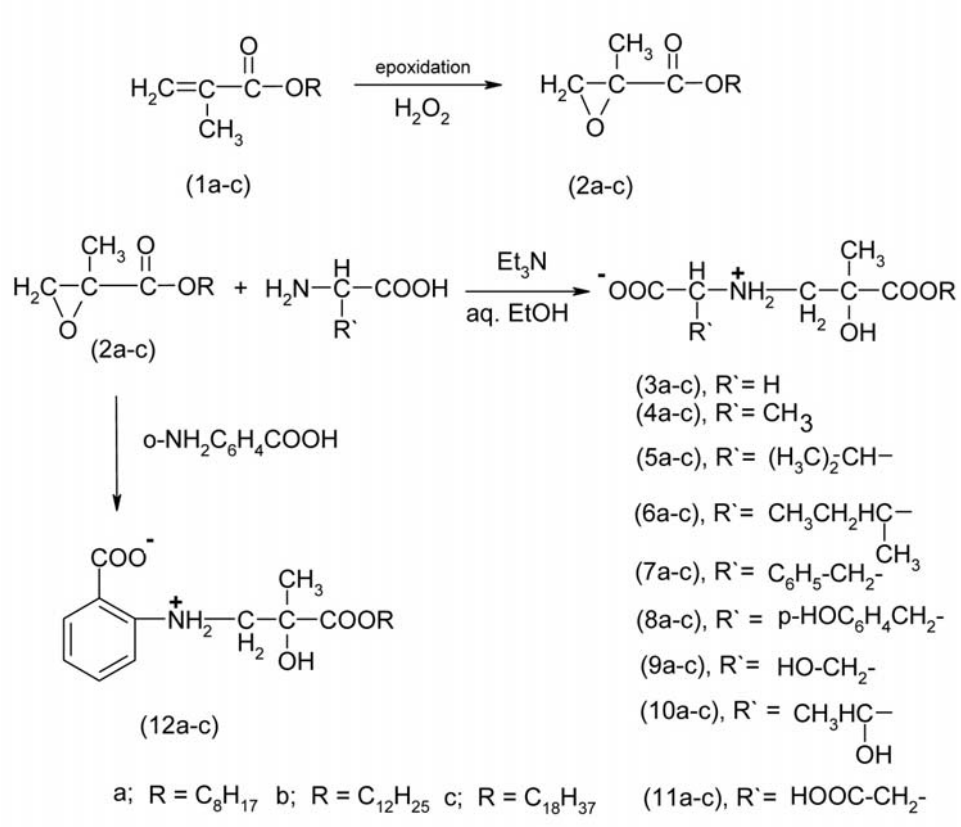

Figure 1
Scheme of the synthesis of the different compounds.

Table 2.
Spectral data of some synthesized compounds

Compds	IR: υ (cm^{-1})	^1H NMR ($CDCl_3$): δ	MS: m/z (%)
2b	2920-2850 (CH_2 aliphatic), 1720 (C=O) and 1270 (C-O)	0.9 (t, 3H, CH_3 terminal of alkyl chain), 1.2 (m, 20H, CH_2 of alkyl chain), 1.4 (s, 3H, CH_3 of α-substituted ester), 3.1 (s, 2H, CH_2 of oxirane ring) and 4.2 (t, 2H, $COO\underline{CH_2}$)	
3c	3420(OH), 3320 (NH), 2910-2830 (CH_2 aliphatic), 1730 (C=O) and 1300 (C-O)	0.96 (t, 3H, CH_3 terminal CH_3 of alkyl chain), 1.3 (m, 32H, CH_2 of alkyl chain), 1.6 (s, 3H, CH_3 of α-substituted esters), 2.0-2.2 (broad, 2H, NH and alcoholic OH), 2.9 (s, 2H, $NH\underline{CH_2}$), 3.5 (s, 2H, $\underline{CH_2}COOH$), 4.1 (t, 2H, $COO\underline{CH_2}$) and 11.2 (s,1H, COOH)	M^++1= 430 (30)
4a	3450 (OH), 3340 (NH), 2950-2820 (CH_2 aliphatic), 1710 (C=O) and 1280 (C-O)	0.9 (t, 3H, CH_3 terminal CH_3 of alkyl chain), 1.3 (m, 12H, CH_2 of alkyl chain), 1.5 (s, 3H, CH_3 of α-substituted ester), 1.7 (d, 3H, CH_3 of α-substituted carboxylic acid), 2.2 (broad, 2H, NH and alcoholic OH), 2.7 (s, 2H, $NH\underline{CH_2}$), 3.6 (q, 1H, α-hydrogen to COOH), 4.1 (t, 2H, $COO\underline{CH_2}$) and 11.2 (s,1H, COOH)	M^+ = 303 (25)
5b	3420 (OH), 3300 (NH), 2950-2820 (CH_2 aliphatic), 1700 (C=O) and 1270 (C-O)	0.9 (t, 3H, CH_3 terminal CH_3 of alkyl chain), 1.1 (d, 6H, $2CH_3$ of isopropyl moiety), 1.3 (m, 20H, CH_2 of alkyl chain), 1.4 (s, 3H, CH_3 of α-substituted ester), 2.2 (broad, 2H, NH and alcoholic OH), 2.5 (m, 1H, $\underline{CH}(CH_3)_2$), 2.8 (s, 2H, $NH\underline{CH_2}$), 3.5 (d, 1H, α-hydrogen to COOH), 4.0 (t, 2H, $COO\underline{CH_2}$) and 11.0 (s,1H, COOH)	
6c	3450 (OH), 3340 (NH), 2950-2820 (CH_2 aliphatic), 1710 (C=O) and 1275 (C-O)	0.9-1.1 (m, 9H, 3 CH_3 (terminal CH_3 of alkyl chain and 2 CH_3 of amino acid residue), 1.2 (m, 32H, CH_2 of alkyl chain), 1.3 (m, 2H, CH_2 of alkyl chain of amino acid residue), 1.5 (s, 3H, CH_3 of α-substituted ester), 2.2 (broad, 2H, NH and alcoholic OH), 2.5 (m, 1H, $\underline{CH}$ of alkyl chain of amino acid), 3.0 (s, 2H, $NH\underline{CH_2}$), 3.5 (d, 1H, α-hydrogen to COOH), 4.1 (t, 2H, $COO\underline{CH_2}$) and 11.0 (s,1H, COOH)	M^+-2= 483 (35)
7a	3430 (OH), 3220 (NH), 3006 (CH aromatic), 2930-2810 (CH_2 aliphatic), 1730 (C=O) and 1285 (C-O)	0.9 (t, 3H, CH_3 terminal CH_3 of alkyl chain), 1.3 (m, 12H, CH_2 of alkyl chain), 1.5 (s, 3H, CH_3 of α-substituted esters), 2.1 (broad, 2H, NH and alcoholic OH), 2.7 (s, 2H, $NH\underline{CH_2}$), 3.1 (d, 2H, $\underline{CH_2}$-Ph), 3.7 (t, 1H, α-hydrogen to COOH), 4.1 (t, 2H, $COO\underline{CH_2}$), 7.1-7.3 (m, 5H, ArH) and 11.0 (s,1H, COOH)	M^+= 379 (22)
8c	3450 (OH), 3200 (NH), 3050 (CH aromatic), 2950-2820 (CH_2 aliphatic),1720 (C=O) and 1300 (C-O)	0.9 (t, 3H, CH_3 terminal CH_3 of alkyl chain), 1.3 (m, 32H, CH_2 of alkyl chain), 1.5 (s, 3H, CH_3 of α-substituted ester), 2.1 (broad, 2H, NH and alcoholic OH), 2.7 (s, 2H, $NH\underline{CH_2}$), 3.1 (d, 2H, $\underline{CH_2}$-Ph), 3.7 (t, 1H, α-hydrogen to COOH), 4.1 (d, 2H, $COO\underline{CH_2}$), 5.2 (br s, 1H, phenolic OH), 6.7-7.0 (d.d, 4H, ArH) and 11.1 (s,1H, COOH)	M^++2=537 (42)
9b	3430-3250 (OH) and (NH), 2950-2820 (CH_2 aliphatic), 1710 (C=O) and 1270 (C-O)	0.9 (t, 3H, CH_3 terminal CH_3 of alkyl chain), 1.3 (m, 12H, CH_2 of alkyl chain), 1.5 (s, 3H, CH_3 of α-substituted esters), 2.2 (broad, 3H, NH and 2 (alcoholic OH), 3.0 (s, 2H, $NH\underline{CH_2}$), 3.6 (t, 1H, $\underline{CH}COOH$), 3.9 (d, 2H, $\underline{CH_2}$-OH), 4.2 (t, 2H, $COO\underline{CH_2}$), and 11.2 (s,1H, COOH)	
10a	3420-3230 (OH) and (NH), 2950-2820 (CH_2 aliphatic), 1710 (C=O) and 1270 (C-O)	0.9 (t, 3H, CH_3 terminal CH_3 of alkyl chain), 1.1 (d, 3H, CH_3 in amino acid residue), 1.4 (m, 12H, CH_2 of alkyl chain), 1.6 (s, 3H, CH_3 of α-substituted ester), 2.1 (broad, 3H, NH and alcoholic 2 OH), 3.0 (s, 2H, $NH\underline{CH_2}$), 3.4 (d, 1H, α-hydrogen to COOH), 3.9 (m, 1H, CH_3-$\underline{CH}$-OH of amino acid part), 4.2 (t, 2H, $COO\underline{CH_2}$) and 11.1 (s,1H, COOH)	M^+= 333(32)
11c	3420-3220 (OH) and (NH), 2940-2830 (CH_2 aliphatic), 1710 (C=O) and 1280 (C-O)	0.9 (t, 3H, CH_3 terminal CH_3 of alkyl chain), 1.3 (m, 32H, CH_2 of alkyl chain), 1.6 (s, 3H, CH_3 of α-substituted ester), 2.1 (broad, 2H, NH and alcoholic OH), 2.6 (d, 2H, $\underline{CH_2}$-COOH), 2.9 (s, 2H, $NH\underline{CH_2}$), 3.8 (t, 1H, α-hydrogen to other COOH), 4.1 (t, 2H, $COO\underline{CH_2}$) and 11.2 (br s, 2H, 2 COOH)	
12b	3430 (OH), 3220 (NH), 3006 (CH aromatic), 2930-2810 (CH_2 aliphatic), 1730 (C=O) and 1285 (C-O)	0.9 (t, 3H, CH_3 terminal CH_3 of alkyl chain), 1.3 (m, 20H, CH_2 of alkyl chain), 1.6 (s, 3H, CH_3 of α-substituted ester), 2.1 (s, 1H, alcoholic OH), 3.3 (s, 2H, $NH\underline{CH_2}$), 3.8 (br s, 1H, NH), 4.1 (t, 2H, $\underline{CH_2}$-COOH), 6.5-7.8 (m, 4H, ArH) and 11.0 (s, 1H, COOH)	

solution was shaken and heated for 2 hr, then allowed to stand overnight at room temperature. Water was then added and the separated solid was crystalized using a suitable solvent to give **(2a-c)**.

2.2. General procedure of the reaction of epoxy fatty ester with amino acids

Triethylamine (1 mmole) dissolved in an aqueous ethanol solution (65 wt % ethanol) was added to amino acid (1 mmole) to protect (as a salt) the carboxyl group of the amino acid. The mixture was stirred at room temperature for 20 min. Subsequently, epoxy fatty ester 2 (1 mmole) was added using a dropper, and the mixture was stirred at 50 °C for 8hr or at 60 °C for one night. Then the triethylamine and ethanol were evaporated. The residue obtained was washed with water and petroleum ether, then dried under vacuum and crystalized using a suitable solvent to obtain (**3a-c** to **11a-c**).

2.3. Surface active properties

2.3.1. Surface and interfacial tension

Surface and interfacial tension were measured using Du-Nouy tensiometer (Findly., 1963) (Kruss,Type 8451),with 0.1 wt % aqueous solution at room temperature (25 °C).

2.3.2. Kraft point

The prepared amphoteric surfactants were measured as the temperature where 1 % dispersion becomes clear under gradual heating (Wiel et al., 1963).

2.3.3. Wetting time

Wetting time was determined by immersing a sample of cotton fabric in a 1.0 wt % aqueous solution of surfactants (Masuyama et al., 1987).

2.3.4. Foaming power

Foaming power was measured according to (Somaya et al., 1998). In this procedure a 25 ml solution (1.0 wt %) was shaken vigorously for 10 seconds in a 100 ml glass stopper, graduated cylinder, at 25 °C. the solution was allowed to stand. The foam height and foaming stability were measured.

2.3.5. Emulsification stability

Emulsification stability was tested using 10 ml of a 20 m mol. aqueous solution of surfactant and 5 ml of toluene at 40 °C. The emulsifying property was determined as the time its took for an aqueous volume separating from the emulsion layer to reach 9 ml counting from the moment of the cession shaking (El-Sawy et al., 1991).

2.3.6. Critical micelle concentration

(CMC) values for the prepared surfactants were determined by the electrical conductivity method (Takeshi., 1970).

2.3.7. Ca^{++} stability

Calcium stability of compounds was determined as described according to (Bristiline et al., 1980).

2.4. Biodegradability test

Biodegradability Die-away test in river water of the prepared surfactants (1.0 wt %) was determined by the surface tension method (Eter et al., 1974) using Du Nouy Tensiometer (Kruss type 8451). Samples taken daily or even more frequently were filtered through Wattmann filter paper number (1) before measuring the surface tension. Surface tension measurements were made periodically each day, on each sample during the degradation test. Biodegradation percent (D) for each sample was calculated using the equation, $D = [(\gamma_t-\gamma_o)/(\gamma_{bt}-\gamma_o)] \times 100$ where γ_t = surface tension at time t, γ_o = surface tension at zero time, γ_{bt}= surface tension of blank experiment at time t (without samples).

2.5. Antimicrobial activity

The antimicrobial activities of the surfactants were evaluated by the agar dilution method (El-Sukkary et al., 1987). Three kinds of Gram-positive bacterial strains, *Stophylococcus Aureus; Bacillus Subtiles* and *Sarcina Lutea,* three kinds of Gram-negative bacteria strains*, Escherichia Coli, Salmonella Trphi and Pseudomonas Aeruginosa* and six kinds of fungal strains, *Candida Albicans, Saccharomyces Cerevisiae, Alternaria Humicala, Fusazium Oxysporum, Aspergillus Flavus* and *Microsporium Gypseum* were used for the testes. Nutrient agar and Sabouraud dextrose agar were used for bacteria and fungi, respectively. In the screening test for antimicrobial activity, 0.4 % stock solutions were prepared by dissolving 40 mg of the test compound in 10 ml of distilled water or ethanol. The stock solutions were diluted in an orderly manner by successive piping of the solution in water containing nutrient agar or sabouraud dextrose agar to obtain 400, 200, 100, 50, 25, 10, 5, 2.5 and 1 ppm concentrations of the compound. After sterilization of the agar, the solutions were poured into sterile Petri dishes, allowed to harden, and were then individually inoculated with one drop of each suspension, each containing a separate test microorganism. The inoculated dishes were then inoculated at 37 °C for two days with bacteria strains and 25 °C for five days with fungal strains, and examined for the presence or absence of

Table 3.
Surface properties of amphoteric compounds

Compd	Surface Tension (dyne/cm) 0.1 %	Interfacial Tension (dyne/cm) 0.1 %	Kraft Point °C 1%	Wetting time (sec.) 1%	Emulsion stability (min.sec)	Foam power (mm) 1%		Ca^{++}stability (ppm)	Cmcx10^{-3} mole / l
						Intial	After 5 min		
3a	31	7.5	17	65	260: 40	200	190	450	3.9
3b	32	8.0	20	90	300: 30	220	205	380	3.7
3c	36	8.5	25	123	350: 50	225	210	350	3.4
4a	33	8.2	16	90	280: 55	155	149	1460	3.8
4b	35	8.7	19	115	320: 25	170	158	1350	3.6
4c	37	9.0	23	132	360: 20	190	173	1200	3.3
5a	34	9.5	26	80	250: 10	180	165	1240	3.6
5b	37	10.3	27	110	290: 25	200	189	1150	3.4
5c	39	11.0	35	135	340: 22	220	205	900	3.2
6a	33	11.4	42	100	286: 40	185	163	1230	4.3
6b	35	12.0	43	115	305: 55	200	184	1260	4.2
6c	37	12.5	48	126	330: 30	230	210	850	3.5
7a	31	6.7	48	85	254: 45	148	155	560	6.1
7b	33	7.0	50	100	280: 26	165	176	500	5.6
7c	35	7.5	55	120	320: 10	188	185	450	4.6
8a	32	8.7	17	36	227: 21	145	164	1530	4.9
8b	34	9.2	22	42	260: 39	159	179	1420	4.7
8c	36	10.0	22	48	300: 38	183	196	1300	4.1
9a	30	8.5	15	30	230: 47	210	190	1260	4.1
9b	32	9.0	16	31	270: 50	220	205	1150	3.8
9c	34	9.5	20	36	310: 11	235	214	950	3.7
10a	33	7.0	13	32	222: 50	190	174	1360	3.9
10b	36	7.5	19	35	265: 11	200	183	1240	3.6
10c	38	8.0	22	35	300: 38	205	191	1120	3.3
11a	28	8.6	16	35	226: 17	180	166	1420	3.8
11b	30	9.0	20	40	263: 33	200	182	1360	3.5
11c	32	9.4	23	45	290: 22	210	190	1300	3.1
12a	32	8.7	19	39	227: 21	155	168	1530	5.7
12b	34	9.2	22	45	260: 39	160	172	1420	5.2
12c	36	10.0	25	48	300: 38	165	206	1300	4.6

Error of measurements was:
Surface and interfacial tensions = ± 0.1 dynes/cm.
Kraft point = ± 1 °C
Foam height = ± 2 mm
Wetting time = ± 1 sec
Emulsion stability = ± 1 min

growth. Antimicrobial activities are represented in terms of minimum inhibitory concentrations (MIC).

3. RESULTS AND DISCUSSION

A number of amphoteric surfactants was synthesized by the reaction of alkyl ester epoxides and amino acids (glycine, alanine, valine, isoleucine, phenylalanine, tyrosine, serine, threonine, aspartic and anthranilic acid). This group can be prepared from readily accessible starting materials without expensive reagents or special equipment. In general, the synthetic procedures gave relatively high yields in two simple synthetic steps.

3.1. Surface active properties

The surface properties (surface and interfacial tension, Kraft point, wetting power, foaming properties, emulsifying power and critical micelle

concentration) of well purified compounds were investigated in distilled water. These surfactants show relatively high surface activity and a comparative study between the structure and the result was made.

3.1.1. Surface and interfacial tension

The measurements of the individual compounds are listed in (Table 3).The results indicated that, a linear relationship was observed between surface and interfacial tension and alkyl chain length (as the number of carbon atoms in the alkyl chain increases the surface and interfacial tension increases).

3.1.2. Kraft point

The Kraft point of a surfactant molecule is the temperature at which 1% dispersion solution becomes clear under gradual heating. The Kraft points of all synthesized amphoteric surfactants are also summarized in (Table 3). Although the Kraft points increase in the order $C_{18} > C_{12} > C_8$ no remarkable difference among three homologues was observed. However, as the molecular weight of an amino acid increases the Kraft point increases.

3.1.3. Wetting power

The wetting time of the tested amphoteric surface active agents was determined by calculating the

Table 4.
Biodegradability of Amphoteric Products

Compds.	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
3a	44	52	60	71	84	–	–
3b	41	49	45	65	77	92	–
3c	38	45	51	62	70	84	–
4a	45	55	62	69	78	88	97
4b	41	50	58	66	75	85	–
4c	37	45	54	63	71	83	91
5a	41	51	64	75	90	–	–
5b	38	46	58	72	87	93	–
5c	35	42	53	67	82	90	–
6a	43	50	63	75	92	–	–
6b	39	47	58	71	88	93	–
6c	37	44	54	68	75	89	94
7a	40	49	58	69	82	98	95
7b	37	46	55	64	79	84	92
7c	35	42	51	60	74	86	92
8a	41	55	66	77	89	–	–
8b	39	53	62	72	86	93	–
8c	33	50	58	68	83	89	95
9a	49	56	67	81	90	–	–
9b	42	52	64	75	87	94	–
9c	39	47	59	69	79	89	96
10a	48	58	69	80	93	–	–
10b	45	54	65	77	89	94	–
10c	41	51	61	73	84	90	–
11a	50	53	64	76	89	–	–
11b	44	50	60	71	86	92	–
11c	38	46	55	66	79	89	97
12a	47	55	66	77	81	89	99
12b	43	53	62	72	79	86	93
12c	39	50	58	68	72	83	90

Error of calculations was: Biodegradation rate = ± 0.5 %.

sinking time in seconds of a grey cotton cloth in the surfactant solution. The synthesized surfactants showed good performance for wetting power (shorter sinking time). Compounds (**8a**, **9a-c**, **10a-c** and **11a**) recorded excellent wetting power which makes them useful for extensive applications in the textile industry.

3.1.4. Foaming power

The foaming properties of all the synthesized surfactants were measured by the Ross and Miles method (Ross et al., 1941). Amphoteric surfactants showed good foam production as well as better foam stability above the (CMC). On the other hand, amphoteric surfactants containing an aromatic ring such as (**7a-c**, **8a-c** and **12a-c**) showed poor foaming properties. Extremely low foaming can probably be ascribed to the low solubility (low hydrophilicity) of the compounds in water. Table 3 shows the foam production and foam stability of all the synthesized amphoteric surfactants.

3.1.5. Emulsifying power

Emulsification is one of the most important properties of surfactants. In many textile processes such as scouring and dyeing, it is necessary to introduce surfactants into a bath to remove oily impurities from the fibers. On the other hand, amphoteric surfactants with good emulsion stability have been used in such fields as shampoos and cosmetics, emulsion paints and in the textile industry. The emulsification power is determined and listed in (Table 3). The results reflect the fact that as the alkyl chain length increases the emulsifying power increases.

3.1.6. Ca^{++} - Stability

The calcium ion stability results of amphoteric surfactants are shown in (Table 3). High calcium stability values show that the prepared surfactants can be used in hard water. The calcium stability

Table 5.
Antimicrobial activity of amphoteric compounds.

Compds	Stophylococcus Aureus *MIC*	Bacillus Subtiles *MIC*	Sarcina Lutea *MIC*	Escherichia Coli *MIC*	Salmonella Trphi *MIC*	Pseudomonas Aeruginosa *MIC*	Candida Albicans *MIC*	Saccharomyces Cerevisiae *MIC*	Alternaria Humicala *MIC*	Fusazium Oxysporum *MIC*	Asperdillus Flavus *MIC*	Microsporium Gypseum *MIC*
3a	> 400	100	50	10	200	50	25	>400	2.5	100	200	400
3b	200	25	50	10	400	100	10	200	10	100	100	400
3c	100	50	100	5	100	100	10	200	10	50	50	>400
4a	400	25	50	10	200	50	25	100	2.5	100	100	400
4b	>400	25	50	10	400	100	10	50	10	100	25	>400
4c	200	50	25	5	100	100	10	50	2.5	50	50	200
5a	100	50	200	10	200	50	2.5	100	2.5	50	50	200
5b	50	25	50	10	200	100	1	50	2.5	25	25	200
5c	25	50	100	5	100	100	2.5	100	2.5	50	50	50
6a	100	100	200	10	50	50	25	100	2.5	100	100	200
6b	25	25	50	10	50	100	10	50	2.5	50	50	200
6c	10	50	100	5	25	100	10	100	1	50	50	50
7a	10	25	50	10	50	50	25	100	10	50	100	200
7b	1	10	50	10	25	100	10	10	2.5	25	50	100
7c	2.5	2.5	25	5	10	100	1	2.5	2.5	10	50	50
8a	100	100	200	10	200	50	25	200	2.5	100	200	50
8b	50	25	50	10	400	100	10	200	2.5	100	100	200
8c	25	50	100	5	100	100	10	100	2.5	50	50	50
9a	> 400	100	200	10	200	50	25	>400	10	100	200	200
9b	200	25	50	10	400	100	10	200	2.5	100	100	200
9c	200	50	100	5	100	100	10	100	2.5	50	50	>400
10a	50	100	200	10	50	50	25	>400	50	100	200	200
10b	50	25	50	10	50	100	10	400	10	100	100	50
10c	25	50	100	5	25	100	10	400	10	50	50	50
11a	50	100	200	10	200	50	25	50	100	100	200	400
11b	50	25	50	10	400	100	10	10	50	100	100	>400
11c	10	50	100	5	100	100	10	2.5	50	50	50	400
12a	> 400	100	200	10	200	50	25	10	2.5	100	200	50
12b	400	25	50	10	400	100	10	2.5	2.5	100	100	200
12c	100	50	100	5	100	100	10	2.5	2.5	50	50	50

Origin of cultures: Botany Department, Faculty of Science, Benha University, Egypt.

decreased with the increase in the molecular weight of the hydrophobic part of the surfactant under the conditions of constant temperature.

3.1.7. Critical micelle concentration (CMC)

The critical micelle concentration values of the prepared amphoteric surfactants were determined using the electrical conductivity method. The results showed that as the hydrophobic part increases the CMC values decrease, this means that aliphatic compounds exhibit larger intermolecular hydrophilic interactions, making it easier for them to form aggregates in water than those which contain an aromatic ring. Also, the results of CMC measurements reflect the fact that as the length of alkyl chain increases the CMC decreases.

3.2. Biodegradability

The biodegradability of the tested compounds after one week was determined and listed in (Table 4). Each experiment was repeated three times, and the results are reported as averages of three values. For example, compound (**9a**) was 100% degraded in 6 days and 81% degraded in 4 days which makes it an excellent biodegradable surface active agent.

3.3. Antimicrobial activity

All the synthesized surface active agents were screened for antimicrobial activity against Gram-positive and Gram-negative bacterial strains and fungal strains. The minimum inhibitory concentrations (MIC) for the compounds tested are given in (Table 5). Compounds (**5a,b**, **6b,c** and **7a,b,c**) showed a broad spectrum of antimicrobial activity. On the other hand compounds (**3c**, **4b**, **9c**, and **11b,c**) showed no significant antimicrobial activities.

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Recibido: Mayo 2005
Aceptado: Marzo 2006