Long Chain Carboxylic Acids Containing Ether Linkage: IV. The Antibacterial Activities of O-(2-Alkylaminoethyl)-3-oxypropionic and N-(2-Alkyloxyethyl)-3-aminopropionic Acids

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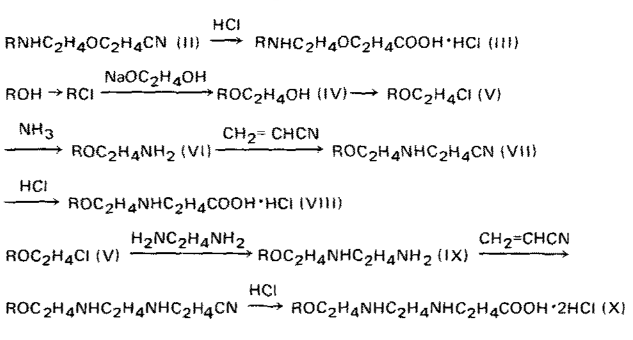
ABSTRACT

The carboxylic amphoteric surfactants containing ether and substituted amine groups such as O-(2-alkylaminoethyl)-3-oxypropionic- and N-(2-alkyloxyethyl)-3-aminopropionic acids were synthesized, and their antibacterial activities in terms of growth inhibition of *Staphylococcus aureus* and *Escherichia coli* were examined. The structural effect, such as bonding position of ether and amino groups, and the chain length effect between undecyloxy-, dodecyloxy- and tridecyloxy radicals were examined, but no definite correlation between positional isomers and chain length and antibacterial activities was observed. Furthermore, to study the effect of the increase of the number of ether or amino groups on the antibacterial activities, N-(2-(2-dodecyloxyethyl)-aminoethyl)-3-aminopropionic acid and N-(2-(2-dodecyloxyethyl)oxyethyl)-3-aminopropionic acid were synthesized. Their antibacterial activities were almost as effective as the corresponding amino or ether acids. Moreover aqueous solution of these substituted propionic acids showed better surface activities at pH 4.0, 7.0 and 10.0.

INTRODUCTION

Previously the authors reported the antibacterial and surface active properties of the amine salts of long chain carboxlic acids containing an ether group (1). But these amine salts are relatively unsatable, especially in acidic solution. To impart better stability, the authors synthesized some amphoteric sufactants containing an ether group by incorporating the substituted amine group into the alkoxy carbon chain. Two types of amphoterics are synthesizable: O-(2-alkylaminoethyl)-3-oxypropionic acid, $RNHCH_2CH_2OCH_2CH_2COOH$; and N-(2-alkyloxyethyl)-3-aminopropionic acid, $ROCH_2CH_2NHCH_2CH_2COOH$.

In this paper the authors describe an experimental study of the antibacterial and surface active properties of these compounds. Undecyloxy, dodecyloxy and tridecyloxy groups were selected, because the largest activities of dodecyloxy group in the even number alkoxy series were previously confirmed (2). Then the effect of the increase of ether or amino groups was also discussed.



$$ROH \rightarrow RCl \xrightarrow{H_2N{\cdot}C_2H_4OH} RNHC_2H_4OH\ (I) \xrightarrow{CH_2=CHCN}$$

$$RNHC_2H_4OC_2H_4CN\ (II) \xrightarrow{HCl} RNHC_2H_4OC_2H_4COOH{\cdot}HCl\ (III)$$

$$ROH \rightarrow RCl \xrightarrow{NaOC_2H_4OH} ROC_2H_4OH\ (IV) \longrightarrow ROC_2H_4Cl\ (V)$$

$$\xrightarrow{NH_3} ROC_2H_4NH_2\ (VI) \xrightarrow{CH_2=CHCN} ROC_2H_4NHC_2H_4CN\ (VII)$$

$$\xrightarrow{HCl} ROC_2H_4NHC_2H_4COOH{\cdot}HCl\ (VIII)$$

$$ROC_2H_4Cl\ (V) \xrightarrow{H_2NC_2H_4NH_2} ROC_2H_4NHC_2H_4NH_2\ (IX) \xrightarrow{CH_2=CHCN}$$

$$ROC_2H_4NHC_2H_4NHC_2H_4CN \xrightarrow{HCl} ROC_2H_4NHC_2H_4NHC_2H_4COOH{\cdot}2HCl\ (X)$$

(Ia) — (Xa): R= $C_{12}H_{25}$

FIG. 1. The preparation of substituted oxy- and aminopropionic acids.

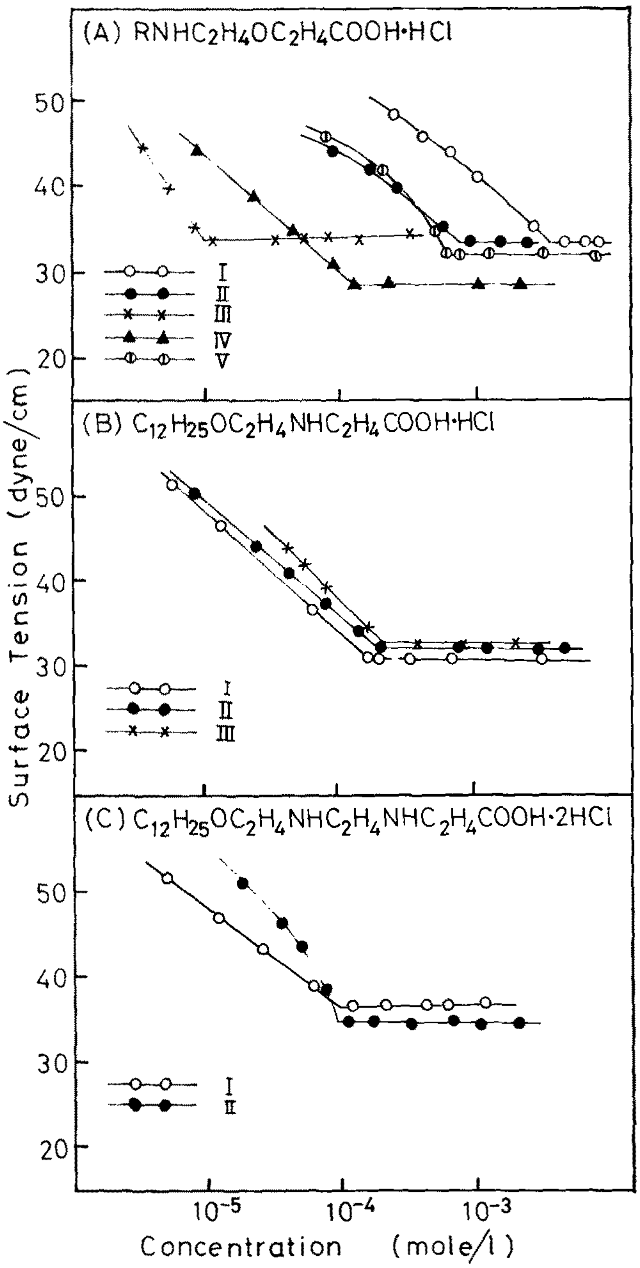


FIG. 2 Surface tension-concentration curves of the substituted oxy- and aminopropionic acids. (A) I: $C_{11}H_{23}$-, pH 7 II: $C_{12}H_{25}$-, pH 7 III: $C_{13}H_{27}$-, pH 7 IV; $C_{12}H_{25}$-, pH 10 V: $C_{12}H_{25}$-, pH 4. (B) I: pH 10 II: pH 7 III: pH 4. (C) I: pH 10 II: pH 4.

TABLE I

Characteristics of 2-Alkylaminoethanols, $R(OCH_2CH_2)_nNHCH_2CH_2OH$

R	*n*	Boiling point, C/mm Hg	Melting point, C[a]	Per cent (calculated) C	H	N
$C_{11}H_{23}$	0	154-159/1	37.0-38.7 ---	72.61 (72.50)	13.50 (13.57)	6.80 (6.50)
$C_{12}H_{25}$	0	125-132/0.7	42.3-45.0 (45)	---	---	---
	1	164-165/0.5	39.0-40.0 ---	70.18 (70.28)	12.68 (12.90)	5.11 (5.12)
	2	190-195/1.5	35.4-36.3 ---	68.32 (68.09)	12.41 (12.38)	4.33 (4.41)
$C_{13}H_{27}$	0	140-144/0.5	49.2-50.5 ---	74.07 (74.01)	13.44 (13.66)	5.78 (5.75)

[a] Reference 7.

TABLE II

Characteristics of Alkyloxyethanols, $R(OCH_2CH_2)_nOH$

R	*n*	Boiling point, C/mm Hg	n_D^t	Per cent (calculated) C	H
$C_{11}H_{23}$	1	113-115/0.5	1.4435^{20}	72.40 (72.17)	13.14 (13.04)
$C_{12}H_{25}$	1	122-123/0.5	1.4434^{25}	72.70 (72.98)	12.96 (13.13)
	2	157-162/1.0	1.4465^{25}	70.20 (70.02)	12.60 (12.49)
$C_{13}H_{27}$	1	128-132/0.5	1.4357^{50}	74.07 (73.71)	13.81 (13.19)

EXPERIMENTAL PROCEDURES

The procedures of the synthesis of these amphoterics are shown in Figure 1.

Material

n-Dodecylalcohol is purified by fractional distillation of commercial alcohol in vacuo. *n*-Undecyl and *n*-tridecylalcohol are prepared by the Bouveault-Blanc reduction of the methyl esters of the corresponding fatty acids. The purity of each alcohol is over 97% by gas liquid chromatography analysis.

From these alcohols, chlorides are prepared by the thionyl chloride method, and the boiling points of the chlorides obtained are: undecyl, 113-116 C/2.0 mm Hg; dodecyl, 99-104 C/0.5 mm Hg; and tridecyl, 94-96 C/0.5 mm Hg. 2-Dodecyloxyethylchloride, bp 110-114 C/0.5 mm Hg, was also prepared from 2-dodecyloxyethanol and thionyl chloride. The synthesis of 2-dodecyloxyethanol is described later. Mono- and diethylene glycols are purified by fractional distillation, and the fractions having satisfactory refractive indices are used for experiments.

Preparative Procedure

2-Alkylaminoethanol(I): Using a modified Pierce method (3), the preparation of 2-dodecylaminoethanol(Ia) is described here as an example. In an autoclave fitted with a stirrer, 0.1 mole dodecylchloride, 0.5 moles ethanolamine and 35 ml dioxane were placed; then the mixture was heated at 150 C for 16 hr. The upper layer of product was extracted with ether, and the extract was washed with water five times. After the evaporation of ether from the extract, the residue was distilled in vacuo, giving 2-dodecylaminoethanol. 2-Undecyl-, 2-tridecyl and 2-(2-dodecyloxyethyl)aminoethanols were also prepared by the same method. Table I shows the analytical results of substituted aminoethanols.

0-(2-Alkylaminoethyl)-3-oxypropionic acid hydrochloride (III): As an example, preparation of 0-(2-dodecylaminoethyl)-3-oxypropionitrile(IIa) is described here. It is prepared by the conventional cyanoethylation of dodecyl-

TABLE III

Characteristics of 2-Alkyloxyethylamine and N-(2-Alkyloxyethyl) ethylenediamine, $ROCH_2CH_2(NHCH_2CH_2)_nNH_2$

R	*n*	Boiling point, C/mm Hg	n_D^t	Per cent (calculated) C	H	N
$C_{11}H_{23}$	0	102-105/1.0	1.4397^{30}	72.80 (72.50)	13.98 (13.57)	6.17 (6.50)
	1	147-148/1.0	1.4555^{20}	69.68 (69.71)	13.20 (13.26)	10.57 (10.84)
$C_{12}H_{25}$	0	125-127/1.0	1.4412^{30}	73.82 (73.30)	14.41 (13.62)	5.95 (6.11)
	1	156-160/0.8	1.4560^{20}	70.80 (70.53)	13.31 (13.32)	10.06 (10.28)
$C_{13}H_{27}$	0	123-130/1.0	1.4421^{30}	74.33 (74.01)	13.58 (13.66)	5.69 (5.76)
	1	155-158/0.5	1.4569^{20}	71.50 (71.27)	13.55 (13.37)	10.02 (9.78)

TABLE IV

Characteristics of Substituted Oxy- and Aminopropionic Acids[a]

R	n	Melting point, C	Per cent (calculated) C	H	Cl	N
A $C_{11}H_{23}$	0	92.1-93.0	59.30 (59.33)	10.84 (10.58)	11.08 (10.95)	4.60 (4.32)
$C_{12}H_{25}$	0	95.0-97.5	61.14 (60.42)	11.02 (10.74)	10.01 (10.49)	4.30 (4.15)
	1	54.0-57.0	59.48 (59.72)	10.92 (10.56)	9.55 (9.29)	4.18 (3.67)
	2	Greasy	59.64 (59.34)	9.98 (10.20)	7.76 (8.34)	3.36 (3.30)
$C_{13}H_{27}$	0	84.5-86.8	61.78 (61.43)	10.86 (10.88)	9.80 (10.07)	4.17 (3.98)
B $C_{11}H_{23}$	---	72.5-74.1	59.93 (59.33)	10.78 (10.58)	10.36 (10.95)	4.53 (4.32)
$C_{12}H_{25}$	---	78.0-80.0	60.67 (60.42)	19.95 (10.74)	10.33 (10.49)	4.34 (4.15)
$C_{13}H_{27}$	---	78.0-78.5	61.59 (61.43)	11.20 (10.88)	9.74 (10.07)	4.15 (3.98)
C $C_{11}H_{23}$	---	156-158	53.72 (53.59)	9.78 (9.99)	17.74 (17.58)	6.84 (6.94)
$C_{12}H_{25}$	---	161-162	55.21 (54.67)	9.96 (10.14)	17.32 (16.98)	7.15 (6.71)
$C_{13}H_{27}$	---	160-162	56.10 (55.67)	10.38 (10.28)	16.10 (16.44)	6.68 (6.49)

[a]A: $R(OCH_2CH_2)_nNHCH_2CH_2OCH_2CH_2COOH \cdot HCl$, B: $ROCH_2CH_2NHCH_2CH_2COOH \cdot HCl$, C: $ROCH_2CH_2NHCH_2CH_2NHCH_2CH_2COOH \cdot 2HCl$.

aminoethanol (Ia) followed by hydrolysis with hydrochloric acid to obtain the substituted propionic acid(IIIa). To the mixture of 0.05 moles of 2-dodecylaminoethanol and 0.5 wt% potassium hydroxide, 0.1 mole acrylonitrile was slowly added at 40 C. After the addition the mixture was stirred at 45-50 C for 4 hr; then the product was hydrolyzed with excess concentrated hydrochloric acid. To remove inorganic salts, the product is recrystallized repeatedly in acetone, and the melting point of the white plate crystals thus purified is 95.0-97.5 C. Other 0-(2-alkylaminoethyl)-3-oxypropionic acids were obtained in the same manner. Analytical data are presented in Table IV.

2-Alkyloxyethanol(IV): The Williamson synthesis was used to prepare these alcohols, using the method of Cooper and Partridge (4). The preparation of 2-dodecyloxyethanol(IVa) is described here as an example. The reaction of 1.5 moles of monosodium glycolate and 1 mole of dodecylchloride, at 170-180 C in an atmosphere of nitrogen for about 6 hr, gave IVa, which was purified by distillation in vacuo. 2-Undecyloxy- and 2-tridecyloxyethanols were also synthesized by the same method. 2-(2-Dodecyloxy)-ethyloxyethanol was prepared from dodecylchloride and monosodium diethylene glycolate in the same way. Analytical data of four alkyloxyethanols are shown in Table II.

2-Alkyloxyethylamine(VI): 2-Dodecyloxyethylamine(VIa) was prepared from 2-dodecyloxyethylchloride (Va) which was derived from IVa by the conventional thionyl chloride method, boiling with excess ammonia at 170 C for 20 hr according to Westphal et al. (5). The amine separated was washed with saturated NaCl aq and distilled in vacuo. Undecyloxy- and tridecyloxyethylamines were similarly synthesized. Analytical data are summerized in Table III.

N-(2-Alkyloxyethyl)-3-aminopropionic acid hydrochloride(VIII): N-(2-Dodecyloxyethyl)-3-aminopropionic acid(VIIIa) was prepared by the cyanoethylation of VIa, followed by hydrolysis of the nitrile(VIIa). The procedure of cyanoethylation and hydrolysis were the same as in the preparation of IIIa. N-(2-Undecyloxyethyl)- and N-(2-tridecyloxyethyl)-3-aminopropionic acids were also prepared (Table IV).

N-(2-(2-Dodecyloxyethyl)aminoethyl)-3-aminopropionic acid(Xa): This was prepared from 2-(2-dodecyloxyethyl)-aminoethylamine(IXa), which was synthesized from Va and ethylene diamine by the method of Linsker and Bruno (6); the cyanoethylation was followed by acid hydrolysis.

Antibacterial Study

Antibacterial evaluations against *Straphylococcus aureus* 209 P and *Escherichia coli* were carried out by a tube dilution method, as described in the previous report, and summerized in Table V.

The Study of Surface Activities

Amphoteric surfactants prepared in this study are all water soluble. The surface tensions of aqueous or Clark-Lub's buffer solutions of amphoterics were measured using a Du-Nouy tensiometer at 20 C, 65% humidity. Figure 2 shows some typical curves of surface tension plotted against concentration.

RESULTS AND DISCUSSION

Melting Point

The melting point of 0-(2-alkylaminoethyl)-3-oxypropionic acids of N-(2-alkyloxyethyl)-3-aminopropionic acids decreases in the order C_{12}, C_{13} and C_{11} in each homolog (Table IV). This trend is the same with other higher alkyl compounds. Table IV also indicates that the melting point of N-(2-alkyloxyethyl)-3-aminopropionic acids is lower than that of the corresponding 0-(2-alkylaminoethyl)-3-oxypropionic acids.

Antibacterial Properties

Generally, amphoteric surfactants tested are more effective against *Staphylococcus aureus* 209 P than against

TABLE V

The Minimum Inhibition Concentration
(γ/ml) of Substituted Oxy- and Aminopropionic
Acids against Staphylococcus aureus 209 P and *Escherichia coli*[a]

R	n	A S. 209P	A E. coli	B S. 209P	B E. coli	C S. 209P	C E. coli
$C_{11}H_{23}$	0	16	31	62	125	31	16
$C_{12}H_{25}$	0	16	62	8	125	8	31
	1	8	31				
	2	8	31				
$C_{13}H_{27}$	0	8	16	8	1000	8	31

[a]A: $R(OCH_2CH_2)_nNHCH_2CH_2OCH_2CH_2COOH \cdot HCl$, B: $ROCH_2CH_2NHCH_2CH_2COOH \cdot HCl$, C: $ROCH_2CH_2NHCH_2CH_2NHCH_2CH_2COOH \cdot 2HCl$.

Escherichia coli. The tridecyloxyacid radical shows the least inhibition against the growth of *E. coli*, but antibacterial effect in terms of carbon chain length of alkyloxy radicals is fairly small. As for structural effect, 0-(2-alkylaminoethyl)-3-oxypropionic acid is more effective than N-(2-alkyloxyethyl)-3-aminopropionic acid, but the difference is very small. Increase in the number of aminoethyl groups does not have a large effect on antibacterial properties, while the addition of one oxyethyl group reinforces the antibacterial effect.

Surface Activities

As shown in Figure 2, some breaking points are located around 10^{-4} mole/liter, deviating to the lower concentration as the number of oxyethylene groups increases, especially in neutral and acidic media which has been previously reported in the case of octylamine salts of β-dodecyloxy- and tetradecyloxypropionic acids (1). The location of ether and amino groups in the substituted propionic acid does not have any influence on the surface activities, but the diaminopropionic acid derivatives are less surface active than the corresponding monoaminopropionic acid derivatives.

As for the effect of carbon chain length of the alkyloxy radical, dodecyloxypropionic acid is more active than undecyloxy- and tridecyloxypropionic acids. There is no direct correlation between surface and biological activities of the amphoteric surfactants reported in this paper, as was the case in our previous paper (1) on soluble amine salts of β-alkyloxypropionic acids.

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