ppm : 2.32 t (6H, CH_3), 3.76 q (4H, CH_2CH_3), 4.96 s (2H, CH_2CO), 7.42-8.13 m (8H, 4-C_6H_5, and 6-C_4H_3S).

EXPERIMENTAL (BIOLOGICAL PART)

Investigation into the tuberculostatic activity of the synthesized compounds was carried out with the method of two-stage serial dilution in a liquid synthetic Soton medium with 10% normal serum. As test strains we used Mycobacterium tuberculosis: strain H37Rv, which is sensitive to antituberculous preparations, and strain 3714, which is highly resistant to isoniazid and streptomycin. The density of the mycobacterial suspension was 50 million microbic cells in 1 ml.

The results of the investigation into the tuberculostatic activity of the tested compounds are summarized in Table 2.

Of all the compound studied the best tuberculostatic activity as regards the strains H37Rv and 3714 is shown by IIIe and IVc, i.e., compounds having a theonyl radical in their structure.

Thus, as a result of the conducted investigations it was established that IIIa-h and IVa, c have a tuberculostatic activity that is comparable with that of the reserve antituberculous preparations.

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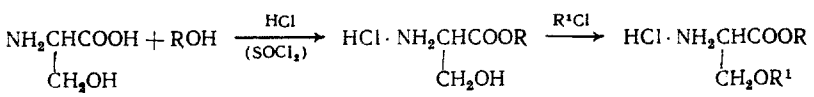
SYNTHESIS AND ANTIMICROBIAL PROPETIES OF ALKYL ESTERS OF O-ACYL SERINE

E. A. Smirnova, Yu. A. Davidovich, M. A. Chernyavskaya, A. S. Belova, and S. V. Rogozhin

UDC (547.466+547.29):542.91:615.281

Cationic surfactants (SA) are used in medicine, pharmacy, and cosmetics as disinfectants and antiseptics [11] and as preservatives in medicinal preparations, ointments, and creams [2, 3]. However, the toxic and skin-irritant effects of some cationic surfactants prevent their widespread use. Consequently, there is considerable interest in amino acid surfactants Hydrochloride salts of higher amino acid alkyl esters have been shown to be effective bacteri ocides with low toxicity in homoiothermal animals [4]. It has also been noted in the literture that alkyl ester salts of higher N-acyl derivatives of diaminomonocarboxylic acids exhibit a high degree of bacteriocidal activity without marked toxic side effects [12, 13]. Base on these findings, and with the purpose of expanding the variety of antibacterial agents with low toxicity, we synthesized a number of cationic surfactants from serine (I-XI) by acylating lower serine alkyl esters with chloroanhydrides of the following higher carboxylic acids: caprylic, caproic, lauric, myristic, palmitic, and stearic. We then investigated the antimicrobial activity and surface active properties of the synthesized compounds.



A. N. Nesmeyanov Institute of Heteroorganic Compounds, Academy of Sciences of the USSR.

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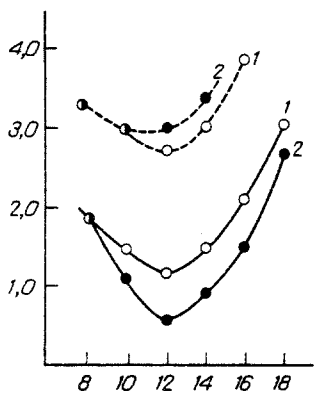

Fig. 1

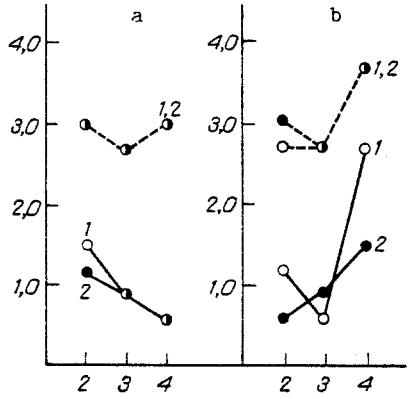

Fig. 2

Fig. 1. Relationship between the antimicrobial activity of the synthesized compounds and the length of the acyl radical R^I where R = C_2H_5. X-axis – number of carbon atoms in acyl group; Y-axis – logarithm of concentration (in μg/ml). Here and in Fig. 2 the broken line represents minimum bacteriocidal concentration; the solid line represents minimum bacteriostatic concentration. 1) E. coli; 2) St. aureus.

Fig. 2. Relationship between the antimicrobial activity of the synthesized compounds and the length of the alkyl radical R, where R^I = $C_9H_{19}CO$ (a) and R^I = $C_{11}H_{23}CO$ (b). X-axis – number of carbon atoms in the alkyl radical; Y-axis – Logarithm of concentration (in μg/ml).

R is the alkyl radical (C_2-C_4); R^1 is the acyl radical with an even number of carbon atoms (C_8-C_{18}).

Serine was esterified in the presence of HCl or thionyl chloride [8] by boiling the reaction mixture for 1.5-2 h. O-Acylation was accomplished by treating the resultant hydrochlorides of serine alkyl esters with the chloroanhydride of the corresponding fatty acid in an inert solvent (CH_2Cl_2, $CHCl_3$, CCl_4). The reaction proceeded in the absence of a base so that only the hydroxyl group would be acylated without a preliminary blocking of NH_2-function as suggested by other investigators [9]. The reaction proceeded for two to four days at room temperature. The actual length of time required depended on the length of the acyl radical and was directly proportional to the increase in the hydrocarbon chain. The progress of the reaction could be visually inspected by the amount of the initial hydrochloride of the serine alkyl ester that dissolved. The characteristics of the compounds obtained are given in Table

The bacteriological and bacteriocidal activity of the synthesized compounds was evaluated by the suspension method in which the test microorganisms used were St. aureus (strain 906) and E. coli (strain 1257). The test results are given in Table 2. All of the tested compounds inhibited bacterial culture growth in the concentration range of 4-125μg/ml and exhibited pronounced bacteriocidal activity. Moreover, the activity of the L-isomer of the O-caprynoyl derivative of serine (VII) was two times more effective against St. aureus than the corresponding racemic compound (II) and four times more effective against E. coli. We found that the antimicrobial activity of the compounds under examination was related to the length of the acyl radical R^1, where R = C_2H_5 (Fig. 1). It should be noted that although this relationship was essentially the same for both microorganisms, the antimicrobial activity against St. aureus was manifested at lower concentrations than it was against E. coli. Thus, the antimicrobial activity of the examined series of compounds is directly proportional to the increase in the size of the acyl radical which determines the surfactant's degree of

TABLE 1. Properties of Alkyl Ester O-Acyl Hydrochloride Derivatives of D,L-Serine

Compound	R	R¹	Yield, %	mp, °C	R_f (A)	R_f (B)	Water solubility, %	Found, %				Empirical formula	Calculated, %			
								C	H	N	Cl		C	H	N	Cl
I	C_2H_5	$C_7H_{15}CO$	91	67—59	0,60	0,58	>40	52,67	8,90	4,70	12,02	$C_{13}H_{26}ClNO_4$	52,78	8,86	4,74	11,99
II	C_2H_5	$C_9H_{19}CO$	72	75—76	0,70	0,80	>35	55,60	9,47	4,60	11,14	$C_{15}H_{30}ClNO_4$	55,63	9,47	4,33	10,95
III	C_2H_5	$C_{11}H_{23}CO$	93	85—86	0,70	0,76	~16	57,87	9,70	4,22	10,16	$C_{17}H_{34}ClNO_4$	58,01	9,73	3,98	10,08
IV	C_2H_5	$C_{13}H_{27}CO$	97	90—92	0,66	0,67	~10	60,29	9,95	3,60	9,56	$C_{19}H_{38}ClNO_4$	60,06	10,08	3,60	9,33
V	C_2H_5	$C_{15}H_{31}CO$	95	94—95	0,71	0,70	~1	61,70	10,28	3,60	9,00	$C_{21}H_{42}ClNO_4$	61,82	10,33	3,43	8,69
VI	C_2H_5	$C_{17}H_{35}CO$	68	94—95	0,66	0,66	~0,5	63,15	10,35	3,02	8,13	$C_{23}H_{46}ClNO_4$	63,45	10,33	3,21	8,13
VII*	C_2H_5	$C_9H_{19}CO$	96	75—76	0,70	0,80	>35	56,02	9,42	4,28	10,90	$C_{15}H_{30}ClNO_4$	55,63	9,37	4,33	10,95
VIII	$n\text{-}C_3H_7$	$C_9H_{19}CO$	90	83—84	0,48	0,71	~10	58,01	9,73	3,98	10,08	$C_{16}H_{32}ClNO_4$	58,08	9,92	3,83	9,69
IX	$n\text{-}C_4H_9$	$C_9H_{19}CO$	90	100—101	0,57	0,68	~10	58,05	9,73	4,19	10,08	$C_{17}H_{34}ClNO_4$	58,01	9,73	3,98	10,08
X	$n\text{-}C_3H_7$	$C_{11}H_{23}CO$	95	96—98	0,40	0,57	—	58,92	9,88	3,77	9,99	$C_{18}H_{36}ClNO_4$	59,08	9,92	3,80	9,69
XI	$n\text{-}C_4H_9$	$C_{11}H_{23}CO$	93	102—103	0,57	0,68	~5	60,20	9,96	3,84	9,33	$C_{19}H_{38}ClNO_4$	60,05	10,01	3,68	9,50

*Given for the L-form of the corresponding serine derivative

TABLE 2. Antimicrobial Activity of Synthesized Compounds (I-XI)

Microorganisms	I		II		III		IV		V		VI*		VII		VIII		IX		X		XI	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
E. coli, strain 1257	62	2000	31	1000	16	500	31	1000	125	3000	1000	—	8	500	8	500	4	1000	4	500	500	5000
St. aureus, strain 906	62	2000	16	1000	4	1000	8	2000	31	—	500	—	8	500	8	500	4	1000	8	500	31	5000

*Preparation's solubility limited to 0.08%

Note: 1) minimum bacteriostatic concentration, μg/ml; 2) minimum bacteriocidal concentration, μg/ml

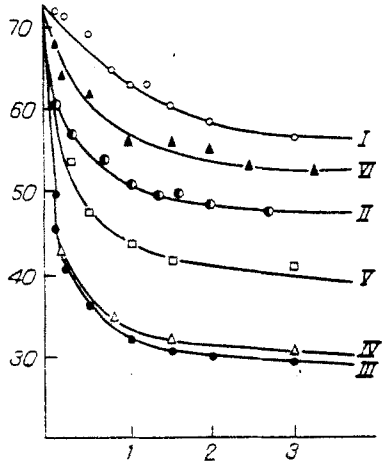

Fig. 3

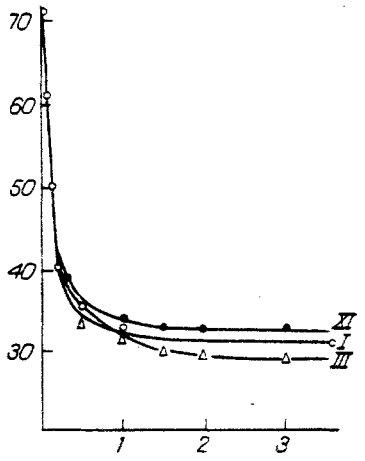

Fig. 4

Fig. 3. Surface tension isotherm of various O-acyl derivatives of serine ethyl ester. Here and in Fig. 4: X-axis – is the substance concentration (in mole /liter × 10^{-4}); Y-axis is the surface tension (in dynes/cm). The curves are marked by the compound numbers.

Fig. 4. Surface tension isotherm of serine O-lauroyl esters.

hydrophoby. The compound reaches its maximum antimicrobial activity when the radical is C_{12}. Subsequent growth in the hydrocarbon chain results in lowered activity. Apparently, compound III has an optimal ratio of hydrophobic to hydrophilic factors which accounts for its maximum activity. The results we obtained agree with the literature data on the antibacterial activity of serveral other amino acid surfactants. The compound with a C_{12} radical in the series of ethyl ester hydrochlorides of N-acyl arginine was shown to have the greatest antibacterial activity [13]. The decyl and dodecyl esters of the higher alkyl esters of amino acids were also shown to have the greatest activity [4, 10].

Figure 2 illustrates the relationship between the antimicrobial activity of O-caprinoyl (see Fig. 2, a) and O-lauroyl (see Fig. 2, b) of serine derivatives and the alkyl radical length in the series C_2-C_4. In compounds II, VIII, and IX this relationship is expressed as an increase in bacteriostatic activity as the number of carbon atoms increases. In compounds II, X, and XI, that relationship is more complex.

It is generally recognized that the biological action of organic compounds is related to their physicochemical properties [6]. In that connection, we investigated the surface activity of the synthesized compounds as measured by their ability to reduce the surface tension of aqueous solutions on the water-air surface. As is demonstrated in Fig. 3, surface activity is significantly dependent upon the length of the acyl radical R^I. The C_8 compound hardly reduces the surface tension of aqueous solutions. The compound with 12 carbon atoms in the hydrophobic radical was observed to have the greatest effect. This corresponds to the data on the bacteriostatic activity of this series of compounds. At the same time, an increase in the length of the alkyl group within the C_2-C_4 range in the same acyl radical practically has no effect on surface activity (Fig. 4), although antimicrobial activity in this case is significantly altered.

EXPERIMENTAL CHEMICAL PART

D,L-Serine manufactured by the Czechoslovak Wojkow Plant and L-serine produced by the Reanal company were used in our study. The higher fatty acid chloroanhydrides were obtained by the method in [1]. The mp of the synthesized compounds was determined in sealed capillary tubes. TLC was performed on Silufol (Czechoslovakia) plates in a 4:1:1 n-BuOH – AcOH – water

system (A) and a 4:1:1 iso-C_3H_7OH—pyridine—water system (B). Infrared spectra were read on a UR-20 (GDR) instrument (samples of the tested compounds were first pelletized with KBr).

D,L-Serine Butyl Ester Hydrochloride. HCl vapor was passed into a suspension of 4 g (0.033 mole) of D,L-serine in 140 ml of butanol up to the saturation point. The mixture was then stored at room temperature for 20 h and boiled for for four h. The product was separated by the addition of 150 ml of diethyl ether. The entire operation was repeated to assure complete esterification. Yield 6.9 g (92%), mp 85-87°C. Found, %: C 42.4; H 7.94; N 7.22; Cl 10.08. $C_7H_{16}ClNO_3$. Calculated, %: C 42.54; H 8.16; N 7.09; Cl 10.08. Rf 0.49 (A), 0.5[illegible] (B). Infrared spectrum, ν_{max}, cm^{-1}: 1750-1735 (C=O), 3030-2500 (NH_3^+).

D,L-Serine O-Myristoyl Ethyl Ester Hydrochloride (IV). A 7.86 g portion (0.036 mole) of myristic acid chloroanhydride was added to a 6 g (0.035 mole) suspension of D,L-serine ethyl ester hydrochloride. The reaction mixture was agitated for 5 h at 40°C. The solvent was vacuum distilled, 250 ml of absolute ether were added to the residue, the precipitate was filtered, then washed with ether and recrystallized from an acetone-chloroform mixture. Yield 13.1 g (97%) of ester IV, mp 90-92°C. Found, %: C 60.29; H 9.95; N 3.60; Cl 9.56. $C_{19}H_{38}ClNO_4$. Calculated, %: C 60.06; H 10.08; N 3.60; Cl 9.33. R_f 0.66 (A), 0.67 (B). Infrared spectrum, ν_{max}, cm^{-1}: 1750-1735 (C=O), 3030-2500 (NH_3^+).

EXPERIMENTAL BIOLOGICAL PART

The bacteriostatic and bacteriocidal activity of the compounds under examination was tested by the suspension method which employed a microbial load of 1×10^8 microbial cells (MC) per ml. A sterile 0.005 M Tris-SO_4 buffer at pH 7.5 was used to prepare the initial microbial suspension (2×10^8 MC) and to dilute the preparations. The test cultures were incubated on casein agar at 37°C for 18 h. After exposing the bacterial cells to the compounds for 30 min at 37°C, 0.1 ml of the mixture was transferred to 5 ml of a sterile casein broth. The tests were repeated 24-32 times. Test microorganism growth was determined by changes in the optical density of the broth samples on a photoelectric colorimeter as compared to a control during the first five to six h, and then after 24 h, 48 h, and seven days [7].

The surface tension of the aqueous solutions of the synthesized substances was measured by the ring-pulling method on a torsion balance [5].

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