SYNTHESIS, ANTIMICROBIAL, AND SURFACTANT ACTIVITY OF OCTAMERIC CYCLIC ESTERS OF ALKYLPHOSPHONIC ACIDS

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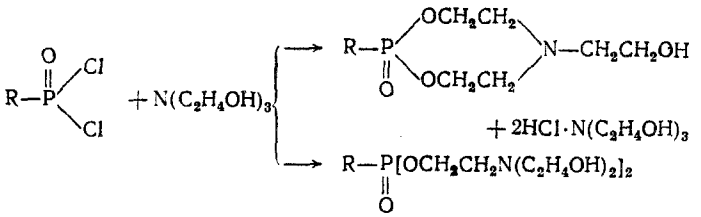
UDC 615.281:547.241].
012.1

It is generally known that many cationic and anionic surfactants (SA) exhibit antimicrobial activity [4, 8, 9]. The phosphororganic surfactants containing biologically active fragments are also of interest as antimicrobial compounds [5, 10].

We investigated the interaction between triethanolamine and alkylphosphonic acid (APA) dichloroanhydrides and found that the resultant octameric cyclic esters of APA exhibit antimicrobial activity [1].

In consideration of triethanolamine's structure one might presume that its reaction with APA dichloroanhydrides can proceed along two different directions:



In the given reaction triethanolamine is the reagent and HCl acceptor and is therefore taken in excess. In studying this reaction we found [1] that the interaction between APA dichloroanhydrides and triethanolamine resulted in the formation of a cyclic product only, regardless of the molar correlation of the starting reagents. This is apprently related to the fact that the nucleophilic reagent's attack on the tetrahedral phosphorus atom is more dependent upon spatial factors than an attack by planar carbonyl compounds. We know that the reaction rate depends not only on the magnitude of the pK_a of the acid corresponding to the substituted group, but also upon its volume [6]. The rates at which two chlorine atoms in APA dichloroanhydrides are replaced are different so that as soon as one chlorine atom has reacted the approach of the other molecule of the nucleophile will be sterically hindered. The replacement of one chlorine atom in the dichloroanhydride molecule by an alkoxy group results in a greater electron density on the phosphorus atom which lowers the molecule's reactivity. Thus, the steric and electron factors enhance the formation of a cyclic product regardless of the molar ratio of the starting reagents.

The cyclic structure of the products was proven by IR and mass spectra as well as by element analysis. A few of the physical constants and yields of the synthesized octameric cyclic esters of APA are presented in Table 1.

The IR spectra (ν_{max}, cm^{-1}) exhibited bands corresponding to the vibrations of the newly formed bonds: 1030-1050 (p-O-alkyl), 3320 (OH) and the retained bonds; 1230 (P=O), 940, 780 (P-C).

The mass spectrum of 2-decyl-2-oxo-1,3-dioxa-6-(β-oxyethyl)aza-2-phosphacyclooctane, i.e., the octameric ester of decylphosphonic acid, contained a molecular ion with m/z 335 and ions with m/z 317, 304, 276, 248, 222, 194, 149, 135, 113, 69, and 56. The most characteristic of those was the ion with m/z 194 which was apparently formed with a rupture of the C-P bond.

Tashkent Pharmaceutical Institute, Institute of Chemistry, Academy of Sciences of the Uzbek SSR. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 21, No. 11, pp. 1332-1335, November, 1987. Original article submitted July 29, 1986.

0091-150X/87/2111-0785$12.50

On the basis of the data presented (IR and mass spectra) as well as element analysis, the second reaction path for the interaction between triethanolamine and APA dichloroanhydrides is completely excluded and we have confirmation of the formation of the octameric cyclic esters.

The most favorable temperature range for the reaction to proceed is 0-20°C. At higher temperatures both the starting reagents and the newly obtained product undergo resinification and partial polymerization.

The octameric cyclic esters of APA constitute light brown oily liquids that are soluble in water, the lower alcohols, halogen-containing hydrocarbons, but are insolube in ether. When their aqueous solutions are shaken they foam with a resultant pronounced reduction in water surface tension (see Table 1). As can be seen, all of the APA derivatives are surfactants. The obtained isotherms of surface tension are well described by the Shishkovskii equation $\Delta\sigma = a\ln(1 + KC)$ [3], where $\Delta\sigma = \sigma_0 - \sigma$ is the difference between the surface tensions of the solvent and solution; a is a constant, $a = \Gamma_\infty RT$; and 1 is a constant number.

For most of the systhesized surfactants the computed correlation coefficients (R), adsorption constant (K), maximum monolayer capacity (Γ_∞), and surface activity ($G = \Gamma_\infty \cdot K \cdot RT$) were the following: R = 0.98-0.99, ln K = 14.4-22.0 liter/min, $\Gamma_\infty \cdot 10^6 = 1.5$-$0.9$ mole/m^2 and G = 1.2-17 H·m^2/mole.

The antimicrobial tests performed for the synthesized surfactants showed that the octameric ester of decylphosphonic acid (TEM-10) at a concentration of 5 mg/ml completely suppressed the growth of *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Shigella flexneri*, and inhibited the gowth of *Shigella Grigor'ev — Shiga*, *Shigella sonnei*.

One might assume on the basis of the literature data [7, 9] that the antimicrobial action of compound TEM-10 is due to its ability to disturb cytoplasm membrane permeability and cellular osmotic equilibrium with the denaturation of proteins and the disturbance of enzymatic equilibrium. Apparently, the sorption of surfactant molecules or ions on the negatively charged cell surface and transfer across the membrane play a definitive role in the antibacterial activity of surfactants.

EXPERIMENTAL (CHEMICAL)

IR spectra were recorded on a UR-20 spectrophotometer (GDR) with LiF and NaCl prisms in petroleum jelly. Mass spectra were recorded on a MX-1303 instrument equipped with a system for directly placing specimens in an ion source at 388 K and an ionizing electron energy of 40 eV.

Surfactant samples were purified on an Al_2O_3 column (second degree Brockmann activity; 0.3-0.5 mesh), compound purity was controlled by TLC on a floating Al_2O_3 layer in systems 1 (70:5:20:5 benzene-acetone-methanol-AcOH) and 2 (8.5:1.5 toluene—ethanol). Iodine vapor was used as the developer.

The surface tension of the aqueous surfactants was determined by the maximum bubble pressure method at a temperature of 293 K.

The starting APA dichloroanhydrides were obtained by the oxidative chlorophosphonylation of the corresponding hydrocarbons in the presence of PCl_3 and oxygen [11].

General Method for Synthesizing Octameric Cyclic Esters of APA. A 0.1 mole portion of APA dichloroanhydride in 50 ml of $CHCl_3$ was added dropwise at a temperature of 20°C over a period of 1.5 to 2 h to a solution of 44.75 g (0.3 mole) of freshly distilled triethanolamine in 50 ml of absolute $CHCl_3$. The reaction mixture was left overnight. Then the triethanolamine HCl was filtered off (37.1 g, mp 176-177°C) and the precipitate was washed with $CHCl_3$, and the final product was separated from the filtrate after the solvent was removed by vacuum.

EXPERIMENTAL (BIOLOGICAL)

Compound solutions were prepared on a beef extract broth at a concentration of 0.2, 1.0, and 5 mg/ml. Sterilization was accomplished by autoclaving. All the strains (18) of the microorganisms were cultivated on stands with nutrient agar. Daily cultures were washed off and the suspensions were standardized with respect to turbidity [12]. The microbial suspensions containing 1000 microbe bodies were diluted to 1 ml, and 0.1 ml of this suspension

TABLE 1. Yield, Element Analysis Data, and Assay of Surface Tension (σ) for Aqueous Solutions of 2-Alkyl-2-oxo-1,3-dioxa-6-(β-oxyethyl)aza-2- phosphacylcylooctanes

Alkyl	Yield, %	Found, %			Empirical formula	Calculated, %			$\sigma \cdot 10^3$, J/m^2				
									concentration, %				
		C	H	P		C	H	P	1	0,5	0,25	0,125	0,0625
C_6H_{13}	90,3	51,51	9,22	11,01	$C_{12}H_{26}NPO_4$	51,61	9,31	11,11	31,9	35,0	37,2	40,9	41,7
C_7H_{15}	91,0	53,02	10,00	10,38	$C_{13}H_{28}NPO_4$	53,24	9,89	10,58	29,4	32,0	38,6	39,5	45,4
C_8H_{17}	90,0	54,64	10,07	10,24	$C_{14}H_{30}NPO_4$	54,72	9,77	10,09	25,1	25,2	26,3	27,8	29,1
C_9H_{19}	89,0	56,20	9,43	9,52	$C_{15}H_{32}NPO_4$	56,07	9,65	9,65	25,6	25,8	26,5	27,3	29,8
$C_{10}H_{21}$	93,0	57,01	10,30	9,02	$C_{16}H_{34}NPO_4$	57,31	10,14	9,25	25,9	27,1	28,2	30,9	32,2
$C_{11}H_{23}$	92,0	58,35	10,13	8,72	$C_{17}H_{36}NPO_4$	58,45	10,32	8,91	26,3	27,6	29,1	30,0	39,0
$C_{12}H_{25}$	91,0	59,20	10,40	8,38	$C_{18}H_{38}NPO_4$	59,17	10,48	8,58	27,8	30,5	33,3	34,7	37,5
$C_{13}H_{27}$	94,5	59,39	11,90	8,43	$C_{19}H_{40}NPO_4$	59,50	11,90	8,52	30,2	32,0	34,2	36,1	38,5
$C_{14}H_{29}$	88,0	64,01	10,74	7,84	$C_{20}H_{42}NPO_4$	63,93	10,74	7,92	33,7	36,1	37,3	38,5	40,9

was inoculated into a test tube with 3 ml of broth which contained the preparation at the aforementioned concentrations.

The control was a similar broth culture without the test preparation. The control and daily test samples were kept in an incubator at 37°C for 24 h. Then a visual inspection of broth turbidity was made and 0.1 ml of the broth was evenly distributed over the surface of a neutral nutrient agar in Petri dishes. The number of colonies was counted after 24 h of incubation at 37°C.

The toxicity of compound TEM-10 was determined on laboratory animals (white mice) in the form of a 10% aq. solution. The results were computed by the Kerber method as modified by Ashmarin [2].

We found that the preparation belongs to the moderately toxic chemical compounds. The LD_{50} for white mice was 650 mg/kg. A triple immersion of the animal's tail in a 10% solution of the preparation for 4 h did not result in any marked signs of intoxication.

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