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Antimicrobial agents based on new lipophilic alkyl (*N*-alkyl-*N*,*N*-dioctylammoniomethyl)phosphonates

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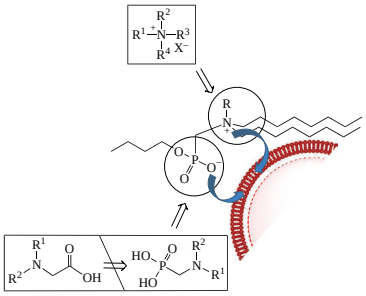
(*N*-Alkyl-*N*,*N*-dioctylammoniomethyl)phosphonates were obtained by alkylation of amino phosphonate potassium salt with alkyl iodides or benzyl chloride. All synthesized compounds demonstrated high antibacterial and antifungal activity against Gram-positive bacterial strains *B. cereus* and *S. aureus* and were not active against Gram-negative strains *Ps. aeruginosa*. Some regularity connecting the structure of the obtained compounds with their antimicrobial action has been established.



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Zwitterionic compounds (betaines) contain both positively- and negatively-charged functional groups in the same molecule and represent an important class of compounds with useful properties that find application in industrial chemistry and the life sciences.[1] The first synthetic zwitterionic copolymer material was 'closely analogous to proteins'.[2] The zwitterionic molecules can be roughly divided into few types: sulfo betaines (trialkylammonioalkanesulfonates whose headgroups are the quaternary ammonium and sulfonate groups), carboxy betaines (trialkylammonioalkanoates with carboxylate and ammonium groups), zwitterionic compounds containing imidazolium and phosphonium cations (such as *N*-alkyl-*N*-propanesulfonate imidazolium inner salts, carboxylate phospha betaines), phospha betaines (trialkylammonioalkylphosphates/phosphonates), and amino acid derivatives.

Sulfo and carboxy betaines are commonly studied and used in industry as surfactants due to their unique properties such as low toxicity, good biodegradability and higher environmental compatibility.[3–7] Zwitterionic imidazolium amphiphiles have recently received attention as ionic liquids because of their special properties caused by the imidazole ring and the absence of migration of salt components along the potential gradient.[8–10] Carboxylate phospho betaines are the phosphorus analogues of organic amino acids, with a wide spectrum of potential chemical and biological properties.[11–13]

In recent decades, interest to phosphorus-containing polyzwitterions (polyphospha betaines) has increased since they are recognized as analogues of important biological structures, such as phospholipids which are the major constituents of cell membranes.[14–19] Numerous polyzwitterions have been shown to be highly biocompatible, combining strong hydrophilicity with zero net charge and as they confer excellent resistance to biofouling to a variety of materials.[20] In this regard, alkyl (*N*-alkyl-*N*,*N*-dioctylammoniomethyl)phosphonates, on the one hand, are derivatives of α-amino phosphonic acids (phosphorus analogues of amino carboxylic acids),[21] on the other hand, phosphorylated analogues of quaternary ammonium salts (QASs) whose molecules contain a negatively charged phosphonate group and a positively charged quaternary nitrogen atom (Figure 1).

Thus, phosphorylated betaines (amino phospha betaines) are engrossing and unique compounds. Amino phospha betaines contain a phosphoryl group, so they can act as complexing agents of various substrates. Previously, we studied the complexing ability of phosphorylated betaines by Fourier Transform InfraRed (FTIR) spectroscopy and UV–(VIS) spectroscopy[22–24] and showed their selectivity to the membrane transport of rare-earth metal ions through impregnated liquid membranes,[25] and studied the structure of their complexes by X-ray diffraction analysis.[23,26]

Shelton studying the biological activity of a series of simple aliphatic quaternary ammonium salts $Me_3N^+R\ Hal^-$, where R was an alkyl from C_6 to C_{18} found that bactericidal activity

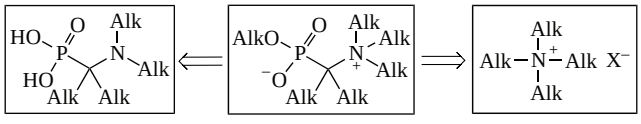


Figure 1 General structure of phosphorylated betaines of alkyl (*N*-alkyl-*N*,*N*-dialkylammoniomethyl)phosphonate chemotype (centered).

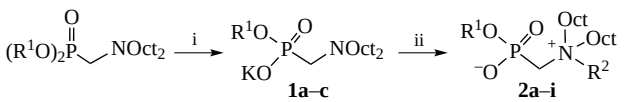
† Deceased.

increased significantly as the alkyl group was lengthened reaching a maximum with cetyltrimethylammonium bromide.[27] Moreover, in combination with pyridine, the cetyl substituent had high bactericidal properties.[28] Currently, QASs containing two methyl substituents such as *N*-benzyl-*N*,*N*-dimethyl-*N*-(3-myristoylaminopropyl)ammonium and benzalkonium chlorides are used in antiseptic preparations. In this regard, we have shown that phosphorylated betaines, as well as QASs containing one higher alkyl substituent at nitrogen atom exhibit antimicrobial activity against pathogenic human and animal bacteria and fungi *Candida albicans*.[29–32]

In this work, the properties of zwitterionic phosphorylated betaines containing two long-chain substituents at the nitrogen atom were explored. We have performed the synthesis and study of the antimicrobial properties of a series of lipophilic phosphorylated betaines with the $^{-}OP(O)(R^1O)CH_2N^{+}Oct_2R^2$ general formula (Scheme 1). These compounds have an optimal hydrophilic–lipophilic balance and can also act as complexing agents, membrane carriers and extractants of organic and inorganic substrates. The synthetic strategy is based on the alkaline mono-hydrolysis of dialkyl α-dioctylamino phosphonates to give intermediate potassium salts **1a**–**c**. The following alkylation occurred exclusively at the nitrogen atom to afford the target compounds **2a**–**i** (see Scheme 1).

The hydrolysis (see Scheme 1, step i) was carried out in 1,4-dioxane using a 10% excess of aqueous alkali with monitoring by FTIR and ^{31}P NMR spectroscopy. The reaction proceeded rather slowly (see Online Supplementary Materials, Figure S1). After the completion of the reaction, potassium salts **1a**–**c** floated in the upper organic phase, so they were isolated and dried in a vacuum. In the final step, for the alkylation of compound **1a** with methyl iodide several solvents such as DMF, propan-2-ol and MeCN were tested (60 °C, 3 h, see Online Supplementary Materials, Figure S2). According to the ^{31}P NMR data, the reaction did not occur in MeCN while in DMF it proceeded quickly giving much side products. Propan-2-ol was recognized as the optimal solvent, the reaction proceeded slowly but smoothly. Phosphorylated betaines **2a**–**i** manifest in propan-2-ol a signal in ^{31}P NMR spectra in the range of 3.6–5.2 ppm. For the purification of such lipophilic compounds **2a**–**i**, they were dissolved in chloroform for separating the precipitated potassium halide by centrifugation. After removal of the solvent from the supernatant, the residue was washed by decantation with light petroleum and then acetonitrile, followed by drying in a vacuum desiccator. The yields of products **2a**–**i** were about 80%. The structures of all new compounds were established using FTIR, ^{1}H, ^{13}C, ^{31}P NMR spectroscopy, and CHN analysis.

The *in vitro* antibacterial and antifungal activities of compounds **2a**–**i** against the Gram-positive bacterial strains *Bacillus cereus* and *Staphylococcus aureus*; Gram-negative bacterial strains *Escherichia coli* and *Pseudomonas aeruginosa* were evaluated (Table 1, Figure 2). The test substances were



a R^1 = Bu, 82%
b R^1 = Bu^i, 75%
c R^1 = Hex, 72%

a R^1 = Bu, R^2 = Me, 83%
b R^1 = Bu, R^2 = Et, 75%
c R^1 = Bu, R^2 = Bn, 81%
d R^1 = Bu^i, R^2 = Me, 86%
e R^1 = Bu^i, R^2 = Et, 83%
f R^1 = Bu^i, R^2 = Bn, 80%
g R^1 = Hex, R^2 = Me, 81%
h R^1 = Hex, R^2 = Et, 80%
i R^1 = Hex, R^2 = Bn, 77%

Scheme 1 *Reagents and conditions*: i, KOH (50%, 1.2 equiv.), 1,4-dioxane, reflux, 10 h; ii, AlkI or BnCl, Pr^{i}OH, reflux ($T \leqslant 60$ °C), 3 h.

Table 1 Antibacterial activity of alkyl (*N*-alkyl-*N*,*N*-dioctylammoniomethyl)phosphonates **2a**–**i** (1% in DMSO) and reference compounds.[a]

Compound	Zone of inhibition, *d*/mm				log*P*
	E. coli	*B. cereus*	*Ps. aeruginosa*	*S. aureus*	
2a	14	30	–	30	4.58
2b	10	30	–	26	4.93
2c	–	22	–	23	6.30
2d	9	30	–	29	4.54
2e	10	31	–	28	4.89
2f	–	27	–	23	6.26
2g	11	28	–	27	5.42
2h	8	26	–	28	5.77
2i	–	23	–	21	7.14
Chlorhexidine	15	14	13	16	
Miramistin	–	13	–	11	
Benzalkonium chloride	8	15	11	13	

[a] Zone of inhibition 22 to 33: highly significant, between 15 to 21 mm: less significant, below 14 mm: poor activity.

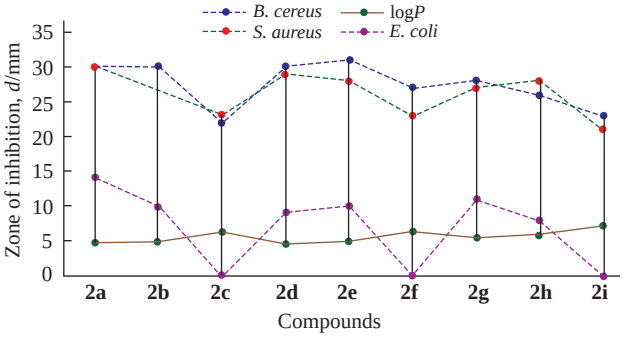


Figure 2 Correlation 'Zone of inhibition – log*P*'.

added to the wells of the agar nutrient medium, the magnitude of bactericidal activity was judged by the presence of a zone of growth inhibition of test cultures, which is formed around the wells. Miramistin, Chlorhexidine and Benzalkonium chloride were examined as the control substances. The choice of control compounds is due to the similarity of their structure with the obtained compounds. According to the obtained data, lipophilic phosphorylated betaines **2a**–**i** are not active against gram-negative bacteria *Ps. aeruginosa* and are slightly active against *E. coli*. Previously, we showed that hydrophilic phosphorylated betaines containing only one higher alkyl substituent at the nitrogen atom also did not exhibit activity against gram-negative bacteria, which was associated with the zwitterionic structure of their molecules.[29,31,32]

In contrast to the phosphorylated betaines, phosphorylated quaternary ammonium salts, are highly active against both Gram-negative and Gram-positive bacteria.[33] It is interesting that compound **2i** containing three lipophilic substituents is more active than the hydrophilic phosphorylated betaine of structure $^{-}OP(O)(Pr^iO)CH_2N^{+}OctBnMe$ containing one short methyl substituent.[30]

Based on data in Table 1, the dependence of lipophilicity on the size of the inhibition zone was plotted. It was found that antibacterial activities of amino phospha betaines containing butoxy and isobutoxy substituents at the phosphorus atom differ slightly; compounds **2g**–**i** containing hexyloxy substituent are inferior to them in activity, so the introduction of a lipophilic substituent leads to a decrease in the activity of the molecule (see Figure 2).

In the obtained series of compounds, amino phospha betaines having short methyl and ethyl substituents at the nitrogen atom

are highly active against bacteria *B. cereus* and *S. aureus* in comparison with their analogues. The introduction of a benzyl substituent on the nitrogen atom (compounds **2c**,**f**,**i**) leads to an increase in the lipophilicity of the molecule (log*P*) and a decrease in activity against all studied bacterial strains up to its complete absence against *E. coli*. In the studied series of amino phospha betaines, compound **2a** is the most promising for further research, as it showed high activity against gram-positive bacteria, exceeding the activity of control compounds.

Since the obtained compounds can be used as carriers and extractants, we were interested to test whether they would inhibit the growth of microorganisms on the membrane surface, where the membrane is in direct contact with the aqueous phase. We evaluated the antimicrobial activity of bacteria that are often found in running water (Table 2). Additionally, the bactericidal activity of 1,2-dichlorobenzene (1,2-DCB) as the membrane solvent was also measured.[25,34] Initially, we have obtained data on the antimicrobial activity of amino phospha betaine **2g** in different percentages in 1,2-DCB.

Based on the data obtained (see Table 2), the membrane solvent has a sufficiently high activity against pathogenic strains of microorganisms, against which the activity of phosphorylated betaine **2g** is insignificant. Regarding the bacteria *Ps. aeruginosa*, there is no activity both in the studied compounds and in the membrane solvent. The concentration of carriers used in membrane transport is 0.1 M, which corresponds to a reagent content of 2.9–3.2%, depending on the structure of the compound. Table 3 presents data for some phosphorylated betaines of 2 and 3% in 1,2-DCB. Thus, the membrane solvent 1,2-DCB makes the main contribution to the suppression of the growth of microorganisms, and at a content of 3 and 2% of the active substance, the data of microbiological activity practically do not change for the bacteria *E. coli* and *Kl. pneumoniae* with some exceptions. Higher values of the zone of inhibition are observed towards *S. aureus*.

In conclusion, we have developed a new efficient synthesis of zwitterionic alkyl (*N*-alkyl-*N*,*N*-dioctylammoniomethyl)-phosphonates **2a**–**i** with two long alkyl chains at nitrogen atom. The synthetic strategy is based on the *N*-quaternization of potassium salts **1a**–**c** with methyl iodide or benzyl chloride. All compounds **2a**–**i** demonstrate a remarkable antibacterial activity against *B. cereus* and *S. aureus* bacterial strains, which is higher than that of Chlorhexidine, Miramistin and Benzalkonium chloride.

Table 2 Antibacterial activity of compound **2g** in 1,2-dichlorobenzene.

C_{2g} (%)	Zone of inhibition, *d*/mm				
	E. coli	*B. cereus*	*Ps. aeruginosa*	*S. aureus*	*Kl. pneumoniae*
1	30	22	–	27	20
2	22	23	–	29	21
3	30	25	–	30	21
4	24	26	–	31	23
0[a]	21	20	–	20	19

[a] Run in pure 1,2-DCB.

Table 3 Antibacterial activity of compounds **2a**,**b**,**d**,**e**,**g**–**i** in 1,2-dichlorobenzene.

Compound	Zone of inhibition, *d*/mm[a]				
	E. coli	*B. cereus*	*Ps. seruginosa*	*S. aureus*	*Kl. pneumoniae*
2a	20/22	25/23	–	**27/27**	15/16
2b	20/25	**32/30**	–	**26/27**	19/16
2d	17/23	23/27	–	22/26	22/15
2e	20/24	24/20	–	**27/19**	20/18
2g	22/30	23/25	–	**29/30**	21/21
2h	24	**28**	–	**30**	14
2i	**33**	17	–	17	20
1,2-DCB	21	20	–	20	19

[a] Slashed values stand for 3 and 2% concentrations, respectively; the single value stands for 2% solution.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.09.012.

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