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SHORT COMMUNICATION

Synthesis and antimicrobial activity of phosphorylated betaines

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ABSTRACT

Target phosphorylated betaines (**1**–**3**) were obtained by the quaternization reaction between potassium salt of amino phosphonate and alkyl halides. The structures of the compounds were investigated by IR and NMR spectroscopy. The molecular structure of isopropyl [(N-octyl-N,N-diethylammonio)methyl]phosphonate (**2**) was determined by a single crystal X-ray analysis. Compound **2** demonstrates a high antibacterial activity against bacterial strain *Staphylococcus epidermidis*.

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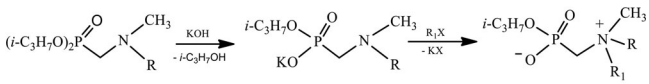
Synthesis; phosphorylated betaines; amino phospha betaines; amino phosphonates; antimicrobial activity; X-ray diffraction

GRAPHICAL ABSTRACT



R = CH_3, $R_1 = CH_2C_6H_5$, X = Cl **1**
R = CH_3, $R_1 = C_8H_{17}$, X = Br **2**
R = C_8H_{17}, $R_1 = CH_2C_6H_5$, X = Cl **3**

Introduction

Microbial pathogens causing infections and diseases have been a threat to human health. In addition, the widespread use of antibiotics and disinfectants has provoked the emergence of new strains of antimicrobial-resistant microorganisms. Therefore, nowadays the search and synthesis of new antimicrobial drugs is attracting the attention of chemists. Quaternary ammonium salts (QASs) have been used for many years on a wide scale in medicine as disinfectants, drugs and in agriculture and forestry as insecticides, fungicides and biocides.[1–3] The prominent representatives of this class are benzalkonium chloride, cetylpyridinium chloride, and miramistin.

Organophosphorus compounds are a wide class of chemical compounds, have largely been used worldwide in agricultural, industrial, medicinal or veterinary applications.[4,5] In this family of compounds, phosphorus-containing betaines, alkylaminophosphonic acids and their derivatives exhibit antibacterial, carcinostatic, cytotoxic and other pharmacological activity.[6–8] Phosphorylated betaines (amino phosphabetaines) are derivatives of QAS, which contain a negatively charged phosphonate group and a positively charged quaternary nitrogen atom.[9–11] In this work, we reported of the synthesis of new phosphorylated betaines and the study of their antimicrobial activity.

Results and discussion

We reported the synthesis strategy of isopropyl [(N-methyl-N-alkyl -N-benzyl(alkyl)ammonio)methyl]phosphonates which was based on the preparation of O,O-dialkyl-α-aminophosphonates with their subsequent involvement in the alkaline hydrolysis reaction to obtain the corresponding potassium salts (Scheme 1).

The Kabachnik–Fields reaction was carried out in a three-component system in benzene; p-toluenesulfonic acid was used as a catalyst. The process was monitored using IR spectroscopy and ^{31}P NMR spectroscopy. The hydrolysis reaction was carried out in a 1,4-dioxane medium with a 10% excess of aqueous alkali. Further, according to the quaternization reaction between the obtained potassium salts of aminophosphonic acids and alkyl halides, we obtain the target aminophosphabetaines **1**–**3**.

Compound **2** was isolated as a crystal and its molecular structure was studied by X-ray analysis (Figure 1). The crystal packing is stabilized by strong hydrogen bonds between the water molecule and the oxygen of the phosphoryl group.

In vitro antibacterial activities of the isopropyl [(N-methyl-N-alkyl -N-benzyl(alkyl)ammonio)methyl]phosphonates against the Gram-positive bacterial strains *Staphylococcus epidermidis* and *Staphylococcus aureus*; Gram-negative bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa* and fungi strains *Candida albicans* were evaluated (Table 1). Benzalkonium chloride was examined as the control.

The compounds **1** and **3** exhibit low microbiological activity. In this group of substances, amino phospha betaine **2**, containing a long-chain octyl substituent at the nitrogen atom, exhibits high antimicrobial activity against the *S.*

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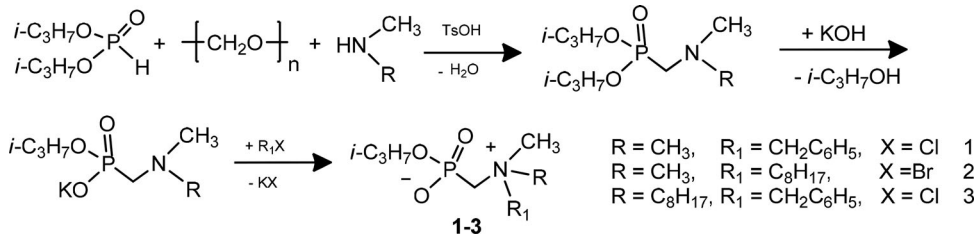

Scheme 1. Synthesis of phosphorylated betians **1–3**

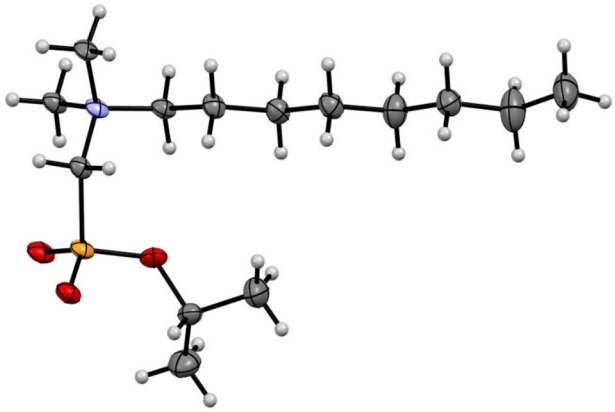

Figure 1. ORTEP representation of isopropyl [(N-octyl-N,N-dimethylammonio)-methyl]phosphonate **2** showing 50% probability thermal ellipsoids. C atoms—gray, N atoms—blue, O atoms—red, P atoms—orange.

epidermidis and can be considered as a potential antimicrobial agent.

Materials and methods

NMR spectra were recorded on the Bruker Avance III instrument with an operating frequency of 162 MHz for ^{31}P spectra, an operating frequency of 400 MHz for ^{1}H spectra and an operating frequency of 100.6 MHz for ^{13}C spectra in a solution of $CDCl_3$. The Fourier Transformed InfraRed (FT-IR) spectrum of the sample was recorded using Perkin Elmer UATR Two FT-IR Spectrometer (Spectrum Two) with an ATR (attenuated total reflectance) diamond crystal. Spectra were baseline corrected and normalized. The mass spectra were obtained on an AB Sciex 5600 Triple TOF mass spectrometer with positive electrospray ionization.

Commercially available alkyl halides were used for the preparation of isopropyl [(N-alkyl-N,N-dimethylammonio)-methyl]phosphonates **1–3**. Commercially available solvents (C_6H_6, *iso*-C_3H_7OH, petroleum ether) were purified by standard procedures. p-toluenesulfonic acid 97.5% chemical grade was used.

Synthesis of alkyl [(N-alkyl-N,N-dialkylammonio)methyl]phosphonates is described previously.[8]

Isopropyl [(N-benzyl-N,N-dimethylammonio)methyl] phosphonate 1

IR (cm^{-1}): 1073 (P-O-C), 1238 (P=O). ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.17 (d, J = 6.17 Hz, 6H), 3.28 (s, 6H), 3.55 (d, J_{P-H} = 12.37 Hz, 2H) 4.50–4.60 (m, 1H), 4.85 (s, 2H), 7.30–7.60 (m, 5H). ^{13}C{^{1}H} NMR ($CDCl_3$, 102 Hz), δ (ppm): 24.33 (d, J_{C-P} = 4.19 Hz), 51.89 (d, J_{C-P} = 2.98 Hz), 60.53 (d, J_{C-P} = 134.92 Hz), 69.64 (d, J_{C-P} = 4.06 Hz), 69.34 (d, J_{C-P} = 6.27 Hz), 127.59, 129.07, 130.62, 133.25. ^{31}P{^{1}H} NMR ($CDCl_3$, 162 MHz): δ (ppm) 4.3. HRMS-ESI (positive) *m/z*: calcd for $C_{13}H_{22}NO_3P^+$ $[MH]^+$ 272.1416, found 272.1417.

Table 1. Antibacterial activity of isopropyl [(N-methyl-N-alkyl-N-benzyl(alkyl) ammonio)methyl]phosphonates **1–3**.

	The MIC of the tested substances, μg/ml			
Strains	1	2	3	Benzalkonium chloride
S. aureus	>64	32	>64	1
S. epidermidis	>64	2	>64	2
E. coli	>64	16	>64	8
Ps. aeruginosa	>64	32	>64	1

Isopropyl [(N-octyl-N,N-dimethylammonio)methyl] phosphonate 2

IR (cm^{-1}): 1067 (P-O-C), 1207 (P=O). ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0,86 (t, J = 6.69 Hz, 3H), 1.23 (d, J = 6.08 Hz, 6H), 1.23–1.40 (m, 12H), 3.34 (s, 6H), 3.50 (d, J_{P-H} = 12.58 Hz, 2H), 3.51–3.59 (m, 2H), 4.50–4.60 (m, 1H). ^{13}C{^{1}H} NMR ($CDCl_3$, 102 Hz), δ (ppm): 13.90, 24.45 (d, J_{C-P} = 4.16 Hz), 22.41, 22.92, 26.07, 28.91, 29.04, 31.49, 52.72 (d, J_{C-P} 2.48 Hz), 61.28 (d, J_{C-P} = 122.55 Hz), 66.89 (d, J_{C-P} = 3.73 Hz), 68.34 (d, J_{C-P} = 6.07 Hz). ^{31}P{^{1}H} NMR ($CDCl_3$, 162 MHz): δ (ppm) 4.2 HRMS-ESI (positive) *m/z*: calcd for $C_{14}H_{33}NO_3P^+$ $[MH]^+$ 294.2198, found 294.2199.

Isopropyl [(N-methyl-N-benzyl-N-octylammonio)methyl] phosphonate 3

IR (cm^{-1}): 1073 (P-O-C), 1238 (P=O). ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.86. (t, J = 6.67 Hz, 3H), 1.22 (d, J = 6.17 Hz, 6H), 1.23–1.40 (m, 12H), 3.35 (s, 3H), 3.55 (d, J_{P-H} = 12.63 Hz, 2H), 3.56–3.66 (m, 2H), 4.50–4.60 (m, 1H), 4.86. (s, 2H), 7.30–7.60 (m, 5H). ^{13}C{^{1}H} NMR ($CDCl_3$, 102 Hz), δ (ppm): 13.95, 24.40. (d, J_{C-P} = 4.17 Hz), 22.48, 22.96, 26.12, 28.96, 29.08, 31.54, 51.89. (d, J_{C-P} = 2.69 Hz), 60.41 (d, J_{C-P} = 133.59 Hz), 66.89. (d, J_{C-P} = 3.62 Hz), 69.30 (d, J_{C-P} = 6.25 Hz) 69.83. (d, J_{C-P} = 4.06 Hz), 127.63, 129.15, 130.68, 133.29. ^{31}P{^{1}H} NMR ($CDCl_3$, 162 MHz): δ (ppm) 4.3. HRMS-ESI (positive) *m/z*: calcd for $C_{20}H_{37}NO_3P^+$ $[MH]^+$ 370.2511, found 370.2511.

X-ray analysis

Data set for single crystal isopropyl [(N-octyl-N,N-dimethylammonio)methyl]phosphonate **2** was collected on a Rigaku XtaLab Synergy S instrument with a HyPix detector and a PhotonJet microfocus X-ray tube using Cu Kα (1.54184 Å) radiation at low temperature. Images were indexed and integrated using the CrysAlisPro data reduction package. Data were corrected for systematic errors and absorption using the ABSPACK module: numerical absorption correction based on Gaussian integration over a multifaceted crystal model and empirical absorption correction based on spherical harmonics according to the point group symmetry using equivalent reflections. The GRAL module was used for analysis of systematic absences and space group determination. The structure was solved by direct methods using SHELXT[12] and refined by the full-matrix least-squares on F^2 using SHELXL.[13] Non-hydrogen atoms were refined anisotropically. The hydrogen atoms were inserted at the calculated positions and refined as riding atoms. The figures were generated using Mercury 4.1[14] program. Crystals were obtained by slow evaporation method in isopropanol media.

Crystal data for $C_{14}H_{34}NO_4P$ ($M = 311.39$ g/mol): monoclinic, space group $P2_1/n$ (no. 14), $a = 6.0865(2)$ Å, $b = 37.8555(16)$ Å, $c = 7.7523(3)$ Å, $\beta = 96.515(4)°$, $V = 1774.65(12)$ Å^3, $Z = 4$, $T = 99.99(10)$ K, μ(Cu Kα) = 1.476 mm^{-1}, $Dcalc = 1.165$ g/cm^3, 10887 reflections measured ($4.668° \leq 2\Theta \leq 152.496°$), 3571 unique ($R_{int} = 0.0656$, $R_{sigma} = 0.0586$) which were used in all calculations. The final R_1 was 0.0938 ($I > 2\sigma(I)$) and wR_2 was 0.2788 (all data). CCDC number 2104184.

Antibacterial activity

The MIC of compounds was determined by the broth micro-dilution method in 96-well microtiter plates (Eppendorf) according to the EUCAST rules for antimicrobial susceptibility testing.[15]

Conclusions

Thus, we have synthesized new phosphorylated betaines **1–3** with various substituents at the nitrogen atom. The study of antimicrobial activity of the compounds against pathogenic bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* showed that compound **2** is highly active and can be considered as leader among these compounds.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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