Synthesis and characterization of 1-carbalkoxymethyl-4-hydroxy-1-methylpiperidinium chlorides

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Dedicated to Professor Jan Epsztajn on his 75th birthday

Abstract

The synthesis of new 1-carbalkoxymethyl-4-hydroxy-1-methylpiperidinium chlorides derived from 4-hydroxy-1-methylpiperidine and alkyl chloroacetates was described. Salts were characterized by MS, FTIR, ^{1}H and ^{13}C NMR spectroscopies. Compounds investigated were tested against *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger.*

Keywords: Quaternary piperidinium salts, FTIR, ^{1}H and ^{13}C NMR, MS, microbial activity

Introduction

The antibacterial properties of quaternary ammonium compounds were first observed at the end of the 19-th century. The effects of substituents, structures and anions of quaternary salts on the antimicrobial activity have been extensively studied.[1] The germicidal activity is the greatest in the compounds containing a long alkyl chain from 12 to 16 carbon atoms.[1] To increase solubility of the quaternary salts an ester group is introduced to compounds of a series of N-methyl-morpholinium, N,N'-dimethyl-piperazinium, N-methyl-piperidinium salts and they were tested against same bacteria. A series of piperidinium salts has been shown to have maximum activity.[2]

In recent years a permanent increase in the number of bacterial strains resistant to disinfectants has been observed. Cationic surfactants are of interest because of their membrane-disruptive properties, rapid antimicrobial activities, and activity against a broad range of bacteria and other cell.[3] This is the reason why the compounds belonging to this group are still synthesized.

Recently we have synthesized N-carbalkoxylmethyl-N-alkyl-piperidinium chlorides and tested them against nine bacteria species and two Candida-type yeasts.[4] The most active are N-carbdodecyloxymethyl-N-methylpiperidinium chloride and N-carbethoxymethyl-N-dodecylpiperidinium chloride, whose activity is greater than that of dodecyl-trimethylammonium chloride, usually used as a reference substance in the microbiological tests. The promising pharmacological properties of this type of salts have prompted a synthesis of a series of

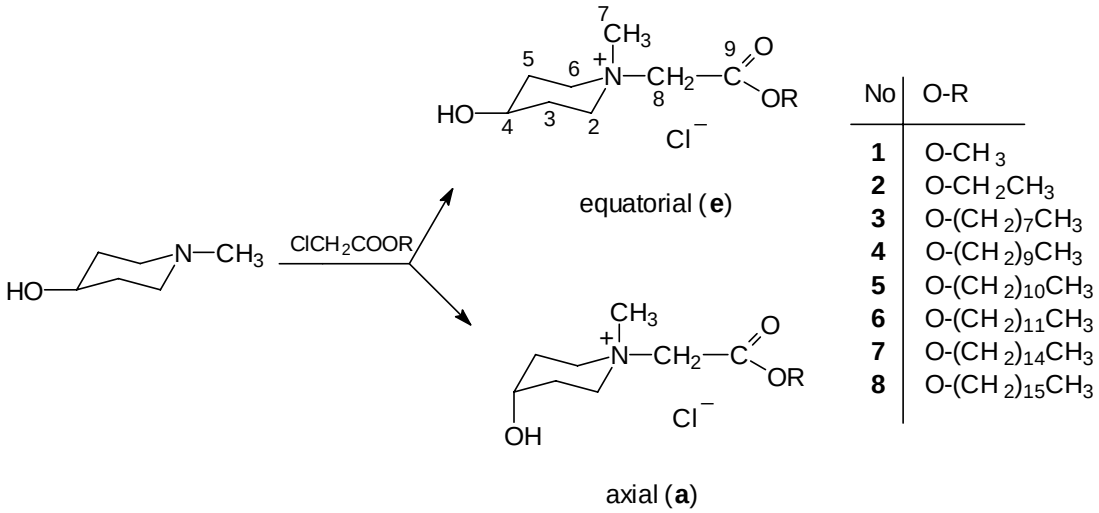
piperidinium salts in which the hydroxyl group is at the piperidinium ring. Different substituted 1,1-dialkyl-4-hydroxypiperidinium salts reveal also interesting pharmacological properties and can be incorporated as building blocks in artificial phospholipids to play an important role as surfactants and biological membranes.[5-12]

The aim of this work is the synthesis of new 1-carbalkoxymethyl-4-hydroxy-1-methylpiperidinium chlorides (Scheme 1) and their spectral characterization by FTIR, MS, ^{1}H and ^{13}C NMR. The hydroxyl group at the piperidinium ring and a long alkyl chain in the ester group are expected to increase the solubility of salts and their antimicrobial properties.

In the previous work, we have studied the structures of two stereoisomers of 1-carboxymethyl-4-hydroxy-1-methylpiperidinium chloride.[13] According to the X-ray analysis these stereoisomers differ in the conformation of the hydroxyl group at the C(4) atom, which can be in the axial or equatorial position, while the methyl group attached to the nitrogen atom is in the axial position and the carboxymethyl substituent in the equatorial one.

Results and Discussion

Starting from 4-hydroxy-1-methylpiperidine and the corresponding alkyl chloroacetates a series of eighth 1-carbalkoxymethyl-4-hydroxy-1-methylpiperidinium chlorides (**1**–**8**) was synthesized (Scheme 1). A mixture of stereoisomers, with the OH group in the axial (**a**) and the equatorial (**e**) positions was obtained. Only esters **1e** and **2a** were isolated as the pure stereoisomer by a fractional crystallization.[14] Esters **3**-**8** are very soluble in water and in organic solvents, hence a separation of stereoisomers (**a**) and (**e**) by a multiple recrystallization from acetonitrile was not successful. The melting points of **3**-**8** were constant and could not be raised by further treatment. The ^{1}H NMR spectra show that the stereoisomer with the equatorial OH group is predominant (60 %) in the reaction mixture. It has been shown earlier that in the quaternization of piperidine derivatives the ratio of equatorial to axial isomers depends on solvent, alkylation agent and substrate structure.[15]



No	O-R
1	O-CH_3
2	O-CH_2CH_3
3	O-$(CH_2)_7CH_3$
4	O-$(CH_2)_9CH_3$
5	O-$(CH_2)_{10}CH_3$
6	O-$(CH_2)_{11}CH_3$
7	O-$(CH_2)_{14}CH_3$
8	O-$(CH_2)_{15}CH_3$

Scheme 1

The proton chemical shifts of the compounds investigated are listed in Table 1. The numbering of atoms is shown in Scheme 1. The significant difference in the ^{1}H NMR spectra of isomers (**e**) and (**a**) appears in the region of the resonance signals assigned to the protons at the C(2,6) carbon atoms. The spectrum of the stereoisomer with the axial OH group reveals two multiplets, while that with the equatorial OH group only one broad multiplet.[14]

The X-ray diffraction study of **1** confirmed the chair conformation of the piperidinium ring with the OH and CH_2COOCH_3 groups in the equatorial positions and the CH_3 group in the axial one.[16] The ^{1}H NMR spectrum of **1(e)** is characterized by a broad multiplet at 3.71 ppm, attributed to the equatorial and axial protons attached to C(2,6) atoms (Figure1a).

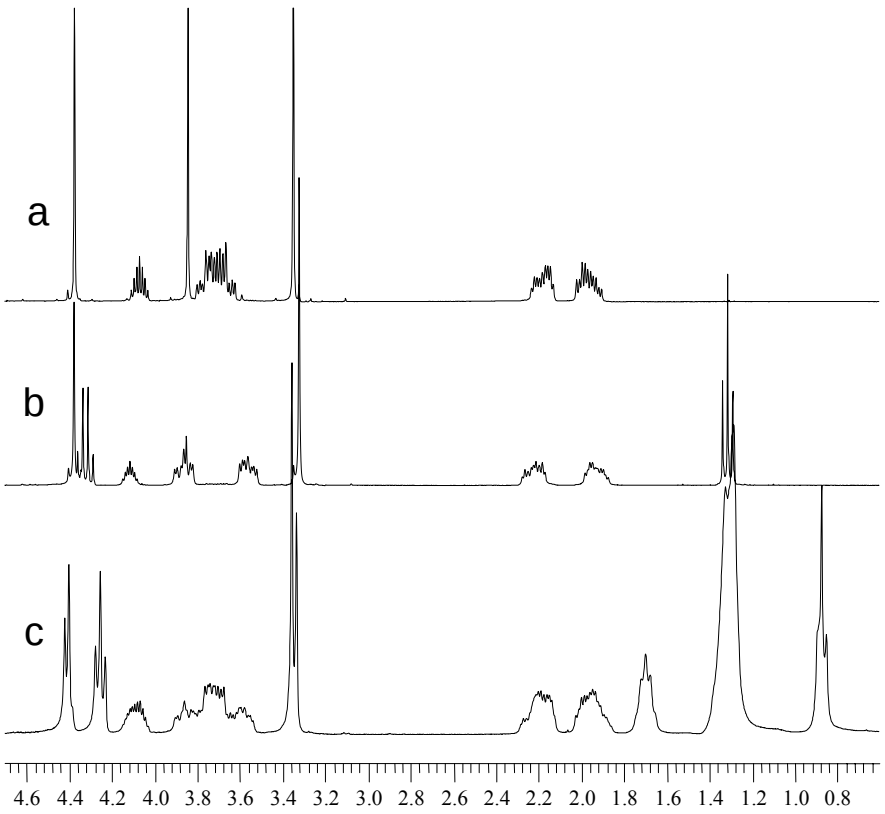


Figure 1. ^{1}H NMR spectra (in D_2O) of (a) 1-Carbmethoxymethyl-4-hydroxy-1-methylpiperidinium chloride (**1e**); (b) 1-Carbethoxymethyl-4-hydroxy-1-methylpiperidinium chloride (**2a**); (c) 1-Carboctyloxymethyl-4-hydroxy-1-methylpiperidinium chloride (**3e** and **3a**).

Table 1. ^{1}H chemical shifts (ppm) of 1-carbalkoxymethyl-4-hydroxy-1-methylpiperidinium chlorides (**1** – **8**) in D_2O

No	R	Piperidinium protons			N^+CH_3	N^+CH_2	O-$(CH_2)_{0-15}$	CH_3
		2,6	3,5	4	7	8		
1e	CH_3	3.71	2.07 1.92	4.07	3.35	4.40	-	3.86
2a	C_2H_5	3.86 eq* 3.56 ax*	2.22 eq 1.93 ax	4.11	3.32*	4.38	4.42[a]	1.31
3	C_8H_{17}	3.85 eq* 3.72 ax,eq 3.59 ax*	2.16 eq 1.95 ax	4.08	3.36 3.34*	4.42* 4.40	4.25[a], 1.70[b], 1.31	0.86
4	$C_{10}H_{21}$	3.85 eq* 3.74 ax,eq 3.63 ax*	2.21 1.97	4.08	3.37 3.35*	4.46* 4.45	4.23[a], 1.69[b], 1.28	0.86
5	$C_{11}H_{23}$	3.85 eq* 3.73 ax,eq 3.61 ax*	2.14 1.95	4.07	3.37 3.35*	4.46* 4.45	4.21[a], 1.70[b], 1.28	0.85
6	$C_{12}H_{25}$	3.86 eq* 3.73 ax,eq 3.61 ax*	2.21 1.97	4.08	3.37 3.35*	4.48* 4.46	4.22[a], 1.70[b], 1.28	0.87
7	$C_{15}H_{31}$ [c]	4.00	2.12	4.12	3.56 3.53*	4.78	4.16[a], 1.65[b], 1.26	0.88
8	$C_{16}H_{33}$ [d]	3.57 3.67 3.76 3.79	2.16 1.95	4.07	3.35 3.32*	4.62	4.23[a], 1.70[b], 1.29	0.88
8	$C_{16}H_{33}$ [c]	3.97	2.11	4.07	3.57 3.52*	4.83* 4.78	4.16[a], 1.64[b],1.26	0.88

[a] O-CH_2-C. [b] O-C-CH_2. [c] in $CDCl_3$. [d] concentration of sample 0.03 mol.dm^{-3}

* The piperidinium ring with the hydroxyl group in the axial position

Two separate multiplets corresponding to the equatorial and axial protons at 3.86 and 3.56 ppm are observed in the spectrum of **2** (Figure1b). Such behavior of the equatorial and axial protons at C(2,6) is similar to that observed in the spectrum of α-4-hydroxy-1-methylpiperidine betaine hydrochloride, in which the orientation of the hydroxyl group was estimated by X-ray analysis.[13,14] However, three multiplets are observed in the region of the resonance of protons attached to C(2,6) atoms in the spectra of the **3** – **8** esters (Figure 1c). Two of these at ca. 3.85 and 3.61 ppm are due to the isomers with the axial OH group, **3(a)** – **8(a)**, while the more intensive multiplet at ca. 3.73 ppm is attributed to the stereoisomers with the equatorial OH group, **3(e)** – **8(e)**. This behavior of the resonance signals suggests that the compounds **3** – **8** exist as a mixture of stereoisomers (**e**) and (**a**) (Scheme 1).

As follows from the ^{1}H NMR spectra, the steroisomers with the equatorial OH group, **3(e)** – **8(e)** are predominant in the reaction mixture and their population has been determined as ca. 60

%, from the intensities of the resonance signal of the N^+CH_3 protons. This signal appears in the lower magnetic field in the spectra of stereoisomers (**e**), with the equatorial OH group (compare Figures 1a and 1b).

Table 2. ^{13}C chemical shifts (ppm) of 1-carbalkoxymethyl-4-hydroxy-1-methylpiperidinium chlorides (**1** – **6**) in D_2O

No	R	Piperidinium carbons			N^+CH_3	N^+CH_2	COO	O-$(CH_2)_{0-15}$	CH_3
		2,6	3,5	4	7	8	9		
1e	CH_3	61.8	30.0	64.8	51.4	63.1	168.1	-	56.1
2a	C_2H_5	61.1	29.7	64.3	50.9	64.0	167.6	66.2[a]	16.0
3	C_8H_{17}	61.3 60.7*	29.5 29.3*	64.2	51.1 50.5*	62.8* 63.2	167.1 167.0*	69.2[a], 33.6, 30.9, 30.8, 30.0[b], 27.5, 24.5	
4	$C_{10}H_{21}$	61.2 60.5*	29.5 29.3*	64.0	51.0 50.4*	62.6* 63.3	166.9 166.8*	68.7[a], 33.9, 31.6, 31.3, 31.2, 30.1[b], 27.6, 24.6	15.9
5	$C_{11}H_{23}$	61.0 60.3*	29.4 29.1*	63.9	50.7 50.1*	62.4* 63.2	166.8 166.7*	68.5[a], 34.0, 31.8, 31,7, 31.5, 31.4, 31.2, 30.1[b], 27.6, 24.6	15.8
6	$C_{12}H_{25}$	60.9 60.2*	29.4 29.1*	63.9	50.7 50.1*	62.3* 63.2	166.9 166.7*	68.4[a], 34.0, 32.0, 31.9, 31.8, 31.6, 31.4, 31.3, 30.1[b], 27.6, 24.7	15.9
7	$C_{15}H_{31}$ [c]	58.6 57.8*	28.3 28.2*	61.2	49.4 46.1*	58.9* 60.4	164.5 164.4*	66.7[a], 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 27.8, 25.7, 22.7	14.1
8	$C_{16}H_{33}$ [c]	58.5 58.6*	28.2 28.1*	61.1	49.3 45.8*	59.1* 60.3	164.7 164.6*	66.7[a], 31.8[b], 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 27.6, 22.2	14.0

[a] O-CH_2-C. [b] O-C-CH_2. [c] in $CDCl_3$.

* The piperidinium ring with the hydroxyl group in the axial position

The carbon chemical shifts of the compounds investigated in D_2O are listed in Table 2 and the ^{13}C NMR spectrum of **6** is shown in Fig. 2. The assignments are based on the ^1H-^{13}C two dimensional correlation.

In the carbon-13 spectra the double signals attributed to the ring carbon atoms C(2,6), C(3,5) and to the carbon atoms of N^+CH_3, N^+CH_2 and COO groups confirm the existence of two stereoisomers in the reaction between 4-hydroxy-1-methylpiperidine and alkyl chloroacetate (Figure 2) (Scheme 1). The resonance signals of the carbon atoms of the N^+CH_3 and N^+CH_2 groups are less intense than the signals of the ring carbon atoms and the alkyl chain.

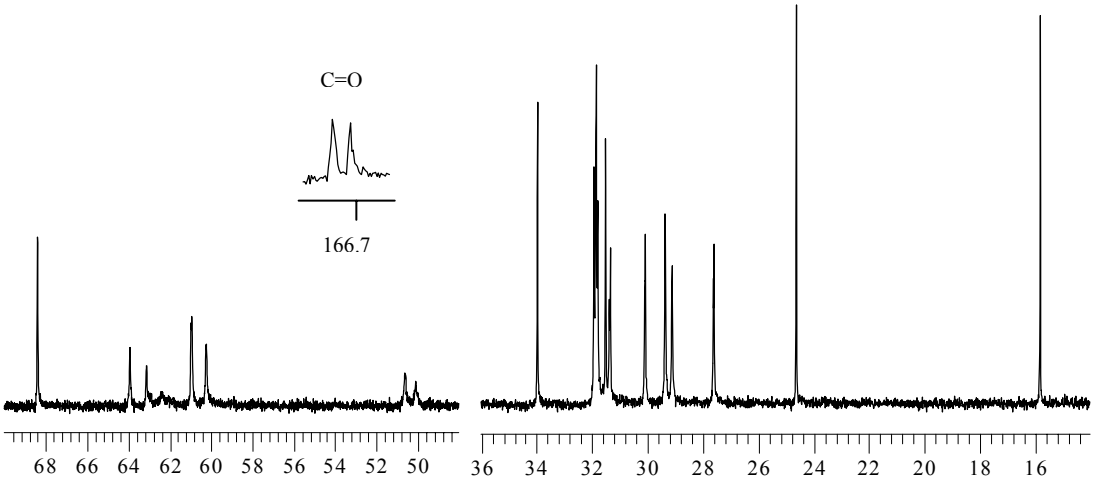


Figure 2. The ^{13}C NMR spectrum of 1-carbdodecyloxymethyl-4-hydroxy-1-methylpiperidinium chloride (**6**) in D_2O.

The solid state FTIR spectra of **1**, **2** and **4** are shown in Figure 3. The strong νOH band appears at ca. 3250 cm^{-1}, which suggests that the hydroxyl group is engaged in the O-H···Cl hydrogen bond. A similar situation has been observed in the infrared spectrum of bis(4-hydroxy-1-methylpiperidine betaine) hydrochloride, in which the O-H···Cl hydrogen bonds are between 2.86 and 3.12 Å, and the νOH···Cl vibration appears at 3320 cm^{-1}.[17] A comparison of these values suggests that the O-H···Cl hydrogen bonds in the compounds investigated are shorter than in bis(4-hydroxy-1-methylpiperidine betaine) hydrochloride. The strong band at 1750 cm^{-1} is attributed to the νC=O vibration of the ester group.

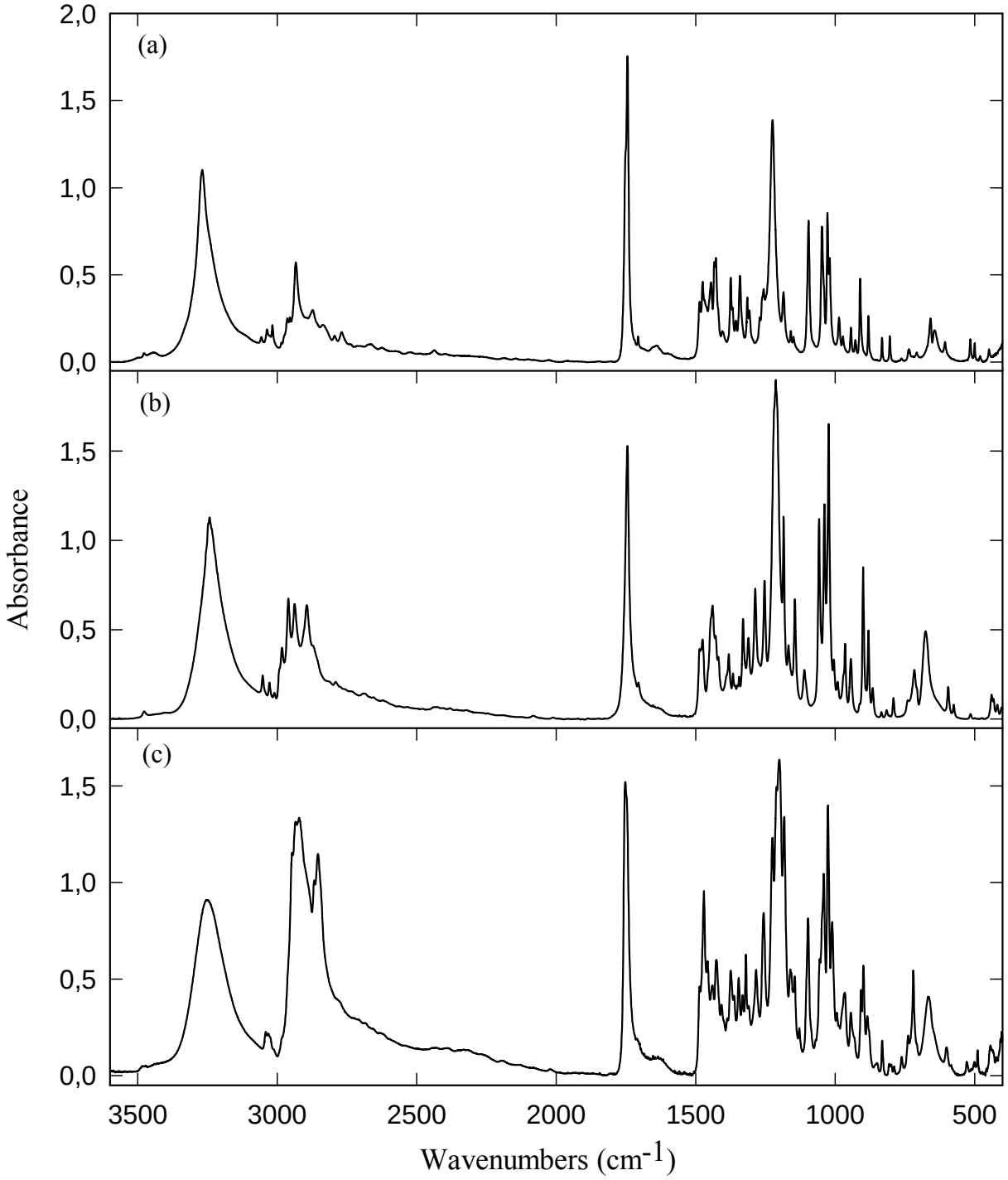


Figure 3. FTIR spectra in nujol-fluorolube suspensions of (a) 1-carbmethoxymethyl-4-hydroxy-1-methylpiperidinium chloride (**1**); (b) 1-carbethoxymethyl-4-hydroxy-1-methylpiperidinium chloride (**2**); (c) 1-carbdecyloxymethyl-4-hydroxy-1-methylpiperidinium chloride (**4**).

The positive electrospray ionization spectra (ESI) for **1**, **2**, **5** and **7** are shown in Figure 4. Each spectrum, instead of the ESI spectrum of **1**, shows at least three peaks. The most intensive peak (100 %) corresponds to the mass of cation $[M]^+$, the second one to the mass of the protonated cation $[M+1]^+$. The third peak at m/z 188, in the ESI spectra of **3-8,** suggests that the fragmentation of cations leads to $[HOC_5H_{10}N(CH_3)CH_2COOCH_3]^+$, because this peak appears in the spectrum of **1**. The intensity of the peak at m/z 188 increases with the elongation of the alkyl chain in the ester group.

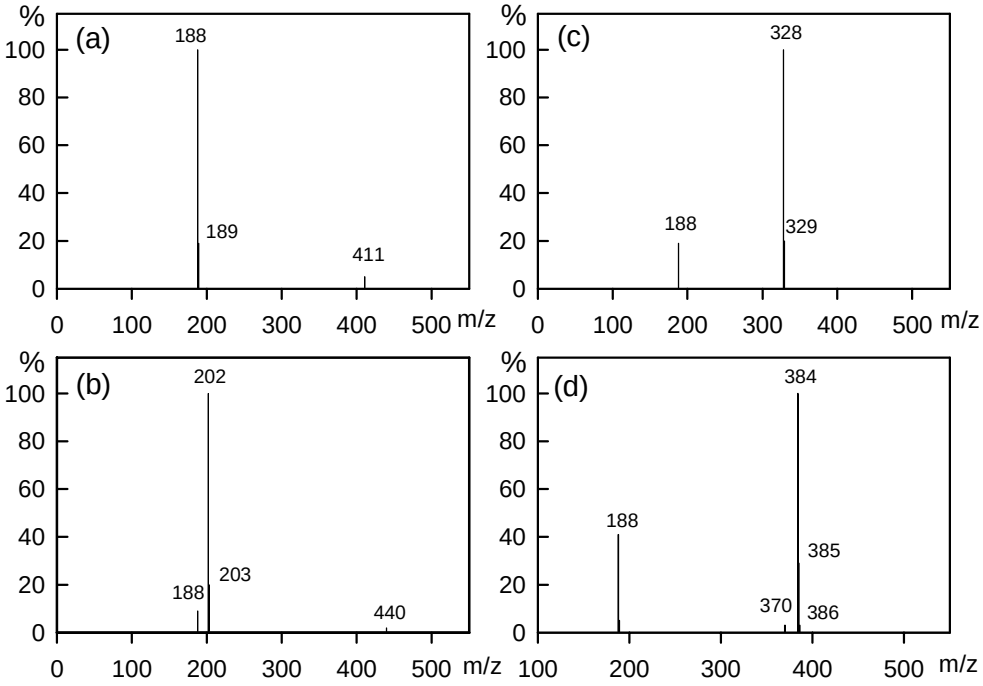


Figure 4. Electrospray isonization spectra of (a) **1**, (b) **2**, (c) **5** and (d) **7**.

To study the antimicrobial activity of the compounds investigated two strains of bacteria: *Escherichia coli* and *Staphylococcus aureus*, one yeast-type: *Candida albicans* and one funge *Aspergillus niger* have been used. The minimum inhibitory concentration (MIC) of the title compounds vary from 0.1% [2.6 mM] to above 5% [119 mM] (Table 3). The lowest values of MIC are observed for the compounds with the decyl, undecyl and dodecyl alkyl chain (Figure 5). The data indicate that the compound **6** shows the highest antibacterial and antifungal activity. The biocidal concentration of **6** is 0.1 % and 0.2 % for *Staphylococcus aureus* and *Escherichia coli,* respectively. Fungicidal concentrations are slightly higher, and both for *Candida albicans* and *Apergillus niger* are 0.5%.

Table 3. Activity of the compounds studied with microorganisms in mM (% w/v)

No	*Escherichia coli*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus niger*
1	89.4 (>2.0)	89.4 (>2.0)	89.4 (>2.0)	89.4 (>2.0)
2	84 (>2.0)	84 (>2.0)	84 (>2.0)	84 (>2.0)
3	3.1 (1.0)	3.1 (1.0)	6.2 (>2.0)	6.2 (>2.0)
4	4.3 (0.15)	5.7 (0.2)	14.3 (0.5)	5.7 (0.2)
5	8.2 (0.3)	2.7 (0.1)	27 (1.0)	8.2 (0.3)
6	5.2 (0.2)	2.6 (0.1)	13.2 (0.5)	13.2 (0.5)
7	119 (5.0)	119 (0.5)	119 (>5.0)	119 (5.0)
8	115.2 (>5.0)	115.2 (>5.0)	115.2 (>5.0)	115.2 (>5.0)

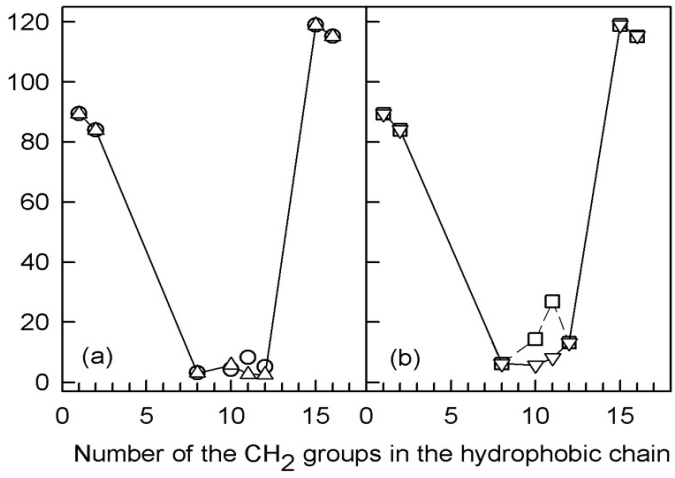


Figure 5. The plot of the minimum inhibitory concentration (MIC) of salts (in mM) on (a) *Escherichia coli* (○) and *Staphylococcus aureus* (Δ) and (b) *Candida albicans* (□) and *Aspergillus niger* (▽), versus the number of the methylene groups in the hydrophobic chain.

The mechanism of the biocidal action of quaternary ammonium compounds is based on the adsorption of positively charged alkylammonium salt moiety on the negatively charged cell wall and its penetration by alkyl chain which leads to a leak of low molecular components of cell. In a consequence the microorganism cell dies. It has been previously shown that the most effective are the ammonium derivatives with hydrocarbon chain between C_{12}-C_{14}.[18-21] A similar correlations are observed for N-methyl-N-carbalkoxymethylpiperidinium chlorides.[4]

Experimental Section

General Procedures. Elemental analyses were carried out with a Vario EL III instrument and the values found were within ±0.2 % of theoretical values. Mass spectra were run on a Waters and Micromass Spectrometer 2Q, using direct inlet system under positive ion electrospray

ionization source in methanol. ^{1}H and ^{13}C NMR spectra were recorded on a Varian-Gemini 300VT spectrometer operating at 300.07 (^{1}H) and 75.4614 (^{13}C) MHz, respectively. The spectra were measured in D_2O relative to an internal standard of 3-(trimethylsilyl)propionic-d_4 acid sodium salt and in $CDCl_3$ relative to TMS. The 2D spectra were obtained with standard Varian software. FTIR spectra were measured on a Bruker IFS 113v instrument, evacuated to avoid water and CO_2 absorption. Solid state spectra were recorded in Nujol and Fluorolube suspensions using KBr plates. Each spectrum consists of 128 scans.

Materials. Microorganisms *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16484 were taken from Collection of Pure Cultures (ŁOCK 105) of Institute of Fermentation Technology and Microbiology of Technical University of Łódź, Poland.

General procedure A

Alkyl chloroacetates were prepared by refluxing chloroacetic acid (0.5 mol) with corresponding alkyl alcohol (0.5) in the presence of sulfuric acid (5 dcm^3) and benzene (100 dm^3) as solvent for 7 h. The mixture was extracted with two 50 portions of 25% sodium carbonate. The benzene layer was dried over sodium sulfate and the solvent distilled off. The esters of chloroacetic acid were purified by vacuum distillation and obtained in yields from 65 to 95%.[2,22] The esters as colorless liquids were further characterized by comparison of their boiling point with reported data from the literature and their NMR spectra. Octyl chloroacetate, b.p. 110-111 ^{o}C/2 mm Hg,[23]; 91-93 ^{o}C/5 mm Hg;[24] decyl chloroacetate, b.p. 148-150 ^{o}C/10 mm Hg,[22] 141-143 ^{o}C/6 mm Hg,[24] undecyl chloroacetate, b.p. 151-154 ^{o}C/5 mm Hg;[24] dodecyl chloroacetate, b.p. 149-150 ^{o}C/3 mm Hg;[2] 190 ^{o}C/25 mm Hg;[23] 163-166 ^{o}C/8 mm Hg;[24] pentadecyl chloroacetate, b.p. 205-207 ^{o}C/2 mm Hg;[25] hexadecyl chloroacetate, b.p 218-220 ^{o}C/10 mm Hg, m.p. 31 ^{o}C.[2,26] Pentadecyl chloroacetate and hexadecyl chloroacetate crystallize as white wax-like solids.

General procedure B

1-Carbalkoxymethyl-4-hydroxy-1-methylpiperidinium chlorides were prepared by treating 4-hydroxy-1-methylpiperidine (1 M) with the corresponding alkyl chloroacetates (1 M) in diethyl ether solution. The mixture was stirred at room temperature till formation of precipitate and finally left to stand for 24 h. The crude product was filtered off and washed with diethyl ether.

1-Carbmethoxymethyl-4-hydroxy-1-methylpiperidinium chloride (1). This compound was prepared according to the literature method,[14] isolated as stereoisomer with the OH group in the equatorial position at the piperidine ring; recrystallized from methanol, m.p. 184-185 ^{o}C. Anal. calcd. for $C_9H_{18}ClNO_3$ (223.70): C, 48.32; H, 8.11; N, 6.26 %. Found: C, 48.36; H, 8.44; N, 6.23%. MS: *m/z* (%) 188 (100) $[M]^+$, 189 (20) $[M+1]^+$; FTIR: νOH, 3270 cm^{-1}; νC=O, 1745 cm^{-1}; ^{1}H and ^{13}C NMR (ppm, in D_2O) see Tables 1 and 2.

1-Carbethoxymethyl-4-hydroxy-1-methylpiperidinium chloride (2). This compound was prepared according to the literature method,[14] isolated as stereoisomer with the OH group in the axial position at the piperidine ring; recrystallized from ethanol, m.p. 222-224 ^{o}C. Anal. calcd. for $C_{10}H_{20}ClNO_3$ (237.73): C, 50.52; H, 8.48; N, 5.89 %. Found: C, 50.56; H, 8.79; N, 5.92 %. MS: *m/z* (%) 202 (100) $[M]^+$, 203 (19) $[M+1]^+$, 188 (9); FTIR: νOH, 3244 cm^{-1}; νC=O, 1744 cm^{-1}; ^{1}H and ^{13}C NMR (ppm, in D_2O) see Tables 1 and 2.

1-Carboctyloxymethyl-4-hydroxy-1-methylpiperidinium chloride (3). According to the general procedure B from 11.5 g (0.1 M) 4-hydroxy-1-methylpiperidine and 20.5 g (0.1 M) octyl chloroacetate to obtain **3** (22.8 g, 71%); m.p. 151 ^{o}C from acetonitrile; colorless plates. Anal. calcd. for $C_{16}H_{32}ClNO_3$ (321.89): C, 59.70; H, 10.02; N, 4.35 %. Found: C, 59.62; H, 10.31; N, 4.35 %. MS: *m/z* (%) 286 (100) $[M]^+$, 287 (22) $[M+1]^+$, 188 (9); FTIR: νOH, 3260 cm^{-1}; νC=O, 1753 cm^{-1}; ^{1}H and ^{13}C NMR (ppm, in D_2O) see Tables 1 and 2.

1-Carbdecyloxymethyl-4-hydroxy-1-methylpiperidinium chloride (4). According to the general procedure B from 11.5 g (0.1 M) 4-hydroxy-1-methylpiperidine and 23.5 g (0.1 M) decyl chloroacetate to obtain **4** (25.4 g, 72.4 %); m.p. 157-158 ^{o}C from acetonitrile; colorless needles. Anal. calcd. for $C_{18}H_{36}ClNO_3$ (349.94): C, 61.78; H, 10.37; N, 4.00 %. Found: C, 61.77; H, 10.68; N, 3.94 %. MS: *m/z* (%) 314 (100) $[M]^+$, 315 (20) $[M+1]^+$, 188 (10); FTIR: νOH, 3252 cm^{-1}; νC=O, 1752 cm^{-1}; ^{1}H and ^{13}C NMR (ppm, in D_2O) see Tables 1 and 2.

1-Carbundecyloxymethyl-4-hydroxy-1-methylpiperidinium chloride (5). According to the general procedure B from 6.9 g (0.06 M) 4-hydroxy-1-methylpiperidine and 14.7 g (0.06 M) undecyl chloroacetate to obtain **5** (19.2 g, 60 %); m.p. 160-162 ^{o}C from acetonitrile; white powder. Anal. calcd. for $C_{19}H_{38}ClNO_3$ (363.97): C, 62.70; H, 10.52; N, 3.85 %. Found: C, 62.59; H, 10.70; N, 3.85 %. MS: *m/z* (%) 328 (100) $[M]^+$, 329 (21) $[M+1]^+$, 188 (15); FTIR: νOH, 3260 cm^{-1}; νC=O, 1752 cm^{-1}; ^{1}H and ^{13}C NMR (ppm, in D_2O) see Tables 1 and 2.

1-Carbdodecyloxymethyl-4-hydroxy-1-methylpiperidinium chloride (6). According to the general procedure B from 8.2 g (0.07 M) 4-hydroxy-1-methylpiperidine and 18.7 g (0.07 M) dodecyl chloroacetate to obtain **6** (22.65 g, 84 %); m.p. 162-163 ^{o}C from acetonitrile; colorless prisms. Anal. calcd. for $C_{20}H_{40}ClNO_3$ (378.00): C, 63.55; H, 10.67; N, 3.71 %. Found: C, 63.47; H, 10.89; N, 3.67 %. MS: *m/z* (%) 342 (100) $[M]^+$, 343 (22) $[M+1]^+$, 188 (19); FTIR: νOH, 3250 cm^{-1}; νC=O, 1752 cm^{-1}; ^{1}H and ^{13}C NMR (ppm, in D_2O) see Tables 1 and 2.

1-Carbpentadecyloxymethyl-4-hydroxy-1-methylpiperidinium chloride (7). According to the general procedure B from 1.7 g (0.015 M) 4-hydroxy-1-methylpiperidine and 4.5 g (0.015 M) pentadecyl chloroacetate to obtain **7** (5.2 g, 85 %); m.p. 161-162 ^{o}C from acetonitrile; white powder. Anal. calcd. for $C_{23}H_{46}ClNO_3$ (420.08): C, 65.76; H, 11.04; N, 3.33 %. Found: C, 65.77; H, 11.11; N, 3.31 %. MS: *m/z* (%) 384 (100) $[M]^+$, 385 (25) $[M+1]^+$, 188 (40); FTIR: νOH, 3257 cm^{-1}; νC=O, 1753 cm^{-1}; ^{1}H and ^{13}C NMR (ppm, in $CDCl_3$) see Tables 1 and 2. **7** is not soluble enough in D_2O to take ^{1}H and ^{13}C NMR spectra.

1-Carbohexadecyloxymethyl-4-hydroxy-1-methylpiperidinium chloride (8). According to the general procedure B from 3.5 g (0.03 M) 4-hydroxy-1-methylpiperidine and 10 g (0.03 M) hexadecyl chloroacetate to obtain **8** (11.7 g, 89 %); m.p. 162-163 ^{o}C from acetonitrile; white

powder. Anal. calcd. for $C_{24}H_{48}ClNO_3$ (433.10): C, 66.41; H, 11.15; N, 3.23 %. Found: C, 66.51; H, 11.35; N, 3.18 %. MS: *m/z* (%) 398 (100) $[M]^+$, 399 (27) $[M+1]^+$, 188 (85); FTIR: νOH, 3239 cm^{-1}; νC=O, 1752 cm^{-1}; ^1H NMR (ppm, in D_2O and $CDCl_3$; see Table 1) and ^{13}C NMR (ppm, in $CDCl_3$; see Table 2) **8** is not soluble enough in D_2O to take a ^{13}C NMR spectrum.

Microbiological experiment. Minimum inhibitory concentration (MIC) was determined by diffusion method with inoculum to give 10^6 microorganisms per mL. The microorganism suspension (0.2 mL) was spread evenly on an agar plate. Solutions of microbiocides were placed into small wells prepared in the agar plate. After 1 hr the agar plates were incubated for 24 hr at $37^oC \pm 1^oC$ (bacteria) and $30^oC \pm 1^oC$ (fungi) and a zone of inhibition on the agar was observed.

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