Synthesis and Antibiotic Activity of 1-Cycloalkoxymethyl-4-dimethylaminopyridinium and 1-[(1-Alkoxy)ethyl]-4-dimethylaminopyridinium Chlorides

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DMAP reacts readily with chloromethylcycloalkyl ethers and α-chloroethyl alkyl ethers to give stable (**1a–1e**) and unstable (**2a–2h**) pyridinium chlorides. Reaction of these chlorides with NaOH produces the corresponding 4-pyridones. All the chlorides synthesized showed antibiotic activity. Particularly high activity against microbes representing cocci, rods, fungi, and bacilli was shown by 1-cyclododecyloxymethyl-4-dimethylaminopyridinium chloride **1d** and 1-[(1-dodecyloxy)ethyl]-4-dimethylaminopyridinium chloride **2f**.

Synthese und Antibiotische Aktivität von 1-Cykloalkoxymethyl-4-dimethylaminopyridinium- und 1-[(1-Alkoxy)ethyl]-4-dimethylaminopyridiniumchloriden

DMAP reagiert leicht mit Chlormethylcyklo- und α-Chloroethylalkyl-ether unter Bildung stabiler (**1a–1e**) bzw. instabiler (**2a–2h**) Pyridiniumchloride. Die Umsetzung dieser Chloride mit NaOH ergibt die entsprechenden 4-Pyrolidone. Alle synthetisierten Chloride weisen antimikrobielle Wirkung auf. 1-Cyklododecyloxymethyl-4-dimethylamin- **1d** und 1-[(1-Dodecyloxy)ethyl]-4-dimethylaminopyridiniumchloride **2f** zeigen gegenüber Kokken, Stäbchen, Pilzen und Bazillen die größte Aktivität.

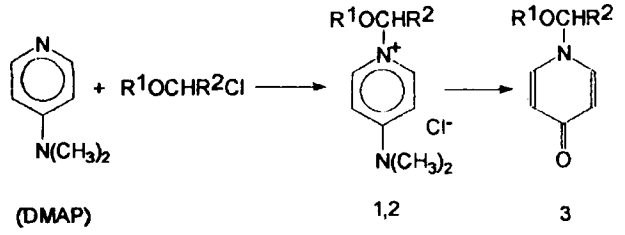
The most common method of preparing pyridinium salts continues to be the *Menschutkin* reaction–electrophilic attack by an alkyl or aryl halide on the pyridine nitrogen. 4-Dimethylaminopyridine (DMAP), a so-called 'super nucleophile' is very effective at catalyzing acylation [1]. DMAP reacts vigorously with a variety of electrophiles at room temperature to give quite stable products.

We have previously demonstrated the synthesis and antibiotic activity of 1-alkoxymethyl- and 1-alkylthiomethyl-4-dimethylaminopyridinium chlorides. We found these pyridinium compounds to have very high biological activity (4-dimethylamino-1-dodecyloxymethylpyridinium chloride: MICs–mean value of rods 1.6, of cocci. 0.007, of fungi 0.24, and of bacilli 0.003 mg/L) [2].

In this paper we extend our investigation to new 1-substituted products of DMAP.

Results and Discussion

Reaction of DMAP with chloromethylcycloalkyl ethers, α-chloroethyl alkyl ethers, and α-chloro-3-nitrobenzyl butyl ether produces the corresponding 1-cycloalkoxymethyl-4-dimethylaminopyridinium, (**1a-1e**), 1-[(1-alkoxy)ethyl]-4-dimethylaminopyridinium, (**2a-2g**), and 1-(α-butyloxy-3-nitrobenzylidene)-4-dimethylaminopyridinium (**2h**) chlorides (Table 1 and Scheme) in very good yields. All the pyridinium chlorides prepared are hygroscopic.



(DMAP) 1,2 3

Scheme

Table 1. Preparation of pyridinium chlorides **1** and **2** and 4-pyridones **3**.

Compound	R^1	R^2	Yield [%]	mp [°C]
1a	cycloC_5H_9	H	81	187–90
1b	cycloC_6H_{11}	H	93	138–41
1c	cycloC_7H_{13}	H	89	solid [a]
1d	cyclo$C_{12}H_{23}$	H	90	176–8
1e	cyclo4-$CH_3C_6H_9$	H	83	172–4
2a	C_2H_5	CH_3	72	oil [b]
2b	C_4H_9	CH_3	80	solid [b]
2c	C_6H_{13}	CH_3	89	solid [b]
2d	C_8H_{17}	CH_3	85	solid [b]
2e	$C_{10}H_{21}$	CH_3	80	solid [b]
2f	$C_{12}H_{25}$	CH_3	85	solid [b]
2g	$C_{14}H_{29}$	CH_3	80	solid [b]
2h	C_4H_9	3-$NO_2C_6H_4$	79	solid [b]
3a	$C_{14}H_{29}$	CH_3	65	oil
3b	cyclo$C_{12}H_{23}$	H	85	73–75

[a] highly hygroscopic; [b] highly hygroscopic with hydrolytic cleavage

1-Cycloalkoxymethyl-4-dimethylaminopyridinium chlorides are quite stable at room temperature. If 1-cyclododecyloxymethyl-4-dimethylaminopyridinium chloride was heated at its melting point for 10 min., however, the dicyclododecyl acetal of formaldehyde **4** could be isolated. 1-[(1-Alkoxy)ethyl]-4-dimethylaminopyridinium chlorides are particularly highly hygroscopic, and are labile when they come into contact with water.

The new quaternary chlorides were characterized by their ^1H and ^{13}C NMR spectra (Tables 2 and 3). The ^{13}C spectra show the absorption peak for the carbon atom directly attached to the nitrogen atom at 83.1– 92.7 ppm. Examination of the ^1H NMR spectra shows that the NCH_2O protons resonated at δ = 5.72–5.61 ppm as a singlet and the NCHO proton appears in the spectrum as a multiplet at 6.10–6.02 ppm. Both ^1H and ^{13}C NMR chemical shifts are in accordance with literature data for this class of compounds [6,7].

The prepared pyridinium chlorides can be easily transformed into the corresponding 4-pyridones **3** by treatment

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Table 2. ^{1}H NMR spectral data (δ, J in Hz) of pyridinium chlorides **1** and **2** and 4-pyridones **3** [a]

Cpd	CH_2 or $CHCH_3$	$N(CH_3)_2$	R^1	Pyridine
1a	5.72 (s, 2H)	3.34 (s)	4.2 (m, 1H); 1.8 (m, 8H)	8.71 (d, J=7, 2H); 7.11 (d, J=7, 2H)
1b	5.61 (s, 2H)	3.22 (s)	3.48 (m, 1H); 1.76 (m, 10 H)	8.51 (d, J=7, 2H); 7.02 (d, J=7, 2H)
1c	5.69 (s, 2H)	3.34 (s)	3.78 (m, 1H); 1.90 (m, 12H)	8.62 (d, J=7.5, 2H); 7.11 (d, J=7.5, 2H)
1d	5.70 (s, 2H)	3.34 (s)	3.72 (m, 1H); 1.72 (m.22H)	8.65 (d, J=7.5, 2H); 7.11 (d, J=7.5, 2H)
2d	6.10 (m, 1H); 1.65 (d, J=6, 3H)	3.33 (s)	1.62 (m, 2H); 1.35 (m, 12H); 0.88 (t, J=6, 3H)	8.73 (d, J=7, 2H); 7.12 (d, J=7, 2H)
3a	5.05 (q, J=7, 1H); 1.58 (d, J=6, 3H)	–	1.35 (m, 26H); 0.90 (t, J=7, 3H)	7.50 (d, J=7, 2H); 6.42 (d, J=7, 2H)
3b	5.11 (s, 2H)	–	3.54 (m, 1H); 1.66 (m, 2H); 1.46 (m, 20 H)	7.50 (d, J=7, 2H); 6.39 (d, J=7, 2H)

[a] $CDCl_3$

Table 3. ^{13}C NMR spectral data of pyridinium chlorides **1** and **2** and 4-pyridones **3** [a]

Cpd	CH_2 or $CHCH_3$	$N(CH_3)_2$	R^1	Pyridine
1a	84.3	40.4	81.6; 32.3; 23.0	156.8; 141.4; 107.7
1b	83.1	40.1	77.3; 31.6; 24.8; 23.1	156.5; 140.7; 107.5
1c	83.3	40.1	79.5; 33.5; 27.8; 21.9	156.3; 140.6; 107.4
1d	83.7	40.4	78.1; 28.3; 24.7; 24.4; 22.7; 22.5; 20.0	156.8; 141.1; 107.7
2d	92.7; 14.0	40.5	70.0; 31.6; 29.15; 29.04; 25.8; 23.3; 22.5	156.9; 138.8; 108.0
3a	91.3; 14.0	–	69.1; 31.8; 29.53; 29.44; 29.4; 29.24; 29.17; 29.08; 25.9; 22.62; 22.58	179.5; 136.0; 118.3
3b	82.6	–	75.5; 28.8; 23.9; 23.0; 22.9; 20.5	179.0; 138.7; 118.1

[a] $CDCl_3$

Table 4. Minimum inhibitory concentrations of compounds **1** and **2** [a]

Strain [b] Compound	**1a**	**1b**	**1c**	**1d**	**1e**	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**
Rods													
Pseudomonas aeruginosa	500 [c]	500 [c]	500 [c]	187.5	500 [c]	500 [c]	500 [c]	187.5	93.7	23.4	46.9	187.5	375
Proteus vulgaris	500 [c]	500 [c]	375	187.5	187.5	500 [c]	375	187.5	46.9	5.9	11.7	46.9	187.5
Escherichia coli	187.5	187.5	93.7	2.9	93.7	500 [c]	187.5	23.4	11.7	0.75	2.9	23.4	46.9
Cocci													
Staphylococcus aureus	375	187.5	93.7	0.75	46.9	375	93.7	2.9	1.5	0.38	0.38	2.9	5.9
Staphylococcus epidermidis	375	187.5	93.7	0.75	46.9	187.5	46.9	1.5	0.75	0.19	0.38	1.5	5.9
Micrococcus luteus	375	187.5	46.9	0.75	46.9	187.5	46.9	2.9	1.5	0.75	0.75	2.9	5.9
Fungi													
Candida albicans	375	375	187.5	23.4	187.5	500 [c]	500 [c]	93.7	23.4	1.5	5.9	11.7	375
Bacilli													
Bacillus subtilis	500 [c]	500 [c]	375	11.7	187.5	500 [c]	500 [c]	187.5	46.9	5.9	5.9	46.9	187.5

[a] The incubation time was 24 h; MICs are expressed as mg/L. [b] The number of microorganisms/mL ranged from 2.5×10^3 to 4.9×10^4. [c] MICs > 500

with aqueous alkali solution at elevated temp. The structures of two new 4-pyridones **3a** and **3b** (Table 1) are supported by their spectral data (Tables 2 and 3).

The minimum inhibitory concentration of the examined pyridinium chlorides against various microorganisms for cocci, rods, fungi, and bacilli after 24h incubation are shown in Table 4. Chlorides **1a-1e** and **2a-2h** are all active against microorganisms. The antibiotic activity correlates well with the length of alkyl chains. The dodecyl derivatives are the best antimicrobial agents of all the chlorides tested.

Because it was noted that chlorides **2a-2h** are labile, two compounds, **2d** and **2c**, were tested against two microorganisms after storage of their aqueous solution. Results are shown in Table 5. The antimicrobial activity of chlorides **2** decline when the compounds are stored in aqueous solution.

Table 5. Minimum inhibitory concentrations of compounds **2d** and **2e**.

Strain	Time of storage of the aqueous solution [days]	Compound **2d**	**2e**
Proteus vulgaris	0	46.9	5.9
	1	187.5	11.7
	14	375	46.9
Escherichia coli	0	11.7	0.75
	1	23.4	1.5
	14	46.9	5.9

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Experimental Part

Melting points: hot-stage microscope, uncorrected.– NMR spectra: Varian Model XL 300 spectrometer, 300 MHz for ^1H, 75 MHz for ^{13}C, relative to TMS as standard.- Elemental analyses: A. Mickiewicz University, Poznan; satisfactory microanalyses were obtained for compounds **1a–1e**, **2a–2h**, and **3a** and **3b**: C ± 0.36, H ± 0.37, N ± 0.32.

Chloromethylcycloalkyl ethers, α-chloroethyl alkyl ethers and α-chloro-3-nitrobenzyl butyl ether were prepared by passing HCl gas into a mixture of the appropriate aldehyde and alcohol [3,4].

The percentage of ether in the crude product was determined by the alkalimetric method. 1 g of crude product was added to 10 mL of acetone at –40 °C. Free HCl was quickly neutralized with 1 % KOH in EtOH and 3 mL of water was added. The mixture was stirred at 30 °C for 15 min. HCl, the hydrolysis product from the estimation of the ether, was neutralized with 2 % KOH in EtOH. The crude product contained 73– 89 % chloromethylcycloalkyl ether and α-chloroethyl alkyl ether and 41 % α-chloro-3-nitrobenzyl butyl ether. α-Chloroethyl alkyl ethers are stable for a few days at –5 °C.

General procedure for pyridinium chlorides

The pyridinium chlorides **1a–1c** and **2a–2h** were prepared by dissolving DMAP in dichloromethane and adding equimolar amounts of the appropriate chloromethylcycloalkyl or α-chloroethyl alkyl ethers. The mixture was stirred and heated under reflux for 5h. The solvent was removed under reduced pressure and the residue extracted with hexane. The pyridinium chlorides were obtained as hygroscopic crystalline solids or oils which were dried *in vacuo* at 60 °C. All compounds were stored over phosphoric anhydride.

Preparation of 4-pyridone **3a,3b**

Aqueous NaOH (4 %, 10 mL) was added to the pyridinium chloride **1d** or **2g** (5 mmol), which was then heated in a water bath. After 1.5h the solution was cooled and neutralized with 2 % HCl to pH 6.5. The product was extracted with chloroform, dried over Na_2SO_4 and the solvent was evaporated. An analytical sample was obtained by recrystallization from acetone.

Dicyclododecyl acetal of formaldehyde **4**

Mp 62–64 °C (acetone).– ^1H NMR ($CDCl_3$) δ = 1.51–1.22 (m, 4 OH); 1.70–1.57 (m, 4H); 3.82–3.74 (m, 2H) 4.71 (s, 2H).– ^{13}C NMR ($CDCl_3$) δ = 20.8, 23.12, 23.20, 24.4, 24.8, 29.1, 73.6, 90.4.

Bactericidal properties

Minimum inhibitory concentrations (MICs) for the test compounds were determined by serial dilution [5] against the organisms: *Pseudomonas aeruginosa* NCTC 6749, *Proteus vulgaris* NCTC 4635, *Escherichia coli* NCTC 8196, *Serratia marcescens* PZH 388, *Staphylococcus aureus* NCTC 4163, *Staphylococcus epidermidis* ATCC 4698, and *Candida albicans* NCTC 1025. The studied microorganisms were all obtained from the State Laboratory of Hygiene (Warsaw, Poland).

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