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Laboratory note

Antimicrobial activities of new analogues of benzalkonium chloride

J. Pernak[a]*, I. Mirska[b], R. Kmiecik[a]

[a]*Poznan University of Technology, Sklodowskiej-Curie 2, 60-965 Poznan, Poland*
[b]*K. Marcinkowski University of Medical Sciences, Sieroca 10, 61-771 Poznan, Poland*

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Abstract – (Alkoxymethyl)dimethyl{2-hydroxy-5-[(4-X-phenyl)azo]benzyl}ammonium chlorides were prepared in high yield. All these chlorides, new analogues of benzalkonium chloride, showed antimicrobial activity. Activity depends on the length and kind of substituent at the quaternary nitrogen atom. © 1999 Éditions scientifiques et médicales Elsevier SAS

analogue of benzalkonium chloride / 4-hydroxyazobenzenes / Mannich bases / chloromethylalkyl ether / antimicrobial activity

1. Introduction

Benzalkonium chloride (BAC) is the product of a nucleophilic substitution reaction of alkyldimethylamine with benzyl chloride [1]. Chemically, it is monoalkyldimethylammonium chloride with one long-chain alkyl group representing a mixture of the alkyls from C_8H_{17} to $C_{18}H_{37}$. Following Domagk's publication in 1935 [2], a large number of application areas were developed for BAC. It is used as a pharmaceutical aid (preservative), cationic surface active agent, germicide, antiseptic (topical), antiseptic for skin preoperatively or for wounds, burns, etc. BAC is often present as a preservative or stabilising agent in nebulizer solutions used to treat asthma and chronic obstructive pulmonary disease [3]. Also it is widely used as an antimicrobial agent in the treatment of common infections of the mouth and throat.

We now report the synthesis and antimicrobial activities of new quaternary ammonium compounds, the analogues of benzalkonium chloride. We plan to find compounds with antimicrobial activity which are diluted in water giving a coloured solution. Commercial products with these compounds will not have to contain any dye.

*Correspondence and reprints

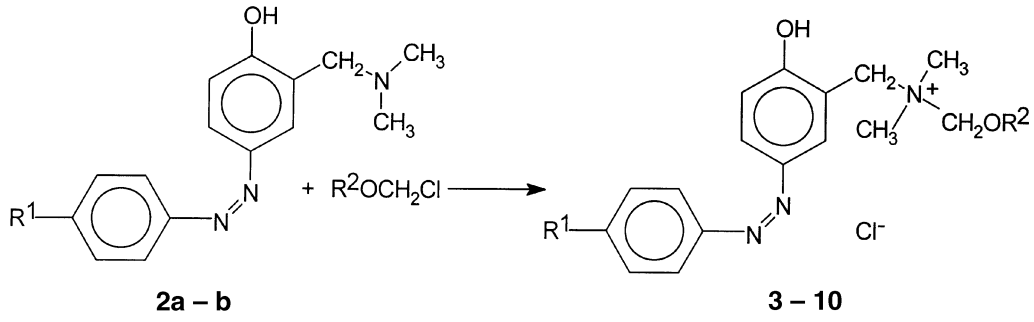
2. Chemistry

Mannich reaction (or aminomethylation) of variously substituted phenols is a well known process and comprehensive reviews have been published [4–7]. A few Mannich bases of phenolic azobenzenes have demonstrated cytotoxicity towards murine and human cancers [8]. Mannich bases methiodides have a promising cytotoxic activity in a wide variety of tumours [9].

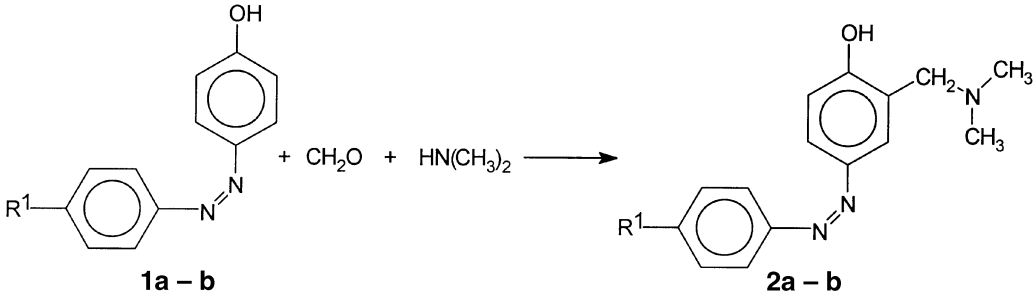
The new analogues of benzalkonium chloride (**3–10**) were prepared by the reaction of 2-[(dimethylamino)-methyl]-4-[(4-chlorophenyl)azo]phenol (**2a**) or 2-[(dimethylamino)methyl]-4-[(4-methylphenyl)azo]phenol (**2b**) with chloromethylalkyl or chloromethylcycloalkyl ethers giving yields between 80–98%. In this case, Mannich base is a compound which is easily transformed into a quaternary ammonium salt.

Chloromethylalkyl and chloromethylcycloalkyl ethers were synthesised from the corresponding alcohols. Mannich bases (**2a**–**b**) were prepared by treatment of the 4-hydroxyazobenzenes with equimolar quantities of formaldehyde and dimethylamine in 75% yield.

The 4-hydroxyazobenzenes (azo dyes) were prepared by several authors in the 19th century [10]. At the present time, only one compound, 4-hydroxyazobenzene (Solvent Yellow 7), is commercially available.



2a – b **3 – 10**



1a – b **2a – b**

3. Antimicrobial activity

All synthesised quaternary ammonium chlorides (**3–10**) were tested for antimicrobial activity against cocci, rods and fungi.

4. Results and discussion

4-Hydroxyazobenzenes react readily with formaldehyde and secondary amines to give Mannich bases. The structures of two prepared mono Mannich bases (**2a–b**) were characterized by their microanalysis CHN and by their ^{1}H and ^{13}C NMR spectroscopy.

Quaternization of **2a** and **2b** with chloromethylalkyl or chloromethylcycloalkyl ethers produced red crystalline quaternary ammonium chlorides **3–10** *(table I)* diluted in water. The water solution of these chlorides is stable and orange in colour. ^{1}H and ^{13}C NMR-spectral analysis of prepared chlorides allowed easy elucidation of their structure.

The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) values determined for all forty chlorides are given in *tables II* and *III*. The chlorides studied were divided into four groups with respect to the kind of substituent: group 1, chlorides with an alkoxymethyl substituent with an even number of carbon atoms (**3** and **7**); group 2, chlorides with the same substituent but with an odd number of carbon atoms (**4** and **8**); group 3, chlorides with a cycloalkoxymethyl substituent (**5** and **8**); and group 4, chlorides with $CH_2O(CH_2)_nC_6H_{11}$ substituent (**6** and **10**). The calculated average MIC values for cocci, rods and fungi are shown in *figures 1* and *2*. As shown by the results in these tables and figures, all the chlorides studied are very active against cocci and active against rods and fungi. The microbial activity depends on the length and kind of substituent at the quaternary nitrogen atom. *Figures 1* and *2* reveal a decrease in the MIC value to the optimum value and these values increase for chlorides from groups 1, 2 and 3. The same correlation is observed for the MBC values. Generally the MBC values are slightly higher than MIC values. To the most active compounds in group 1 belong chlorides which have butoxymethyl, hexyloxymethyl and octyloxymethyl substituent, in group 2 chloride with heptyloxymethyl chain, and in group 3 chlorides which have cyclopentyloxymethyl, cyclohexyloxymethyl and cycloheptyloxymethyl substituent. To the worst compounds belong chlorides with dodecyloxymethyl and cyclododecyloxymethyl chain. Chlorides from group 4 have comparable values of MIC. In this group the microbial activity does not depend on the substituent – $CH_2O(CH_2)_nC_6H_{11}$ where n = 1 or 2.

The most active chlorides against microorganisms were: {5-[(4-chlorophenyl)azo]-2-hydroxybenzyl}dimethyl-(cyclohexyloxymethyl)ammonium (**5b**), {5-[(4-chlorophenyl)azo]-2-hydroxybenzyl}dimethyl(cyclohexylmethyloxymethyl)ammonium (**6a**), {2-hydroxy-5-[(4-methylphenyl)azo]benzyl}dimethyl(hexyloxymethyl)-

Table I. (Alkoxymethyl)dimethyl{5-[(4-chlorophenyl)azo]-2-hydroxybenzyl}ammonium chlorides (**3–6**) and (alkoxymethyl)dimethyl{2-hydroxy-5-[(4-methylphenyl)azo]benzyl}ammonium (**7–10**) chlorides.

Chloride	R^1	R^2	Yield (%)	Chloride	R^1	R^2	Yield (%)
3a	Cl	C_2H_5	80	**7a**	CH_3	C_2H_5	80
3b	Cl	C_4H_9 [a]	96	**7b**	CH_3	C_4H_9 [a]	86
3c	Cl	C_6H_{13} [a]	98	**7c**	CH_3	C_6H_{13} [a]	90
3d	Cl	C_8H_{17} [a]	90	**7d**	CH_3	C_8H_{17} [a]	80
3e	Cl	$C_{10}H_{21}$ [a]	85	**7e**	CH_3	$C_{10}H_{21}$ [a]	80
3f	Cl	$C_{12}H_{25}$ [a]	86	**7f**	CH_3	$C_{12}H_{25}$ [a]	96
4a	Cl	C_3H_7 [a]	90	**8a**	CH_3	C_3H_7 [a]	80
4b	Cl	C_5H_{11} [a]	80	**8b**	CH_3	C_5H_{11} [a]	87
4c	Cl	C_7H_{15} [a]	80	**8c**	CH_3	C_7H_{15} [a]	80
4d	Cl	C_9H_{19} [a]	90	**8d**	CH_3	C_9H_{19} [a]	80
4e	Cl	$C_{11}H_{23}$ [a]	96	**8e**	CH_3	$C_{11}H_{23}$ [a]	90
5a	Cl	C_5H_9 [b]	80	**9a**	CH_3	C_5H_9 [b]	88
5b	Cl	C_6H_{11} [b]	80	**9b**	CH_3	C_6H_{11} [b]	84
5c	Cl	C_7H_{13} [b]	90	**9c**	CH_3	C_7H_{13} [b]	92
5d	Cl	C_8H_{15} [b]	80	**9d**	CH_3	C_8H_{15} [b]	80
5e	Cl	$C_{12}H_{23}$ [b]	80	**9e**	CH_3	$C_{12}H_{23}$ [b]	86
5f	Cl	$C_6H_{10}CH_3$ [b]	80	**9f**	CH_3	$C_6H_{10}CH_3$ [b]	84
6a	Cl	$CH_2C_6H_{11}$ [b]	90	**10a**	CH_3	$CH_2C_6H_{11}$ [b]	80
6b	Cl	$CH_2CH_2C_6H_{11}$ [b]	86	**10b**	CH_3	$CH_2CH_2C_6H_{11}$ [b]	80
6c	Cl	$CH_2CH_2CH_2C_6H_{11}$ [b]	85	**10c**	CH_3	$CH_2CH_2CH_2C_6H_{11}$ [b]	80

[a] linear alkyl, [b] alicyclic.

ammonium (**7c**), {2-hydroxy-5-[(4-methylphenyl)azo]benzyl}dimethyl(cyclopentyloxymethyl)ammonium (**9a**).

The results presented demonstrate that the new BAC analogues are very active against cocci. Their activities are similar to the activity of BAC. The antimicrobial activities of BAC (Aldrich product, in which R represents a mixture of alkyls from C_8H_{17} to $C_{18}H_{37}$) against cocci, *Micrococcus luteus*, *Staphylococcus epidermidis* and *Staphylococcus aureus* as measured in the same MIC test are 1.5, 3.0 and 1.5 mmol/L, respectively. Tomlinson and coworkers [11] reported the antibacterial activities of homologues series (C_8–C_{18}) of alkylbenzyldimethylammonium chlorides against *Pseudomonas aeruginosa*. BAC resistance is a potential problem for application, for example in the food processing industry [12]. We found compounds with large molecular weights, crystalline, diluted in water and the orange water solution is stable.

5. Experimental protocols

5.1. Chemistry

NMR spectra were recorded on a Varian Model XL 300 spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C at 20 °C with tetramethylsilane as internal reference. Satisfactory elemental analyses were obtained: C ± 0.32, H ± 0.29 and N ± 0.24.

Chloromethylalkyl ethers and chloromethylcycloalkyl ethers were prepared via the procedures which were reported earlier [13]. The percentage of ether in a crude product was determined by an alkalimetric method [14].

2-[(Dimethylamino)methyl]-4-(phenylazo)phenols (**2a–b**); general procedure:

Pathway A. To a solution of dimethylamine (30 mmol) in 95% EtOH (10 mL) paraformaldehyde powder (0.9 g, 30 mmol) was added. The mixture was stirred and heated when the paraformaldehyde had dissolved, then the corresponding 4-hydroxyazobenzene (30 mmol) in 100 mL EtOH was added. The reaction mixture was stirred for 1 h at 60 °C.

Pathway B. To 2-[(dimethylamino)methyl]phenol (20 mmol) in 50 mL MeOH was added diazonium salt prepared from dimethylamine (20 mmol). The mixture was stirred at room temperature for 2 h. The solid substrate was filtered and then recrystallized from EtOH.

2-[(Dimethylamino)methyl]-4-[(4-chlorophenyl)azo]-phenol (**2a**): m.p. 120–122 °C, ^{1}H NMR ($CDCl_3$) δ ppm = 11.6 (s, OH), 7.83 (dd, *J* = 6 Hz, 1H), 7.82 (d, *J* = 9 Hz, 2H), 7.61 (d, *J* = 2 Hz, 1H), 7.45 (d, *J* = 8 Hz, 2H), 6.95 (d, *J* = 8 Hz, 1H), 3.72 (s, 2H), 2.36 (s, 6H); ^{13}C NMR δ ppm = 161.8, 151.0, 145.5, 135.7, 129.1, 125.2, 123.6, 122.6, 122.1, 116.6, 62.6, 44.4.

2-[(Dimethylamino)methyl]-4-[(4-methylphenyl)azo]-phenol (**2b**): m.p. 99–100 °C, lit. 103 °C [10].

Table II. The MIC[a] and MBC[a] values of examined chlorides **(3–6)**.

Strains[b]		Chlorides																			
		3a	**3b**	**3c**	**3d**	**3e**	**3f**	**4a**	**4b**	**4c**	**4d**	**4e**	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**6a**	**6b**	**6c**
Cocci																					
M. luteus	MIC	21	10	18	17	32	59	78	37	9	33	61	19	36	18	34	479	9	9	8	16
	MBC	21	10	18	34	32	118	78	37	18	518	61	19	71	18	34	479	9	9	8	16
S. epidermidis	MIC	21	10	9	8	8	30	156	73	9	16	31	19	18	9	8	8	9	18	8	16
	MBC	21	18	18	17	16	30	156	146	18	128	31	19	18	9	17	118	9	18	8	64
S. aureus	MIC	260	38	217	267	252	238	156	146	9	128	245	9	9	9	214	8	18	18	8	16
	MBC	260	76	217	534	252	477	1256	219	36	518	490	19	18	9	214	8	36	36	8	129
Rods																					
P. aeuruginosa	MIC	260	304	284	267	504	1908	2512	1750	275	518	980	73	71	553	429	1915	137	137	133	520
	MBC	520	304	568	534	504	1908	2515	4695	550	2074	980	146	142	553	429	1915	256	553	268	520
P. vulgaris	MIC	81	76	18	132	252	477	628	219	9	259	245	19	18	9	268	16	9	18	8	16
	MBC	161	152	36	267	504	954	2512	438	36	518	490	73	71	137	536	1915	68	137	66	129
K. pneumoniae	MIC	260	304	141	132	504	954	1256	875	9	64	490	38	36	36	133	239	36	36	66	64
	MBC	520	304	284	267	504	954	2512	1750	36	1037	980	146	71	36	133	958	68	36	133	129
E. coli	MIC	81	38	141	132	125	477	628	438	136	259	490	38	71	18	66	958	36	18	34	32
	MBC	326	76	141	267	252	954	628	875	275	1037	980	73	142	18	133	1915	36	36	66	64
S. marescens	MIC	1302	608	568	534	1008	3816	5025	1750	275	1037	1960	73	142	256	1073	3831	1106	68	133	1041
	MBC	1302	1216	568	534	4032	3816	5025	4695	550	2074	1960	146	285	553	2146	3831	256	137	268	1041
Fungi																					
C. albicans	MIC	325	304	70	267	504	3816	1256	875	136	259	490	146	71	68	268	3831	256	68	34	129
	MBC	651	608	141	534	1008	3816	2512	1750	1101	4149	980	146	142	256	536	3831	1106	137	66	260
T. mentagrophytes	MIC	161	76	18	66	125	477	1256	438	36	64	122	73	36	36	268	1915	137	68	34	64
	MBC	161	152	18	132	252	477	1256	875	36	2074	245	73	36	36	286	1915	256	68	34	129
Rh. rubra	MIC	161	152	36	132	252	3816	628	219	36	259	490	38	36	36	268	1915	256	36	17	64
	MBC	161	304	70	267	504	3816	1256	219	68	2074	490	73	71	36	268	3831	256	68	34	64

[a] in mmol/L, [b] the number of microorganisms in mL ranged from 10^4–10^5.

The quaternary ammonium chlorides **3–10** were prepared by dissolving 2-[(dimethylamino)methyl]-4-(X-phenylazo)phenol in CH_2Cl_2 and adding an equimolar amount of the appropriate chloromethylalkyl or chloromethylcycloalkyl ether. The mixture was stirred at room temperature for 24 h. The solvent was evaporated and the crude product was extracted three times with hexane. Finally, the products were crystallized from $CH_3COOC_2H_5$/MeOH and dried in vacuum oven.

{5-[(4-chlorophenyl)azo]-2-hydroxybenzyl}dimethyl-(dodecyloxymethyl)ammonium chloride (**3f**): m.p. 124–126 °C, ^{1}H NMR (DMSO-d_6) δ ppm = 11.9 (s, OH), 8.06 (d, J = 2 Hz, 1H), 7.96 (dd, J = 7 Hz, 1H), 7.86 (d, J = 9 Hz, 2H), 7.66 (d, J = 9 Hz, 2H), 7.46 (d, J = 9 Hz, 1H), 4.82 (s, 2H, CH_2N), 4.58 (s, 2H, NCH_2O), 3.86 (t, J = 7 Hz, 2H), 3.02 (s, 6H), 2.00 (m, 2H), 1.63 (m, 18H), 0.86 (t, J = 7 Hz, 3H); ^{13}C NMR δ ppm = 161.4, 150.5, 144.6, 135.2, 130.5, 129.5, 125.9, 123.8, 117.1, 114.9, 90.0, 73.0 (NCH_2O), 57.9 (CH_2N), 46.3 [$N(CH_3)_2$], 33.7, 31.3, 29.2, 29.0, 28.8, 28.7, 25.3, 22.1, 13.9.

{5-[(4-chlorophenyl)azo]-2-hydroxybenzyl}dimethyl-(cyclohexyloxymethyl)ammonium chloride (**5b**): m.p. 120–123 °C, ^{1}H NMR (DMSO-d_6) δ ppm = 11.5 (s, OH), 8.07 (d, J = 2 Hz, 1H), 7.96 (dd, J = 7 Hz, 1H), 7.85 (d, J = 9 Hz, 2H), 7.58 (d, J = 9 Hz, 2H), 7.38 (d, J = 9 Hz, 1H), 4.86 (s, 2H, CH_2N), 4.60 (s, 2H, NCH_2O), 3.84 (m, 1H), 3.05 (s, 6H), 1.92 (m, 2H), 1.74 (m, 2H), 1.49 (m, 6H); ^{13}C NMR δ ppm = 159.3, 148.7, 143.0, 133.6, 128.3, 127.4, 124.4, 121.8, 115.2, 113.2, 82.5, 78.0 (NCH_2O), 56.1 (CH_2N), 44.4 [$N(CH_3)_2$], 33.4, 27.7, 21.9.

(Cycloheptyloxymethyl)dimethyl{2-hydroxy-5-[(4-methylphenyl)azo]benzyl}ammonium chloride (**9c**): m.p. 152–154 °C, ^{1}H NMR (DMSO-d_6/$CDCl_3$) δ ppm = 11.3 (OH), 8.03 (d, J = 2 Hz, 1H), 7.92 (dd, J = 6 Hz, 1H), 7.76 (d, J = 9 Hz, 2H), 7.34 (d, J = 9 Hz, 3H), 4.82 (s, 2H, CH_2N), 4.59 (s, 2H, NCH_2O), 4.02 (m, 1H), 3.05 (s, 6H), 2.42 (s, 3H), 1.99 (m, 2H), 1.81 (m, 4H), 1.58 (m, 4H), 1.54 (m, 2H); ^{13}C NMR δ ppm = 158.7, 148.4,

Table III. The MIC[a] and MBC[a] values of examined chlorides **(7–10)**.

Strains[b]	Chlorides	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	**8a**	**8b**	**8c**	**8d**	**8e**	**9a**	**9b**	**9c**	**9d**	**9e**	**9f**	**10a**	**10b**	**10c**	**BAC**
Cocci																						
M. luteus	MIC	22	20	9	9	65	61	42	20	18	9	63	20	10	9	9	249	9	9	9	17	1.5
	MBC	22	40	9	9	65	61	82	20	18	34	126	40	19	37	9	249	9	9	9	17	3
S. epidermidis	MIC	22	10	9	9	33	248	42	10	9	34	8	20	19	9	9	8	9	9	9	17	3
	MBC	22	79	9	18	33	248	42	10	9	67	8	40	38	18	18	16	18	9	9	17	3
S. aureus	MIC	11	20	19	9	16	16	42	10	9	134	126	77	19	9	9	249	9	37	9	17	1.5
	MBC	170	40	19	9	262	16	82	20	36	270	126	154	38	18	9	997	9	72	9	17	6
Rods																						
P. aeuruginosa	MIC	344	158	147	138	2103	3972	331	76	143	1083	2043	1239	148	143	139	1994	143	143	280	1088	96
	MBC	688	319	298	279	4206	3972	662	308	288	4333	4086	1239	299	289	1122	1994	289	143	561	1088	192
P. vulgaris	MIC	85	79	19	69	262	496	662	308	9	270	255	20	19	9	280	16	9	18	9	17	12
	MBC	688	319	298	69	262	993	662	154	143	1083	1021	619	299	18	280	249	289	143	139	561	12
K. pneumoniae	MIC	344	79	19	138	262	993	331	39	18	67	63	40	38	18	36	997	18	72	18	67	12
	MBC	344	158	38	138	529	1986	331	76	36	1083	255	154	74	71	139	997	37	72	139	134	24
E. coli	MIC	85	40	19	69	529	1986	82	20	36	270	255	77	19	18	36	498	9	37	36	134	3
	MBC	85	79	19	138	1051	1986	164	76	143	1083	510	154	38	37	70	997	18	37	69	134	6
S. marescens	MIC	688	319	147	279	4206	3972	1324	152	143	2166	4086	1239	148	289	1122	1994	289	143	561	2176	12
	MBC	1376	638	596	279	4206	3972	1324	308	143	2166	2043	2478	299	289	1122	3988	289	143	561	2176	24
Fungi																						
C. albicans	MIC	688	319	38	36	1051	1986	331	152	288	541	1021	309	148	37	36	1994	72	143	139	272	3
	MBC	1376	319	38	69	2103	1986	662	308	576	2166	1021	619	299	143	70	1994	143	289	280	272	3
T. mentagrophytes	MIC	170	40	38	9	262	248	164	39	288	1083	510	154	38	18	9	489	37	72	69	272	3
	MBC	170	79	74	18	529	248	331	152	288	1083	255	308	148	71	9	489	72	143	69	282	6
Rh. rubra	MIC	344	158	19	9	1051	1986	164	20	143	270	255	154	74	37	18	997	72	72	69	272	12
	MBC	344	158	38	36	1051	1986	331	39	143	1083	255	154	74	37	36	1994	72	72	131	272	12

[a] in mmol/L, [b] the number of microorganisms in mL ranged from 10^4–10^5.

143.3, 139.0, 127.9, 127.8, 124.4, 120.5, 115.2, 112.5, 86.6, 81.1, 56.4, 44.5, 31.9, 26.1, 20.3, 19.4 (CH_3).

5.2. Antimicrobial activity

Microorganisms used: eleven standard strains representative of cocci; *Micrococcus luteus* ATCC 9341, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 6538, rods; *Pseudomonas aeruginosa* ATCC 15442, *Proteus vulgaris* NCTC 4635, *Klebsiella pneumoniae* ATCC 4352, *Escherichia coli* NCTC 8196, *Serratia marcescens* ATCC 8100, yeast-like fungi; *Candida albicans* ATCC 10231, *Rhodotorula rubra* PhB, and dermatophytes *Trichophyton mentagrophytes* var. *gypseum* ATCC 9533.

Standard strains were supplied by National Collection of Type Cultures (NCTC), London and American Type Culture Collection (ATCC). *Rhodotorula rubra* (PHB) strain was taken from the Department of Pharmaceutical Microbiology, K. Marcinkowski University of Medical Sciences, Poznan.

Antimicrobial activity was determined by the tube dilution method. The method shows, the lowest concentration of a chloride inhibiting cell multiplication (MIC) or killing them (MBC). Two-fold dilutions of the chlorides were prepared in the Mueller-Hinton broth medium (bacteria) or in the Sabouraud broth medium (fungi). A suspension of the standard microorganisms prepared from 24 h cultures of bacteria in the Mueller-Hinton broth medium and from 5 and 10 day cultures in the Sabouraud agar medium for fungi at a concentration of 10^5 cfu/mL were added to each dilution in a 1:1 ratio. Growth (or its lack) of the microorganisms was determined visually after incubation for 24 h at 37 °C (bacteria) or 5–10 days at 28–30 °C (fungi). The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC.

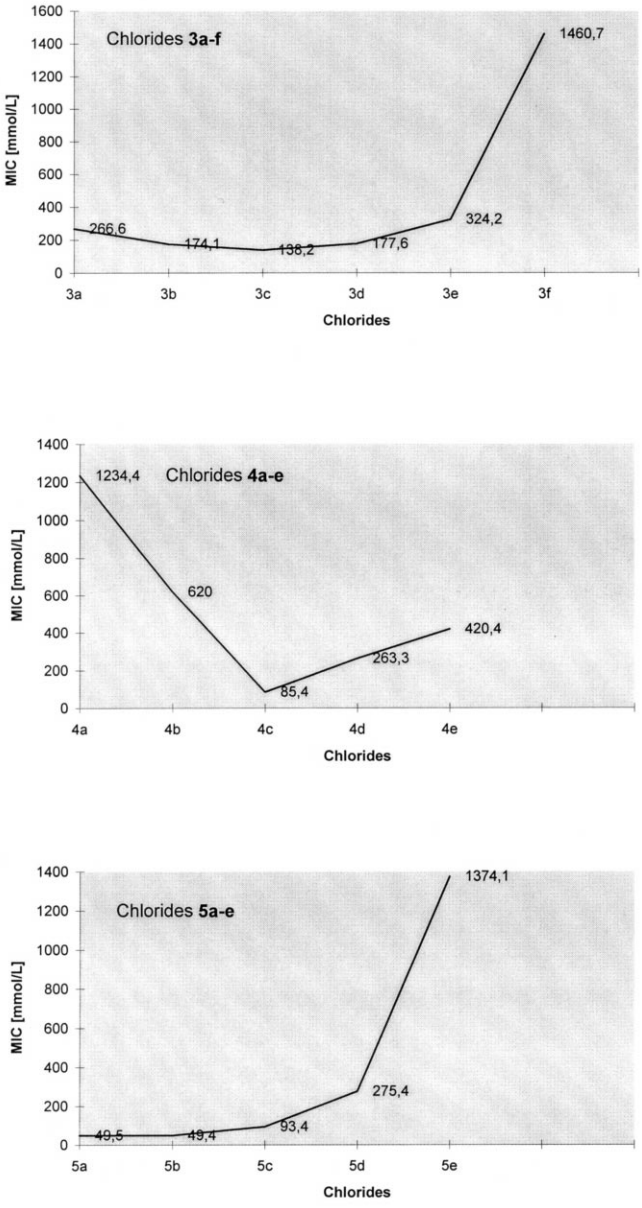


Figure 1. The MIC mean values for (alkoxymethyl)dimethyl-{5-[(4-chlorophenyl)azo]-2-hydroxybenzyl}ammonium chlorides (**3–5**).

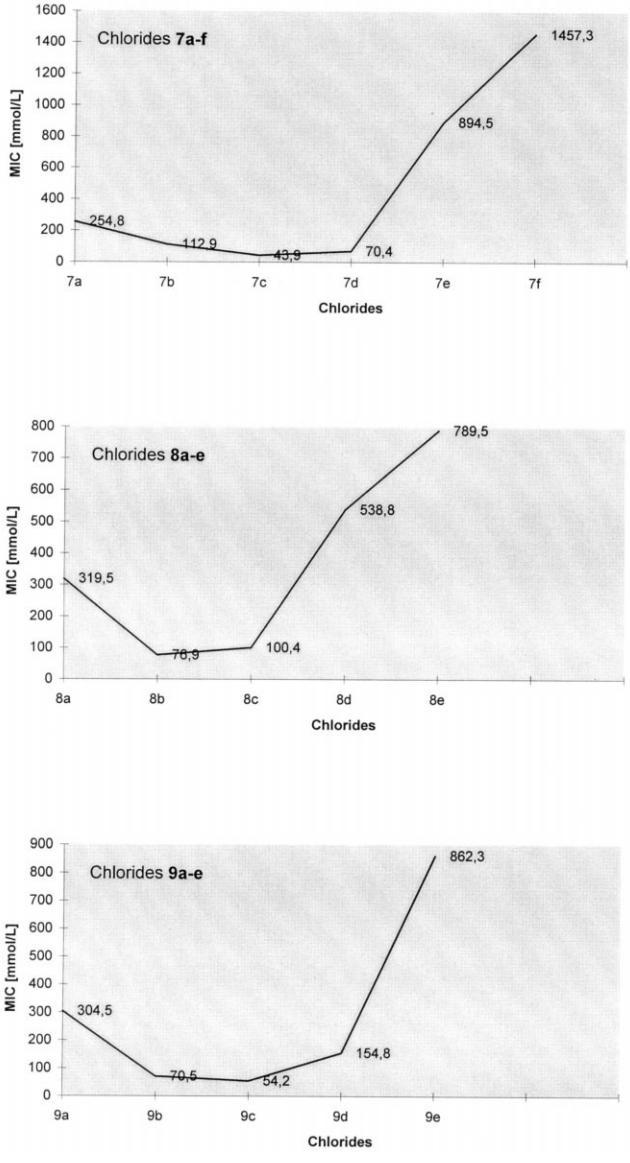


Figure 2. The MIC mean values for (alkoxymethyl)dimethyl-{2-hydroxy-5-[(4-methylphenyl)azo]benzyl}ammonium chlorides (**7–9**).

Then from each tube, one loopful was cultured on an agar medium with inactivates [14] (0.3% lecithin, 3% polysorbate 80 and 0.1% cysteine L) and incubated for 48 h at 37 °C (bacteria) or for 5–10 days at 28–30 °C (fungi). The lowest concentration of the chloride supporting no colony formation was defined as the MBC.

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References

[1] Dunn C.C., Proc. Soc. Exp. Biol. Med. 35 (1936) 427–429.

[2] Domagk G., Dtsch. Med. Wochenschr. 61 (1935) 829–832.

[3] Beasley R., Fishwick D., Miles J.F., Hendeles L., Pharmacotherapy 18 (1998) 130–139.

[4] Tramontini M., Synthesis (1973) 703–775.

[5] Tramontini M., Angiolini L., Tetrahedron 46 (1990) 1791–1837.

[6] Tramontini M., Angiolini L., Mannich-Bases, Chemistry and Uses, CRC, Boca Raton USA, 1994.

[7] Arend M., Westermann B., Risch N., Angew. Chem. Int. Ed. 37 (1998) 1045–1070.

[8] Dimmock J.R., Erciyas E., Kumar P. et al., Eur. J. Med. Chem. 32 (1997) 583–594.

[9] Dimmock J.R., Pandeya S.N., Allen T.M., Koa G.Y., Pharmazie 53 (1998) 201–202.

[10] a. Kimich C., Ber. 8 (1875) 1026–1032; b. Nolting E., Kohn O., Ber. 17 (1884) 351–369; c. Heumann K., Oeconomides L., Ber. 20 (1887) 904–909; d. Krause M., Ber. 32 (1899) 124–127; e. Farmer R., Hantzsch A., Ber. 32 (1899) 3089–3101.

[11] Tomlinson E., Brown M.R.W., Davis S.S., J. Med. Chem. 20 (1977) 1277–1282.

[12] Heir E., Sundheim G., Holck A.L., J. Appl. Bacteriol. 79 (1995) 149–156.

[13] Bedford C.D., Harris R.N., Howd R.A. et al., J. Med. Chem. 32 (1989) 493–516.

[14] Skrzypczak A., Brycki B., Mirska I., Pernak J., Eur. J. Med. Chem. 32 (1997) 661–668.