QUANTITATIVE RELATIONSHIPS BETWEEN STRUCTURE, AGGREGATION PROPERTIES AND ANTIMICROBIAL ACTIVITY OF QUATERNARY AMMONIUM BOLAAMPHIPHILES

Magdalena PAVLIKOVA[a], Ivan LACKO[a], Ferdinand DEVINSKY[a,*] and Dusan MLYNARCIK[b]

[a] *Department of Chemical Theory of Drugs, Comenius University, 832 32 Bratislava, The Slovak Republic*

[b] *Department of Cellular and Molecular Biology, Comenius University, 832 32 Bratislava, The Slovak Republic*

Received September 12, 1994
Accepted December 29, 1994

QSAR analysis employing Kubinyi's bilinear model was applied to examine the relationship between the structure (characterized by the lengths of the terminal hydrocarbon chain, *m*, and of the hydrocarbon spacer chain, *y*), lipophilicity (characterized by the chromatographic parameter R_m and aggregation properties expressed through the critical micellar concentration c_K), and antimicrobial activity (characterized by the minimum inhibition concentration, MIC) of quaternary ammonium bolaamphiphiles. The log $c_K = f(m)$ dependence was found to be linear whereas the log (1/MIC) = $f(m)$, log (1/MIC) = $f(y)$, log (1/MIC) = f (log c_K) and log (1/MIC) = $f(R_m)$ dependences were nonlinear. The effect of the hydrophobic terminal chains (*m*) and of the hydrophobic spacer chain (*y*) on the aggregation properties and on the biological activity of the substances was studied.

Quantitative relationships between the structure and activity of organic monoammonium salts have been described systematically in the literature. Recently, attention has also been paid to bolaamphiphilic compounds[1–5], including bisammonium salts[6–8], which in their molecules possess two hydrophilic groups connected by a hydrophobic chain, called spacer[9–12]. Such compounds can serve as good biomimetic materials for biological membrane modelling. Due to their hydrophobic nature, their solutions organize into multimolecular aggregates – micelles, vesicles and double layers. Some of the compounds, such as *N*,*N*′-bis(trimethyl)-1,12-dodecanediammonium dichloride, can serve to model pores for the diffusion of salts through biological membranes; other compounds can be used as models of ionic channels[2–4,13]. The role of bisammonium salts as catalysts on interfaces has also been documented[14].

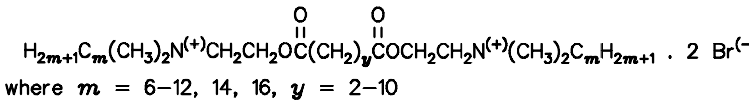
* The author to whom correspondence should be addressed.

Biological activity of bisammonium salts has been recognized and their uses as antimicrobially active substances are known[15]. New types of bolaamphiphilic substances and their potential as antivirotics have been described in ref.[16] The majority of the compounds described are so-called "hard" amphiphiles. This means that after exerting their biological effect, the substances persist in the medium, stressing it by their presence. Their structure involves no grouping that would facilitate their fast deterioration, and their biodegradation proceeds slowly[17–20].

Current efforts are aimed at designing bioactive substances that would meet the condition of being "soft" compounds as defined by Bodor[17,21]: such compounds must have a "weak spot" in their molecules, i.e. a readily degradable bond that, after the molecule has fulfilled its desired biological effect, is easily split, on the action of hydrolytic enzymes for instance. Ester or amide bonds usually serve this purpose. In nature, the substance decomposes in this manner into nontoxic products, which are less harmful to the environment. Among remarkable new properties of such substances – and of a whole group of amphiphiles – is their immunomodulating effect[22–26]. If the compounds involve rapidly degradable components, their effect on the components of the immune system is minimal.

As with monoammonium salts, the mechanism is assumed to be based on a perturbation effect on the bacterial membrane[27,28]. The amphiphilic nature and ability to form micelles are closely related to the hydrophilic–lipophilic balance (HLB) of the molecule, and the critical micellar concentration (c_K) can be employed as a lipophilicity parameter[29,30]. Lipophilicity is an important property affecting not only the pharmacological effect of bisammonium disinfectants but also their ability to penetrate into the microbial membrane where they exert their effect.

This work was aimed to correlate the structure of "soft" bisammonium salts derived from dicarboxylic acids, viz.



with their lipophilicity (see Table I), expressed through their aggregation properties and the R_m values, and with their antimicrobial effect.

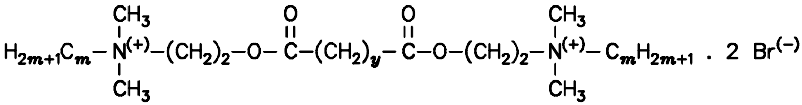
EXPERIMENTAL

Synthesis of Compounds

Seven series of bisammonium salts were synthesized from the corresponding α,ω-alkanedicarboxylic acids. The procedure and spectral characteristics of the compounds have been published previously[31].

TABLE I

Minimum inhibition concentrations (MIC), critical micellar concentrations (c_K) and R_m values of bis-ammonium salts



Compound	*m*	*y*	MIC . 10^6, mol dm^{-3}			–log c_K mol dm^{-3}	R_m
			S. aureus	*E. coli*	*C. albicans*		
I	6	2	4 237.2	21 169.6	8 467.8	–	0.09
II	8	2	483.3	773.3	483.3	1.5450	0.18
III	10	2	3.4	13.8	27.8	1.9480	0.27
IV	12	2	0.4	6.3	6.3	2.8117	0.45
V	14	2	1.5	383.5	30.3	3.5829	0.72
VI	16	2	11.1	5 740.4	179.2	4.0496	0.90
VII	6	3	1 650.0	24 810.0	9 930.0	0.3979	–0.10
VIII	7	3	988.0	7 900.0	988.0	1.1163	0.04
IX	8	3	118.0	946.0	236.0	1.4058	0.14
X	9	3	28.3	113.0	56.7	1.9149	0.23
XI	10	3	1.7	13.5	13.5	2.1372	0.39
XII	11	3	0.4	6.4	0.8	2.4712	0.54
XIII	12	3	0.4	3.1	1.6	3.2472	0.72
XIV	14	3	2.9	94.2	5.8	3.6991	0.99
XV	16	3	5.4	5 650.0	88.2	5.0808	1.14
XVI	6	4	4 041.7	8 083.4	8 083.4	–	0.12
XVII	8	4	57.9	463.2	926.4	1.3132	0.25
XVIII	10	4	1.3	6.6	3.3	2.1700	0.39
IXX	12	4	3.1	24.8	6.2	3.0639	0.52
XX	14	4	23.1	741.4	10.8	3.7547	0.72
XXI	16	4	86.9	2 780.6	86.9	5.0980	1.00
XXII	6	5	1 976.0	7 904.1	7 904.1	–	0.10
XXIII	8	5	28.3	907.5	907.5	1.3351	0.22
XXIV	10	5	1.3	6.4	2.5	2.2559	0.34
XXV	12	5	4.9	48.8	12.1	3.1830	0.46
XXVI	14	5	9.1	729.3	22.8	3.9614	0.58
XXVII	16	5	21.4	5 475.7	85.5	5.1656	0.70
XXVIII	6	6	241.6	7 732.6	1 933.1	0.8323	0.27
IXXX	8	6	13.8	55.6	55.6	1.5465	0.42
XXX	10	6	0.6	6.4	2.6	2.3186	0.51
XXXI	12	6	1.2	23.9	6.4	3.2759	0.62
XXXII	14	6	11.1	1 435.0	44.8	3.5645	0.73
XXXIII	16	6	21.0	5 392.8	337.1	4.2245	0.89

TABLE I
(*Continued*)

Compound	*m*	*Y*	MIC . 10^6, mol dm^{-3}			–log c_K, mol dm^{-3}	R_m
			S.aureus	*E.coli*	*C.albicans*		
XXXIV	6	7	473.0	3 784.2	946.1	1.4654	0.27
XXXV	8	7	13.5	54.5	13.5	1.7426	0.35
XXXVI	10	7	1.3	12.6	6.3	2.5789	0.48
XXXVII	12	7	1.9	47.1	47.1	3.3276	0.60
XXXVIII	14	7	5.4	2 824.6	88.2	4.0656	0.70
IXL	16	7	10.3	5 312.5	6 641.7	5.0151	0.78
XL	6	8	115.8	926.4	231.5	1.2533	0.28
XLI	8	8	13.3	13.3	13.3	1.8741	0.37
XLII	10	8	2.4	6.2	6.2	2.6322	0.49
XLIII	12	8	4.6	185.3	11.5	3.5393	0.60
XLIV	14	8	5.3	2 780.5	21.7	4.1696	0.71
XLV	16	8	10.1	5 234.5	81.8	5.2144	0.81
AJATIN[a]			78.1	625.0	78.1		
SEPTONEX[b]			39.1	78.1	2.4		

[a] Benzyldodecyldimethylammonium bromide; [b] 2-ethoxycarbonylpentadecyltrimethylammonium bromide.

Critical Micellar Concentration

The c_K parameter was determined by measuring changes in the electric conductance (κ) of aqueous solutions of the bisammonium salts at various concentrations[32]; the value was represented by the point of intersection of the two linear segments of the $\kappa = f(c)$ plot, obtained by linear regression. The temperature was 293.15 ± 0.1 K, conductance of redistilled water was 1.5 to 2.2 μS cm^{-1}. The critical micellar concentrations obtained as averages of triplicate measurements are given in Table I.

Determination of the R_m Value

The R_m values (Table I) were determined by thin layer chromatography on Silufol plates (Kavalier, Votice, The Czech Republic) using direct impregnation with a 5% solution of silicone oil in heptane[29]. The chromatograms were developed with a hydrochloric acid–methanol–acetone 1 : 3 : 1 mixture (c_{HCl} = 1 mol dm^{-3}), and Dragendorf's reagent was used for detection.

Determination of the Minimum Inhibition Concentration

The antimicrobial efficiency of the bisammonium salts was determined by using the strains *Staphylococcus aureus* (Mau 29/58), *Escherichia coli* (Ec 377/79), and *Candida albicans* (45/54) from the Czech national collection of cultures. The dilution test was applied using the micromethod[33,34]. The minimum inhibition concentration (MIC) was represented by the lowest concentration of the compound which inhibited the growth of the microorganisms. The MIC values are given in Table I.

TABLE II

Regression coefficients of bilinear dependences between antimicrobial activity, lipophilicity and structure of bisammonium salts derived from bis(2-dimethylaminoethyl) succinate, in the form[a] of log (1/MIC) = $ax + b \log (\beta\ 10^{x} + 1) + c$, where x is m (type I) or log c_K (type II) or R_m (type III)

Equation	Type	a	b	c	log β	n	r	s_D	F	Optimum m, c_K or R_m	MIC_{max}	α, %
					S. aureus							
1	I	0.772 ±0.067	–1.219 ±0.150	–2.460 ±0.614	–11.94	6	0.990	0.286	72.8	12.2	$3.84 . 10^{-7}$	99.5
2	II	1.526 ±0.139	–14.121 ±0.867	11.241 ±0.493	1.71	5	0.997	0.127	170.7	$2.34 . 10^{-3}$	$2.99 . 10^{-7}$	99.9
3	III	58.006 ±9.213	–77.687 ±12.967	20.584 ±2.879	–0.09	6	0.975	0.446	28.9	0.56	$2.91 . 10^{-7}$	97.5
					E. coli							
4	I	0.813 ±0.050	–1.567 ±0.094	–3.266 ±0.444	–11.15	6	0.994	0.185	141.6	11.2	$4.73 . 10^{-6}$	99.5
5	II	1.959 ±0.265	–15.609 ±2.452	10.881 ±0.981	1.55	5	0.982	0.329	27.3	$4.08 . 10^{-3}$	$5.12 . 10^{-6}$	95.0
6	III	61.531 ±10.732	–86.997 ±15.105	22.666 ±3.353	–0.09	6	0.958	0.520	16.6	0.47	$6.62 . 10^{-6}$	97.5
					C. albicans							
7	I	0.625 ±0.002	–1.018 ±0.004	–1.682 ±0.017	–11.50	6	0.999	0.007	58 118.2	11.7	$6.10 . 10^{-6}$	99.9
8	II	1.571 ±0.076	–6.625 ±0.277	–10.175 ±0.273	2.11	5	0.998	0.061	294.0	$2.42 . 10^{-3}$	$5.15 . 10^{-6}$	99.9
9	III	46.466 ±5.375	–63.332 ±7.564	21.125 ±2.451	–0.09	6	0.983	0.260	44.1	0.53	$5.09 . 10^{-6}$	99.0

[a] For symbols see List of symbols.

TABLE III

Regression coefficients of bilinear dependences between antimicrobial activity, lipophilicity and structure of bisammonium salts derived from bis(2-dimethylaminoethyl) glutarate, in the form[a] of log (1/MIC) = $ax + b \log (\beta\, 10^{x} + 1) + c$, where x is m (type I) or log c_K (type II) or R_m (type III)

Equation	Type	a	b	c	log β	n	r	s_D	F	Optimum m, c_K or R_m	MIC_{max}	α, %
					S. aureus							
1	I	0.966 ±0.040	–1.280 ±0.074	–3.905 ±0.352	–11.00	9	0.995	0.174	326.6	11.5	$3.90 \cdot 10^{-7}$	99.9
2	II	0.871 ±0.258	–3.219 ±0.460	9.515 ±0.989	2.74	9	0.972	0.429	51.2	$6.75 \cdot 10^{-4}$	$4.89 \cdot 10^{-7}$	99.9
3	III	16.631 ±0.844	–26.955 ±1.607	6.004 ±0.173	–0.49	9	0.995	0.179	307.4	0.69	$3.98 \cdot 10^{-7}$	99.9
					E. coli							
4	I	0.802 ±0.029	–1.682 ±0.064	–3.333 ±0.267	–11.69	9	0.996	0.144	390.9	11.6	$2.88 \cdot 10^{-6}$	99.9
5	II	1.940 ±0.296	–4.029 ±0.497	12.017 ±1.162	2.89	9	0.961	0.460	35.9	$1.18 \cdot 10^{-3}$	$6.49 \cdot 10^{-6}$	99.9
6	III	11.936 ±0.724	–49.735 ±0.320	3.642 ±0.126	–1.14	9	0.989	0.243	136.5	0.64	$4.03 \cdot 10^{-6}$	99.9
					C. albicans							
7	I	0.701 ±0.023	–1.233 ±0.052	–2.055 ±0.209	–11.89	9	0.997	0.116	474.8	12.0	$1.22 \cdot 10^{-6}$	99.9
8	II	1.207 ±0.154	–3.052 ±0.251	10.150 ±0.608	2.96	9	0.987	0.231	116.9	$7.13 \cdot 10^{-4}$	$2.06 \cdot 10^{-6}$	99.9
9	III	9.640 ±0.286	–35.546 ±1.266	3.795 ±0.049	–1.14	9	0.998	0.096	694.6	0.71	$1.58 \cdot 10^{-6}$	99.9

[a] For symbols see List of symbols.

TABLE IV

Regression coefficients of bilinear dependences between antimicrobial activity, lipophilicity and structure of bisammonium salts derived from bis(2-dimethylaminoethyl) adipate, in the form[a] of log (1/MIC) = $ax + b \log (\beta\, 10^{x} + 1) + c$, where x is m (type I) or log c_K (type II) or R_m (type III)

Equation	Type	a	b	c	log β	n	r	s_D	F	Optimum m, c_K or R_m	MIC_{max}	α, %
					S. aureus							
1	I	0.959 ±0.029	–1.336 ±0.045	–3.381 ±0.243	–10.04	6	0.999	0.085	533.5	10.4	$1.26 \cdot 10^{-6}$	99.9
2	II	0.801 ±0.067	–9.146 ±0.779	8.058 ±0.264	1.31	5	0.994	0.126	78.7	$4.70 \cdot 10^{-3}$	$1.48 \cdot 10^{-6}$	97.5
3	III	99.901 ±14.066	–152.494 ±22.025	34.337 ±4.483	–0.14	6	0.975	0.354	28.5	0.42	$2.17 \cdot 10^{-6}$	97.5
					E. coli							
4	I	0.807 ±0.098	–1.348 ±0.155	–2.867 ±0.822	–10.31	6	0.981	0.301	37.7	10.5	$8.68 \cdot 10^{-6}$	99.0
5	II	1.132 ±0.163	–10.905 ±1.892	8.123 ±0.642	1.31	5	0.980	0.307	24.3	$5.67 \cdot 10^{-3}$	$8.72 \cdot 10^{-6}$	95.0
6	III	99.125 ±21.281	–154.847 ±33.321	34.738 ±6.782	–0.14	6	0.936	0.536	10.7	0.39	$2.07 \cdot 10^{-5}$	95.0
					C. albicans							
7	I	0.798 ±0.120	–1.157 ±0.217	–2.888 ±1.046	–11.00	6	0.970	0.426	23.5	11.4	$2.62 \cdot 10^{-6}$	97.5
8	II	0.665 ±0.104	–11.973 ±1.201	7.518 ±0.408	1.31	5	0.991	0.194	52.0	$2.88 \cdot 10^{-3}$	$2.94 \cdot 10^{-6}$	97.5
9	III	53.717 ±11.625	–135.607 ±31.835	10.518 ±1.980	–0.64	6	0.956	0.511	15.8	0.46	$4.55 \cdot 10^{-6}$	95.0

[a] For symbols see List of symbols.

TABLE V

Regression coefficients of bilinear dependences between antimicrobial activity, lipophilicity and structure of bisammonium salts derived from bis(2-dimethylaminoethyl) pimelate, in the form[a] of log (1/MIC) = $ax + b \log (\beta\ 10^{x} + 1) + c$, where x is m (type I) or log c_K (type II) or R_m (type III)

Equation	Type	a	b	c	log β	n	r	s_D	F	Optimum m, c_K or R_m	MIC_{max}	α, %
					S. aureus							
1	I	0.960 ±0.049	–1.168 ±0.067	–3.074 ±0.386	–9.44	6	0.973	0.360	209.5	10.1	$1.77 . 10^{-6}$	99.9
2	II	0.484 ±0.117	–6.122 ±1.341	7.067 ±0.462	1.33	5	0.977	0.293	20.8	$4.01 . 10^{-3}$	$2.05 . 10^{-6}$	95.0
3	III	60.114 ±12.330	–87.000 ±18.596	23.020 ±4.153	–0.10	6	0.955	0.413	15.7	0.45	$2.14 . 10^{-6}$	95.0
					E. coli							
4	I	0.788 ±0.116	–1.347 ±0.186	–2.823 ±0.981	–10.34	6	0.973	0.360	26.2	10.5	$1.18 . 10^{-5}$	97.5
5	II	1.198 ±0.177	–11.875 ±2.027	8.245 ±0.699	1.33	5	0.979	0.332	23.3	$5.24 . 10^{-3}$	$1.08 . 10^{-5}$	95.0
6	III	73.074 ±18.686	–110.381 ±28.183	27.871 ±6.294	–0.10	6	0.914	0.626	7.7	0.39	$3.05 . 10^{-5}$	90.0
					C. albicans							
7	I	0.752 ±0.147	–1.105 ±0.267	–2.537 ±1.284	–11.00	6	0.949	0.523	13.7	11.3	$3.69 . 10^{-6}$	95.0
8	II	0.659 ±0.156	–11.283 ±1.786	7.354 ±0.615	1.33	5	0.977	0.292	20.6	$2.90 . 10^{-3}$	$4.12 . 10^{-6}$	95.0
9	III	57.909 ±17.099	–88.177 ±27.545	19.196 ±5.177	–0.18	6	0.922	0.645	8.5	0.46	$7.15 . 10^{-6}$	90.0

[a] For symbols see List of symbols.

TABLE VI

Regression coefficients of bilinear dependences between antimicrobial activity, lipophilicity and structure of bisammonium salts derived from bis(2-dimethylaminoethyl) suberate, in the form[a] of log (1/MIC) = $ax + b \log (\beta\, 10^{x} + 1) + c$, where x is m (type I) or log c_K (type II) or R_m (type III)

Equation	Type	a	b	c	log β	n	r	s_D	F	Optimum m, R_m or c_K	MIC_{max}	α, %
						S. aureus						
1	I	0.691 ±0.063	–1.017 ±0.097	–0.570 ±0.526	–10.15	6	0.988	0.187	60.2	10.5	$6.71 \cdot 10^{-7}$	99.5
2	II	1.403 ±0.322	–3.740 ±0.672	10.479 ±1.155	2.36	6	0.962	0.329	18.4	$2.63 \cdot 10^{-3}$	$8.02 \cdot 10^{-7}$	97.5
3	III	49.521 ±14.151	–72.462 ±21.167	12.076 ±2.345	–0.27	6	0.901	0.518	6.5	0.60	$1.57 \cdot 10^{-6}$	90.0
						E. coli						
4	I	1.058 ±0.122	–1.615 ±0.169	–4.226 ±0.968	–9.44	6	0.984	0.296	47.1	9.7	$4.89 \cdot 10^{-6}$	99.0
5	II	2.258 ±0.434	–5.998 ±1.094	11.652 ±1.525	2.09	6	0.954	0.507	15.1	$4.94 \cdot 10^{-3}$	$6.11 \cdot 10^{-6}$	95.0
6	III	69.622 ±19.927	–105.889 ±29.806	15.403 ±3.303	–0.27	6	0.901	0.729	6.5	0.55	$2.23 \cdot 10^{-5}$	90.0
						C. albicans						
7	I	0.779 ±0.003	–1.220 ±0.005	–1.965 ±0.024	–10.24	6	0.999	0.009	3 268.1	10.5	$2.17 \cdot 10^{-6}$	99.9
8	II	2.028 ±0.206	–4.626 ±0.400	11.995 ±0.745	2.47	6	0.989	0.197	71.9	$1.63 \cdot 10^{-3}$	$2.50 \cdot 10^{-6}$	99.5
9	III	61.818 ±11.764	–91.524 ±17.596	13.572 ±1.950	–0.27	6	0.950	0.430	13.8	0.59	$6.08 \cdot 10^{-6}$	95.0

[a] For symbols see List of symbols.

TABLE VII

Regression coefficients of bilinear dependences between antimicrobial activity, lipophilicity and structure of bisammonium salts derived from bis(2-dimethylaminoethyl) azelaoate, in the form[a] of log (1/MIC) = $ax + b \log (\beta\, 10^{x} + 1) + c$, where x is m (type I) or log c_K (type II) or R_m (type III)

Equation	Type	a	b	c	log β	n	r	s_D	F	Optimum m, R_m or c_K	MIC_{max}	α, %
					S. aureus							
1	I	0.788 ±0.016	–0.958 ±0.023	–1.347 ±0.129	–9.60	6	0.999	0.041	1 261.5	10.2	$1.21 . 10^{-6}$	99.9
2	II	0.575 ±0.108	–11.515 ±1.181	7.761 ±0.425	1.46	6	0.987	0.185	60.3	$1.82 . 10^{-3}$	$1.18 . 10^{-6}$	99.5
3	III	68.247 ±10.775	–102.822 ±16.840	16.016 ±2.053	–0.27	6	0.974	0.270	27.3	0.57	$1.15 . 10^{-6}$	97.5
					E. coli							
4	I	0.932 ±0.157	–1.431 ±0.211	–3.170 ±1.228	–9.22	6	0.972	0.356	25.3	9.5	$9.49 . 10^{-6}$	97.5
5	II	1.417 ±0.297	–14.265 ±3.237	8.997 ±1.166	1.46	6	0.941	0.508	11.7	$3.8 . 10^{-3}$	$1.19 . 10^{-5}$	95.0
6	III	90.591 ±21.370	–144.460 ±33.398	21.720 ±4.071	–0.27	6	0.934	0.535	10.3	0.50	$1.76 . 10^{-5}$	95.0
					C. albicans							
7	I	0.941 ±0.187	–1.420 ±0.245	–2.620 ±1.444	–9.02	6	0.941	0.397	19.3	9.31	$3.37 . 10^{-6}$	97.5
8	II	1.433 ±0.241	–13.802 ±2.626	9.494 ±0.946	1.46	6	0.960	0.412	17.8	$4.01 . 10^{-3}$	$3.97 . 10^{-6}$	97.5
9	III	88.732 ±18.372	–141.929 ±28.712	22.031 ±3.500	–0.27	6	0.950	0.460	13.9	0.49	$6.08 . 10^{-6}$	95.0

[a] For symbols see List of symbols.

TABLE VIII

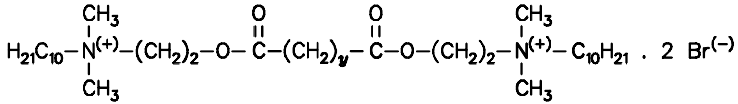
Regression coefficients of bilinear dependences between antimicrobial activity, lipophilicity and structure of bisammonium salts derived from bis(2-dimethylaminoethyl) sebacate, in the form[a] of log (1/MIC) = $ax + b \log (\beta\, 10^{x} + 1) + c$, where x is m (type I) or log c_K (type II) or R_m (type III)

Equation	Type	a	b	c	log β	n	r	s_D	F	Optimum m, R_m or c_K	MIC_{max}	α, %
						S. aureus						
1	I	0.492 ±0.030	–0.598 ±0.042	–0.981 ±0.239	–9.57	6	0.995	0.076	147.6	10.2	$2.74 \cdot 10^{-6}$	99.5
2	II	0.311 ±0.059	–2.578 ±0.248	6.580 ±0.239	2.07	6	0.990	0.106	74.0	$1.18 \cdot 10^{-3}$	$2.99 \cdot 10^{-6}$	99.5
3	III	39.022 ±7.640	–58.104 ±11.843	10.598 ±1.344	–0.28	6	0.960	0.208	17.6	0.59	$2.79 \cdot 10^{-6}$	97.5
						E. coli						
4	I	1.053 ±0.206	–1.544 ±0.253	–3.297 ±1.533	–8.50	6	0.973	0.363	26.6	8.8	$5.90 \cdot 10^{-6}$	97.5
5	II	1.239 ±0.229	–12.339 ±2.838	8.323 ±0.894	1.25	6	0.952	0.478	14.7	$6.27 \cdot 10^{-3}$	$9.38 \cdot 10^{-6}$	95.0
6	III	63.643 ±28.450	–103.964 ±44.099	16.978 ±5.004	–0.28	6	0.868	0.776	4.6	0.48	$2.21 \cdot 10^{-5}$	90.0
						C. albicans						
7	I	0.743 ±0.068	–0.910 ±0.084	–0.850 ±0.507	–8.50	6	0.988	0.120	59.8	9.1	$5.34 \cdot 10^{-6}$	99.5
8	II	0.513 ±0.011	–4.534 ±0.073	6.780 ±0.043	1.66	6	0.999	0.021	1 927.3	$2.79 \cdot 10^{-3}$	$5.86 \cdot 10^{-6}$	99.9
9	III	46.128 ±8.804	–71.118 ±13.647	12.301 ±1.549	–0.28	6	0.948	0.240	16.6	0.55	$6.32 \cdot 10^{-6}$	97.5

[a] For symbols see List of symbols.

TABLE IX

Regression coefficients of bilinear dependences between antimicrobial activity, lipophilicity and structure of bisammonium salts[a] *III*, *XI*, *XVII*, *XXIV*, *XXX*, *XXXVI*, *XLII*, in the form[b] of log (1/MIC) = $ax + b \log (\beta\ 10^x + 1) + c$, where x is y (type I) or log c_K (type II)



log (1/MIC = $f(y)$ *I*

log (1/MIC) = $f(\log c_K)$ *II*

log (1/MIC) = $ax + b \log (\beta\ .\ 10^x + 1) + c$ $x = y$ or log c_K

Equation	Type	a	b	c	log β	n	r	s_D	F	Optimum y c_K	MIC_{max}	α, %
					S. aureus							
1	I	0.159 ±0.032	–0.585 ±0.122	5.212 ±0.137	–6.50	7	0.932	0.102	13.2	6.07	$7.98 . 10^{-7}$	97.5
2	II	10.627 ±2.824	–16.289 ±4.223	27.052 ±8.638	2.62	7	0.900	0.150	8.5	$4.36 . 10^{-3}$	$9.12 . 10^{-7}$	95.0
					C. albicans							
3	I	0.473 ±0.058	–0.666 ±0.096	3.565 ±0.201	–4.68	7	0.973	0.110	35.1	5.07	$2.46 . 10^{-6}$	99.5
4	II	15.915 ±0.011	–24.871 ±0.073	54.479 ±0.043	2.63	7	0.895	0.215	8.1	$4.27 . 10^{-3}$	$2.66 . 10^{-6}$	95.0

[a] For symbols see List of symbols; [b] for compounds see Table I.

TABLE X

Regression coefficients of bilinear dependences between antimicrobial activity, lipophilicity and structure for all bisammonium salts examined (*I–XLV*), in the form[a] of log (1/MIC) = $ax + b \log (\beta\, 10^x + 1) + c$, where x is m (type I) or log c_K (type II) or R_m (type III)

Equation	Type	a	b	c	log β	n	r	s_D	F	Optimum m, R_m or c_K	MIC_{max}	α, %
					S. aureus							
1	I	0.766 ±0.056	–1.039 ±0.092	–1.780 ±0.475	–10.50	45	0.909	0.489	99.5	10.9	$1.00 \cdot 10^{-6}$	99.0
2	II	0.627 ±0.101	–3.239 ±0.300	7.892 ±0.389	2.21	42	0.888	0.466	73.0	$1.49 \cdot 10^{-3}$	$1.54 \cdot 10^{-6}$	99.0
3	III	25.767 ±3.385	–32.940 ±4.724	10.785 ±1.036	–0.09	45	0.828	0.657	45.8	0.64	$2.74 \cdot 10^{-6}$	99.0
					E. coli							
4	I	0.783 ±0.058	–1.375 ±0.093	–2.620 ±0.491	–10.37	45	0.916	0.495	108.9	10.4	$8.04 \cdot 10^{-6}$	97.5
5	II	1.396 ±0.120	–4.361 ±0.366	9.085 ±0.457	2.14	42	0.891	0.544	75.0	$3.42 \cdot 10^{-3}$	$1.23 \cdot 10^{-5}$	99.0
6	III	26.559 ±28.450	–37.079 ±44.099	11.461 ±5.004	–0.09	45	0.646	0.940	15.0	0.49	$6.78 \cdot 10^{-5}$	90.0
					C. albicans							
7	I	0.655 ±0.058	–1.079 ±0.105	–1.403 ±0.504	–11.0	45	0.868	0.561	64.5	11.2	$3.25 \cdot 10^{-6}$	99.0
8	II	0.938 ±0.145	–2.550 ±0.298	8.357 ±0.565	2.61	42	0.820	0.578	40.0	$1.42 \cdot 10^{-3}$	$6.65 \cdot 10^{-6}$	99.0
9	III	20.548 ±3.693	–28.052 ±5.431	8.392 ±1.549	–0.17	45	0.701	0.807	20.4	0.61	$1.53 \cdot 10^{-5}$	99.0

[a] For symbols see List of symbols.

Quantitative Structure–Property–Activity Relationships (QSAR)

The relationships were calculated using Kubinyi's bilinear model[35]. The regression equation coefficients, obtained by the least squares method, are given in Tables II–X. The results obtained for the seven bisammonium salt series were employed to calculate universal QSAR equations (Table X), which allow the antimicrobial activity of analogous compounds to be predicted based on their known c_K values.

RESULTS AND DISCUSSION

QSAR analysis was performed to obtain a mathematical description of the dependence between biological activity (characterized by the minimum inhibition concentration MIC), structure (characterized by the hydrocarbon chain lengths m and y), and lipophilicity (characterized by the R_m values and aggregation properties – the critical micellar concentration c_k).

It has already been demonstrated[29,30] that in addition to the conventional distribution coefficients, the chromatographic R_m values and aggregation properties characterized by the c_K values can also serve to express the structure–activity relationships.

Table I demonstrates that all dependences where MIC is employed as the biological activity parameter are nonlinear, which is consistent with data in literature[36] where this nonlinearity of the structure–activity relationship is referred to as the cut-off effect. The cut-off effect is a general pharmacological phenomenon, which is explained by several theories as summarized in the above mentioned paper[36].

Kubinyi's bilinear model was employed to quantify the log (1/MIC) = $f(m)$, log (1/MIC) = $f(y)$, log (1/MIC) = $f(\log c_K)$ and log (1/MIC) = $f(R_m)$ dependences. (In fact, it has been found in our previous work[30,37,38] that giving a better fit of the experimental data to the statistically obtained regression equations, the bilinear approach is preferable to the parabolic approach.)

The regression equation on its own does not allow us to find how closely the biological activity follows the physico-chemical parameters chosen. Therefore, when evaluating the results, we also performed a statistical analysis indicating the significance of the regression relations obtained.

In addition to the optimum m, y, c_K or R_m values calculated from the correlation equations, the theoretical values corresponding to the highest biological activity were calculated as well. Tables II through VIII demonstrate that the highest biological activity is exhibited by compounds whose c_K values lie within the region of 2–6 mmol dm^{-3}; in particular, the optimum values are 2–4 mmol dm^{-3} for the strains *S. aureus* and *C. albicans*, and slightly more, viz. 4–6 mmol dm^{-3}, for *E. coli*. Hence, the finding that the maximum antimicrobial activity is displayed by ammonium salts within a narrow range of c_K values at the 10^{-3} mol dm^{-3} level[39], is borne out.

The experimental optimum alkyl chain lengths (m values) correlate well with the QSAR data; the optimum numbers of carbon atoms in the terminal hydrocarbon chain

are m = 10–12 for *S. aureus* and *C. albicans* and m = 9–11 for *E. coli* (Tables II–VIII). This decrease in the optimum of the structural parameter again demonstrates the lipophilic contribution of the extending spacer chain to the HLB of the molecules in the various series. Correlation of the antimicrobial activity with the number of carbon atoms in the spacer (y) is given in Table IX. The calculated optimum values correspond to y = 5–6.

The most active compounds of the series tested provide an antimicrobial effect which corresponds to values of MIC = $1 . 10^{-7}$ to $1 . 10^{-6}$ mol dm^{-3} for *S. aureus* and $1 . 10^{-6}$ to $1 . 10^{-5}$ mol dm^{-3} for *E. coli* and *C. albicans*. The results show that the most active compounds surpass substantially conventional disinfectants; for instance, as compared to AJATIN (benzyldodecyldimethylammonium bromide), the activity is hundredfold with respect to all the strains used, and as compared to SEPTONEX (2-ethoxycarbonylpentadecyltrimethylammonium bromide), the activity is hundredfold for *S. aureus* and tenfold for *E. coli*; for *C. albicans* the activities are roughly the same.

Statistical analysis of the regression equations (Tables II–IX) gives evidence that the results are significant at a high confidence level. It also follows from the analysis that c_K is preferable to R_m as the lipophilicity parameter[29] (relations II, III in Tables II through X).

This work was supported by grant No. 1/1223/94 of the Ministry of Education and Science of the Slovak Republic. The authors wish to thank Mrs M. Ruzickova and Mrs M. Olsavska for excellent technical cooperation.

LIST OF SYMBOLS

a, b, c	regression coefficients
c_K	critical micellar concentration (obtained conductometrically)
F	Fischer–Snedecor's test value
m	number of carbon atoms in terminal hydrocarbon chain
n	number of data points
r	correlation coefficient
R_m	thin layer chromatography parameter
s_D	standard deviation of equation
y	number of carbon atoms in spacer
α	significance level
β	nonlinear parameter in bilinear equations
κ	electric conductivity

REFERENCES

1. Menger F. M., Wrenn S.: J. Phys. Chem. *78*, 1387 (1974).
2. Fuhrhop J. H., David H.-H., Mathieu J., Liman U., Winter H. J., Boekema E.: J. Am. Chem. Soc. *108*, 1785 (1986).

3. Fuhrhop J. H., Liman U., Koesling V.: J. Am. Chem. Soc. *110*, 6840 (1988).
4. Thompson D. H., Wong K. F., Humpry-Baker R., Wheeler J. F., Kim J.-M., Rananavare S. B.: J. Am. Chem. Soc. *114*, 9035 (1992).
5. Moss A. R., Li J.-M.: J. Am. Chem. Soc. *114*, 9227 (1992).
6. Devinsky F., Lacko I., Bittererova F., Tomeckova L.: J. Colloid Interface Sci. *114*, 314 (1986).
7. Devinsky F., Lacko I., Imam T.: J. Colloid Interface Sci. *143*, 336 (1991); and references therein.
8. Edebo L., Ahlstrom B., Allenmark S., Bertilsson M., Jenische E., Lange S., Lindsted M., Thompson R. A. in: *Industrial Applications of Surfactants* (D. R. Karsa, Ed.), Vol. III, pp. 185–206. Royal Society of Chemistry, Cambridge 1992.
9. Menger F. M., Littau C. A.: J. Am. Chem. Soc. *113*, 1451 (1991).
10. Menger F. M., Littau C. A.: J. Am. Chem. Soc. *115*, 10083 (1993).
11. Rosen M. J.: CHEMTECH *1993*, 30.
12. Bosch M. P., Parra J. L., Sanchez-Baeza F.: Can. J. Chem. *71*, 2095 (1993).
13. Fuhrhop J. H., Liman U.: J. Am. Chem. Soc. *106*, 4643 (1984).
14. Devinsky F., Lacko I.: Acta Fac. Pharm. *44*, 119 (1990).
15. Merianos J. J. in: *Disinfection, Sterilization and Preservation* (S. Block, Ed.), 4th ed., pp. 225–255. Lea & Febiger, Philadelphia 1991.
16. Jayasuriya M., Bosak S., Regen S. L.: J. Am. Chem. Soc. *112*, 5844 (1990).
17. Bodor N., Kaminsky J. J., Selk S.: J. Med. Chem. *23*, 469 (1980).
18. Cupkova V., Sirotkova L., Mlynarcik D., Devinsky F., Lacko I.: J. Com. Esp. Deterg. *23*, 281 (1992).
19. Cupkova V., Sirotkova L., Mlynarcik D., Lacko I., Devinsky F.: Folia Microbiol. (Prague) *37*, 311 (1992).
20. Cupkova V., Sirotkova L., Mlynarcik D., Devinsky F., Lacko I., Kovackova Z.: Folia Microbiol. (Prague) *38*, 43 (1993).
21. Bodor N.: U. S. 3,989,711 (1976).
22. Ashmann B. R., Ninham W. B.: Mol. Immunol. *22*, 609 (1985).
23. Ashmann B. R., Blanden R. V., Ninham B. V., Evans D. F.: Immunol. Today *7*, 278 (1986).
24. Coy A. E., Reddy A. K., Hostynek J. J., Gleich G. T.: Int. J. Immunopharmacol. *12*, 871 (1990).
25. Ferencik M., Lacko I., Devinsky F.: Pharmazie *45*, 695 (1990).
26. Jahnova E., Ferencik M., Nyulassy S. Devinsky F., Lacko I.: Immonul. Lett. *39*, 71 (1994).
27. Sersen F., Leitmanova A., Devinsky F., Lacko I., Balgavy P.: Gen. Physiol. Biophys. *8*, 133 (1989).
28. Gallova J., Devinsky F., Balgavy P.: Chem. Phys. Lipids *53*, 231 (1990).
29. Devinsky F., Lacko I., Vidlakova J., Gallayova D.: Cesk. Farm. *36*, 141 (1987).
30. Devinsky F., Lacko I., Mlynarcik D. in: *QSAR in Design of Bioactive Compounds* (M. Kuchar, Ed.), p. 233. Prous, Barcelona 1992.
31. Lacko I., Pavlikova M., Devinsky F.: Chem. Papers, in press.
32. Devinsky F., Masarova L, Lacko I.: J. Colloid Interface Sci. *105*, 235 (1985).
33. Mlynarcik D., Lacko I., Devinsky F., Krasnec L.: Pharmazie *31*, 407 (1976).
34. Kneiflova J.: Cesk. Epidemiol., Mikrobiol. Imunolog. *37*, 97 (1988).
35. Kubinyi H.: Ref.[30], p. 321.
36. Devinsky F., Kopecka-Leitmanova A., Sersen F., Balgavy P.: J. Pharm. Pharmacol. *42*, 790 (1990).
37. Devinsky F., Lacko I., Mlynarcik D., Svajdlenka E., Borovska V.: Chem. Papers *44*, 159 (1990).
38. Devinsky F., Lacko I., Mlynarcik D., Svajdlenka E., Masarova L: Acta Fac. Pharm. Univ. Comenianae *44*, 127 (1990).
39. Devinsky F., Lacko I., Mlynarcik D., Racansky V., Krasnec L: Tenside Detergents *22*, 10 (1985).