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Stereoisomeric effect on antimicrobial activity of a series of quaternary ammonium salts

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Abstract

Two homologous series of diastereoisomeric racemic ± *cis* and ± *trans-N,N*-dimethyl-*N*-alkyl-2-benzoyloxycyclohexylmethylammonium bromides with the number of carbon atoms in the alkyl chain from six to twenty ($m = 6,8 \ldots 20$) were synthesised. Their structures have been elucidated by IR, UV and in some cases also with ^{1}H and ^{13}C NMR spectrometry. The title compounds were assayed for their antimicrobial activity on microorganisms *S. aureus*, *E. coli* and *C. albicans*. The highest antimicrobial activity was observed against *S. aureus* (log 1/MIC = 5.5 mol^{-1} dm^{3}) and the lowest against *E. coli* (log 1/MIC = 4.5 mol^{-1} dm^{3}). The ± *cis* and ± *trans* stereoisomers of all eight couples of diastereoisomeric compounds show differences in their physico-chemical characteristics (including partition coefficient and lipophilicity) which is also reflected in the different antimicrobial activity of these diastereoisomers.

Keywords: Diastereoisomeric organic ammonium bromides; Lipophilicity; Antimicrobial activity; Stereoisomerism

1. Introduction

Quaternary ammonium salts belong to the group of compounds which exhibit high antimicrobial activity. This activity, characterised by minimum inhibitory concentration (MIC), is highly effective especially against gram-positive bacteria and fungi [1–4]. Experiments on many homologous series of quaternary ammonium salts show that antimicrobial activity increases with the length of the alkyl chains, but only until a certain limit after which a decrease of activity appears. It is evident that antimicrobial activity of certain homologous series of quaternary ammonium salts is a function of lipophilicity of these compounds. Parameters of lipophilicity are for example partition coefficient, P, R_M values and C_K values (critical micelle concentration). If antimicrobial activity is expressed as log(1/MIC) and parameters of lipophilicity X, e.g. as log C_K, log P, R_M, then log(1/MIC) = $f(X)$ is nonlinearly dependent and it can be expressed by Hansch's parabolic model or Kubinyi's bilinear model which better describes this relationship [5,6]. Applications of these correlations are illustrated in Refs. [7–11].

The influence of spatial structure-stereoisomerism (geometrical isomerism, enantiomerism) on the antimicrobial activity of organic ammonium salts has not been well investigated. It is known [12] that stereoisomers possess different physico-chemical characteristics, including partition coefficient and lipophilicity [13]. Because the lipophilicity has an evident effect on antimicrobial activity, it is logical to suppose that the antimicrobial activity of stereoisomers should also not be the same and the difference in their activity should be proportional to the difference in the lipophilicity of these isomers. Previous investigations have shown [14] that the microbial activity of some compounds also depends on the stereoisomerism.

The aim of this paper is to prepare and test homologous series of diastereoisomeric racemic ± *cis* and ± *trans-N,N*-dimethyl-*N*-alkyl-2-benzoyloxycyclohexylmethylammonium bromides on microorganisms *S. aureus*, *E. coli* and *C. albicans* in order to find out how the stereospecificity of the compounds could influence their activity. The results obtained are presented and discussed herein.

2. Chemistry

2.1. Preparation of cis- and trans-N,N-dimethyl-2-benzoyloxycyclohexylmethylammonium chlorides (cis-2 and trans-2, Scheme 1)

To a solution of 16.5 g (0.105 mol) benzoylchloride in 150 ml of anhydrous benzene was added under stirring and

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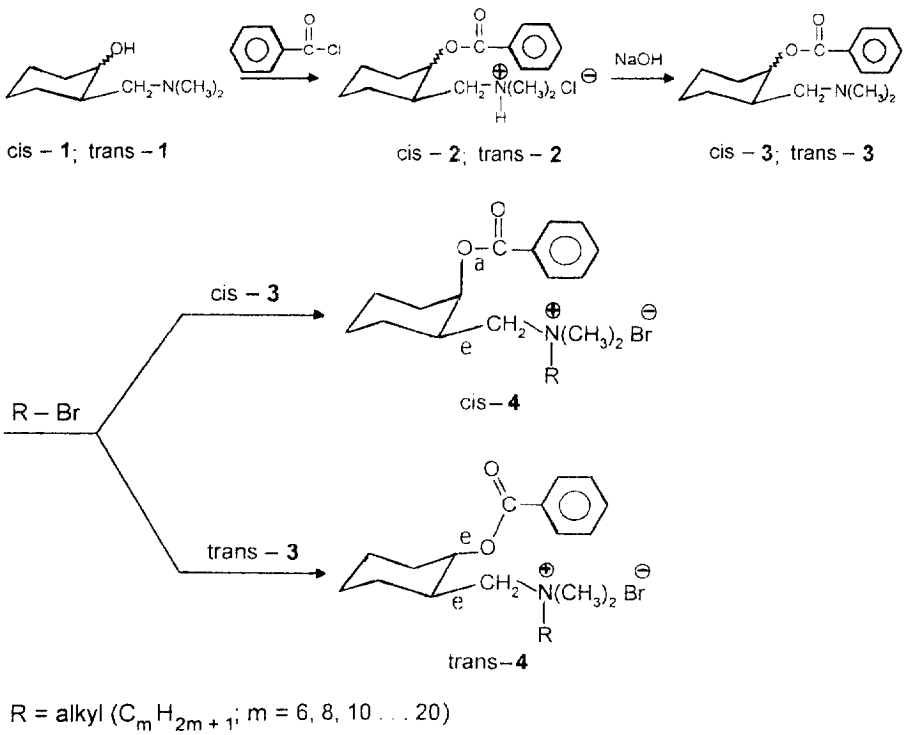


Scheme 1.

cooling a solution of 15.7 g (0.1 mol) aminoalcohol (*cis*-**1** and *trans*-**1**) in 50 ml of anhydrous benzene during 15 min at 20°C. Then the solution was boiled for 5 min. After cooling, the solid product was filtered off, washed with benzene and ether, and dried. Crystallisation from butanone gives 24 g (81%) of *cis*-**2**, m.p. 204°C and 22 g (74%) of *trans*-**2** (Scheme 1). m.p. 228–229°C (according to the literature [11] m.p. is 230–231°C for *trans*-**2**).

2.2. Preparation of cis- and trans-2-dimethylaminomethyl-cyclohexylbenzoates (cis-3 and trans-3, Scheme 1)

To a solution of 29.8 g (0.1 mol) *cis*- or *trans*-*N,N*-dimethyl-2-benzoyloxycyclohexylmethylammonium chlorides (*cis*-2 or *trans*-2) in 100 ml H_2O were added 50 g crushed ice and 20 ml of ether. To this solution a solution of 4.8 g (0.12 mol) NaOH in 20 ml H_2O was added over 1–2 min. The solution was carefully stirred and the organic layer was separated. The water solution was twice extracted into ether. The combined organic layers were dried over anhydrous sodium sulfate. The solution was filtered and ether was distilled off. The distillation residue was purified by distillation under reduced pressure. Colourless liquid, 17 g (65%) of *cis*-**3**, b.p. 152–153°C/0.3 torr, $n_D^{20} = 1.5145$; 16 g (62%) of *trans*-**3**, b.p. 150–151°C/0.3 torr, $n_D^{20} = 1.5148$.

2.3. Preparation of N,N-dimethyl-N-alkyl-(2-benzoyloxy-cyclohexylmethyl)ammonium bromides (cis-4 and trans-4)

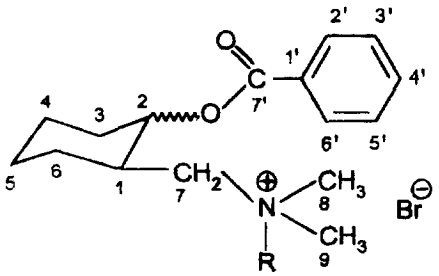
A mixture of 1.6 g (0.01 mol) *cis*- or *trans*-2-dimethylaminomethylcyclohexylbenzoate and 0.012 mol of 1-bromalkane in 10 ml of acetonitrile was heated until smooth boiling over a period of 18 h. The mixture was cooled and 1-bromalkane was removed by extraction into hexane (three times) in a separatory funnel. Moisture from the product was removed by azeotropic distillation with benzene and the product was purified by crystallisation from acetone/methyl-tert-buthylether (3:1). (Products crystallised after 12 h at 0°C.) The characteristics of the prepared compounds are shown in Table 1.

3. Experimental

3.1. Chemistry

All prepared compounds (*cis*-**4** and *trans*-**4**) were characterised by melting points n_D^{20}, R_F values, partition coefficients, elemental analysis, IR and UV spectroscopy, and selected compounds also by ^1H and ^{13}C NMR spectroscopy. Melting points were determined on a Boetius apparatus and are uncorrected. Elemental analyses (elemental analyser model 1102 Carlo Erba) agreed with the theoretical values to within ±0.3% for H, C, N. IR spectra were taken with a Imparck 400D (Nicolet) spectrophotometer in chloroform solution. NMR spectra (^1H at 299.93 MHz and ^{13}C at 75.43 MHz) were measured (in deuterochloroform) at room temperature on a Varian VXR-300 spectrometer using TMS as an internal reference. UV–Vis spectra were determined with a Hewlett Packard 8452A spectrometer in water. The n_D^{20} values were determined on a refractometer (Zeiss). All the compounds were checked for purity by partition TLC on

Table 1
Characteristics of *cis*- and *trans*-*N*,*N*-dimethyl-*N*-alkyl-2-benzoyloxycyclohexylmethylammonium bromides



Compound	R	Yield (%)	M.p. (°C) n_D^{20}	Formula MW	R_f [a]	P [b]	Analyses
Cis-**4a**	$(CH_2)_5CH_3$	61	105–106	$C_{22}H_{36}NO_2Br$	0.60	1.6	C,H,N
Trans-**4a**		56	1.5418	426.44	0.64	2.2	C,H,N
Cis-**4b**	$(CH_2)_7CH_3$	58	112–113	$C_{24}H_{40}NO_2Br$	0.48	6.8	C,H,N
Trans-**4b**		54	1.5340	454.49	0.58	8.6	C,H,N
Cis-**4c**	$(CH_2)_9CH_3$	58	128–130	$C_{26}H_{44}NO_2Br$	0.50	31.3	C,H,N
Trans-**4c**		52	1.5263	482.55	0.51	59.6	C,H,N
Cis-**4d**	$(CH_2)_{11}CH_3$	56	120–121	$C_{28}H_{48}NO_2Br$	0.45	159.7	C,H,N
Trans-**4d**		52	35–36	510.60	0.48	503.3	C,H,N
Cis-**4e**	$(CH_2)_{13}CH_3$	56	109–110	$C_{30}H_{52}NO_2Br$	0.40	427.6	C,H,N
Trans-**4e**		50	48–49	538.65	0.43	1048.1	C,H,N
Cis-**4f**	$(CH_2)_{15}CH_3$	50	102–103	$C_{32}H_{56}NO_2Br$	0.34	1377.5	C,H,N
Trans-**4f**		48	59–60	566.69	0.39	3980.0	C,H,N
Cis-**4g**	$(CH_2)_{17}CH_3$	54	89–90	$C_{34}H_{60}NO_2Br$	0.29	4177.0	C,H,N
Trans-**4g**		48	62–63	594.77	0.34	4666.8	C,H,N
Cis-**4h**	$(CH_2)_{19}CH_3$	50	75–76	$C_{36}H_{64}NO_2Br$	0.22	[c]	C,H,N
Trans-**4h**		47	65–66	622.85	0.28	[c]	C,H,N

[a] 1 M HCl/acetone (1:2), silica gel impregnated with silicon oil.
[b] Partition coefficient P was determined in system octanol–water.
[c] P was not determined because of low solubility of these compounds in water.

silica gel Silufol UV-254 (Kavalier, Czech) plates impregnated with 5% solution of silicon oil in heptane and detected by the Dragendorff's reagent, using 1 M HCl/acetone (1:2) as the mobile phase. Partition coefficients (P) were determined in the system n-octanol/water (pH = 7; 0.01 mol dm^{-1}, potassium phosphate buffer); the relative concentration was determined spectrophotometrically from the absorption bands at 234 and 274 nm.

3.2. Microbiology

MIC, expressed as the lowest concentration of a compound which still hindered the growth of microorganisms, was determined by the dilution test method according to Lacko et al. [15] using strains from the Czecho–Slovak state collection of type cultures: *Staphylococcus aureus* Oxford Man 29/58, *Escherichia coli* 377/79 and *Candida albicans* 45/53. As a standard *N*,*N*-dimethyl-*N*-benzyldodecylammonium bromide (Ajatin) and *N*,*N*,*N*-trimethyl-(ethoxycarbonylpentadecyl)ammonium bromide (Septonex) were used. The toxicity of selected compounds was evaluated after the subcutaneous application of a 1% solution of the compound to white mice and expressed as an estimation of LD_{50} values (determined 24 h after application of the compound).

3.3. Theoretical calculations

The calculations of the selected model structures of *cis* and *trans* isomers of *cis*- and *trans*-2-dimethylaminomethylcyclohexylbenzoate (Fig. 1) were performed using the AM1 [16] semiempirical quantum chemical method as implemented in the Hyperchem [17] series of programs. The

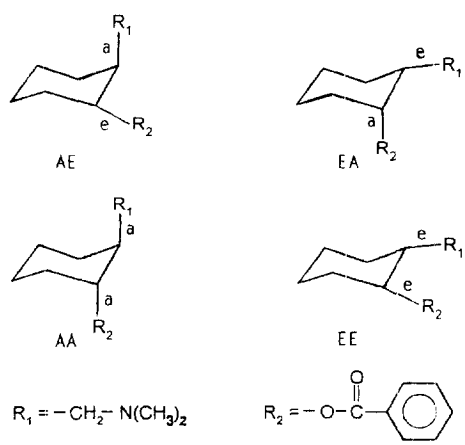


Fig. 1. Possible isomers of *cis*- and *trans*-2-dimethylaminomethylcyclohexylbenzoate.

geometries of all conformers were fully optimised, beginning at idealised atomic arrangements near the structure of interest generated by the molecular modelling program MOLGEN 3.0 [18]. The calculations were performed on isolated molecules possessing no intermolecular interactions, a situation that is most analogous to experimental results from the gas phase. Although this situation is far from the active environment of the drug, information such as that determined here can still be useful. Qualitative arguments can be used to explain how such a molecule would behave in situations closer to a biologically active environment.

4. Results and discussion

The aim of the present work was the investigation of physico-chemical properties and antimicrobial activity in relation with the stereoisomerism and lipophilicity of prepared organic ammonium bromides. Stereoisomerism was realised with 1,2-disubstituted cyclohexane. The presence of two different substituents which do not lie in a symmetry plane of the ring results in the existence of two diastereoisomers (entitled also as geometric isomers *cis* and *trans*). Both of them consist of two enantiomers (racemates). For this purpose racemic *cis*- and *trans*-2-dimethylaminomethylcyclohexanols (*cis*-**1**, *trans*-**1**, Scheme 1) were prepared by stereoselective reactions [19–22]. Characteristics and selected physico-chemical properties of these compounds are shown in Table 1. All prepared *cis*-4 ammonium bromides are solids. The melting point (m.p.) of these compounds varies with the length of the aliphatic chain, increasing steadily from C_6 to C_{10}. Then the m.p. decreases. *Trans*-4 ammonium bromides with $m = 6, 8, 10$ in the alkyl chain are liquid compounds and species with $m = 12, 14 \ldots 20$ are solid compounds. The m.p. values of these structures increase with m (see Fig. 2). The greatest difference in m.p. values between *cis*-4 and *trans*-4 isomeric compounds (a comparison of *cis* and *trans* compounds with the same number of carbon atoms) was found in compounds with a short alkyl chain (Fig. 2). Experiments have shown that diastereoisomers do not only differ in values of melting point, but also in other physico-chemical parameters (solubility and lipophilicity). Values of partition coefficients in both homologous series of *cis*-4 and *trans*-4 compounds increase with the number of carbon atoms m (Table 1). The values of P for *trans* isomers are higher in comparison with values of P in *cis* derivatives (for isomers with the same number of m), and *trans* isomers are always more lipophilic than the corresponding *cis* isomers. R_F values obtained from the TLC measurements decrease in both homologous series of *cis*-4 and *trans*-4 isomers with increasing number of carbon atoms (m) and $R_F(cis) < R_F(trans)$ isomers. This could mean a contrary statement to that resulting from the partition coefficients. However, it is possible to explain this aparent contradiction by the fact that R_F values were obtained from thin-layer silica gel which was impregnated with a 5% solution of silicon oil (non-polar phase). On condition that the impregnation of thin layer silica gel with silicon oil is not applied the course of the R_F values follows the P values. (The results of R_F values obtained in this way are not shown in this work.)

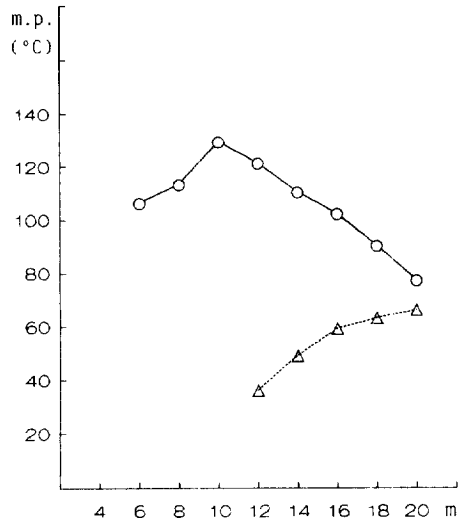


Fig. 2. Dependence of melting points on number of carbon atoms (m) in the alkyl groups for compounds **4a–h**. *Cis* isomers, – ○ –; *trans* isomers (– △ –).

The identity and structure of the prepared compounds were checked by elemental analysis (Table 1) and by interpretation of UV–Vis (Table 2), IR (Table 3), ^1H and ^{13}C NMR spectroscopy (Tables 4 and 5). In the UV spectrum there are three absorption bands (at 198, 234 and the low-intensity 274 nm transition). Values of ε_{max} of all three absorption bands of the *trans* isomers are higher than that of the corresponding *cis* isomers and increase slightly with increasing number of carbon atoms (m). The length of the alkyl chain R, as well as *cis–trans* isomerism, have only a slight influence on the position of characteristic IR absorption bands (several cm^{-1}). The occurrence of quaternary ammonium group

Table 2
UV–Vis spectral data of final compounds **4a–h** [a]

Compound	ε_{max} (198 nm)	ε_{max} (234 nm)	ε_{max} (274 nm)
Cis-**4a**	3398	1199	81
Trans-**4a**	3561	1257	96
Cis-**4b**	3380	1195	76
Trans-**4b**	3536	1207	87
Cis-**4c**	3289	1133	74
Trans-**4c**	3385	1154	81
Cis-**4d**	2934	1094	72
Trans-**4d**	3168	1115	80
Cis-**4e**	2876	1058	67
Trans-**4e**	3105	1107	79
Cis-**4f**	2662	1022	62
Trans-**4f**	2926	1084	75
Cis-**4g**	2613	943	59
Trans-**4g**	2789	1032	68

[a] In $m^2\ mol^{-1}$.

Table 3
IR spectral data (in cm^{-1}) of prepared compounds **4a–h** measured in $CHCl_3$

Compound	$\nu(C-H)_{ar}$	$\nu(C=O)$	$\nu(C=C)_{ar}$	$\delta(CH_2)$ [a]	$\nu(C-O-C)$
Cis-**4a**	3038	1715	1603, 1586	1197	1106, 1097
Trans-**4a**	3037	1716	1602, 1584	1177	1106, 1098
Cis-**4b**	3038	1715	1603, 1586	1190	1105, 1090
Trans-**4b**	3037	1716	1602, 1583	1177	1106, 1084
Cis-**4c**	3039	1715	1603, 1587	1180	1104, 1078
Trans-**4c**	3038	1716	1602, 1580	1177	1106, 1070
Cis-**4d**	3039	1715	1603, 1587	1178	1103, 1074
Trans-**4d**	3038	1716	1602, 1580	1177	1106, 1068
Cis-**4e**	3039	1716	1603, 1587	1177	1102, 1072
Trans-**4e**	3038	1717	1602, 1579	1176	1107, 1066
Cis-**4f**	3039	1716	1603, 1588	1177	1101, 1070
Trans-**4f**	3038	1717	1603, 1579	1176	1106, 1064
Cis-**4g**	3040	1717	1604, 1588	1176	1100, 1070
Trans-**4g**	3039	1718	1603, 1578	1175	1107, 1063
Cis-**4h**	3040	1717	1604, 1588	1176	1100, 1070
Trans-**4h**	3039	1718	1603, 1578	1175	1107, 1062

[a] Cyclohexane ring.

Table 4
^1H NMR chemical shifts (ppm relative to TMS) of selected compounds **4a** and **4f** [a,b]

Compound	1	2	6	7	8	9	2′,6′	3′,5′	4′
Cis-**4a**	2.68 ax.	5.37 eq.		3.50	3.45	3.40	8.04	7.48	7.61
Trans-**4a**	2.43 eq.	4.85 ax.	2.12 eq.	3.50	3.42	3.37	8.00	7.46	7.58
Cis-**4f**	2.63 ax.	5.38 eq.		3.47	3.42	3.36	8.06	7.49	7.61
Trans-**4f**	2.43 ax.	4.86 ax.	2.15 eq.	3.50	3.46	3.39	8.00	7.46	7.59
Alkyl									
	1	2	3	4	5	6	7–15	16	
Cis-**4a**	3.56	1.85	1.52	1.26	1.26	0.85			
Trans-**4a**	3.57	1.86	1.53	1.23	1.23	0.83			
Cis-**4f**	3.53	1.87	1.52	1.31	1.23	1.25	1.25	0.86	
Trans-**4f**	3.58	1.86	1.53	1.30	1.22	1.25	1.25	0.88	

[a] See Table 1 for numbering of the atoms.
[b] Signals for the other cyclohexane protons are unresolved overlapped multiplets in the region 1.3–1.8 ppm.

results in the absence of two absorption bands belonging to the stretching vibration of the methyl group. These bands are typical for tertiary *N,N*-dimethylalkylamines. Because the method of synthesis of all organic ammonium salts (**4a–4h**, Table 1) was the same for the *cis* and *trans* homologous series the NMR measurements were carried out with a few compounds only. Numbering of atoms for the evaluation of NMR spectra of measured compounds (*cis*-**4a**, *trans*-**4a**, *cis*-**4f** and *trans*-**4f**) is given in the formulae of Table 1. From the values of chemical shifts of axial and equatorial hydrogens of the carbon atoms C-1 and C-2 (Table 4), as well as analogous carbon chemical shifts of C-1 and C-2 atoms (Table 5-

Table 5
^{13}C NMR chemical shifts (ppm relative to TMS) of chosen compounds **4a** and **5f** [a,b]

Carbon atom	Compound			
	Cis-**4a**	*Trans*-**4a**	*Cis*-**4f**	*Trans*-**4f**
1	34.91	36.15	35.07	36.16
2	72.71	74.44	72.60	74.42
3	31.21	32.13	31.90	32.10
4	20.39	23.17	20.43	23.15
5	24.15	24.00	24.13	23.98
6	25.83	25.88	26.18	26.22
7	66.34	66.92	66.54	66.99
8	50.69	50.69	50.81	50.70
9	51.47	51.47	51.76	51.43
1′	133.60	133.60	133.69	133.62
2′,6′	129.65	129.54	129.67	129.55
4′	128.80	128.69	129.57	128.73
3′,5′	128.70	128.18	128.74	128.64
7′	166.17	166.11	166.20	166.12
Alkyl				
1	65.32	65.34	65.53	65.36
2	28.61	30.82	28.60	29.16
3	22.85	22.84	26.18	26.22
4	29.70	31.21	26.60	26.58
5	22.32	22.34	29.66	29.66
6	13.82	13.83	29.66	29.66
7–9			29.66	29.66
10			29.41	29.42
11			29.35	29.33
12			29.29	29.42
13			29.14	29.15
14			29.54	29.55
15			22.66	22.65
16			14.09	14.08

[a] See Table 1 for numbering of the atoms.
[b] Signals for the carbon atoms 5–9 in alkyl chain of compounds *cis*-**4f** and *trans*-**4f** are unresolved.

), it follows that the prepared *cis* and *trans* isomers possess the configurations shown in Scheme 1. The configuration on C-1 and C-2 carbon atoms of the compounds under study is analogous to the configuration of the structurally related diastereoisomeric *cis* and *trans* 1,2-disubstituted cyclohexane derivatives [12].

The problem of isomerism on the cyclohexane ring was also studied theoretically using 2-dimethylaminomethylcyclohexylbenzoate as a simpler model of the compounds studied (Fig. 1). The heats of formation as computed by AM1 are −76.2, −75.3, −74.9 and −73.5 kcal mol^{-1} for the EE, AE, EA and AA structures, Fig. 1. Thus, AM1 predicts the EE *trans* isomer to be the most stable. However, the calculated differences in energies of various species are small. The calculated population ratios (at 310 K) for four isomers (EE:AE:EA:AA) are 72:17:10:1. This indicates that, although several conformers with various probability can coexist, the most stable *trans* EE form should predominate in the crystal. All three most stable conformers are stabilised by an intramolecular hydrogen bond formed between the acidic proton of the $-CH_2-N$ group and neighbouring oxygen atoms. The interatomic distances between hydrogen and oxygen atoms are in the range 2.2–2.5 Å, which is less than the sum of the van der Waals radii (2.6 Å) of the oxygen and hydrogen atoms. In the least stable AA isomer this extra stabilisation is, for reasons of stereochemisty, impossible. The stabilisation via an intramolecular hydrogen bond may be further strengthened in the quaternary ammonium salts investigated due to the higher acidity of hydrogens of the $-CH_2-N^{(+)}$ group. The results of our theoretical calculations of model systems are in good agreement with NMR measurements of selected compounds. These studies have shown, that with *cis* derivatives the AE isomer and with *trans* structures the EE isomer is the most stable species.

All prepared compounds were tested for their antimicrobial activity on three microbiological species: *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* (Table 6, Fig. 3). The maximum of antimicrobial activity (expressed as log 1/MIC) for individual microbial species is achieved at a different number of carbon atoms, *m*. The microbial activity (on *S. aureus* and *E. coli*) is also different for *cis* and *trans* isomers.

S. aureus	*cis* isomers: log 1/MIC(max.) = 5.5; *m* = 14
	trans isomers: log 1/MIC(max.) = 5.8; *m* = 12
E. coli	*cis* isomers: log 1/MIC(max.) = 4.5; *m* = 12
	trans isomers: log 1/MIC(max.) = 4.3; *m* = 11
C. albicans	*cis* isomers: log 1/MIC(max.) = 5.5; *m* = 13
	trans isomers: log 1/MIC(max.) = 5.5; *m* = 13

From the comparison of the values of log 1/MIC of quaternary ammonium salts (QAS) **4a–4h** (Table 6, Fig. 3) with the structurally related QAS of the ester type described in the literature [7], we may conclude that our compounds belong among the most active QAS structures investigated up to now. Moreover these compounds have the great advantage of a high effect against *E. coli*.

As our investigation indicates, there are two main differences in biological behaviour of the two homologous series of compounds studied: (i) differences in maxima (Fig. 3) of biological activity for all three microbial species and for *cis* and *trans* isomers; (ii) differences in log 1/MIC values of isomers in every couple of diastereoisomers. As indicated in Fig. 3 the differences in antimicrobial activity caused by diastereoisomerism are comparable with the changes of antimicrobial activity resulting from the changes caused by 1–2 carbon atoms of the homologous series of compounds.

More lipophilic *trans* isomers achieve the maximum of antimicrobial activity against *S. aureus* and *E. coli* (Fig. 3(a)

Table 6
Antimicrobial activity log 1/MIC, lipophilicity (R_M and log *P*) and LD_{50} values in mice after S.C. application of prepared compounds [a]

Compound	*m* [b]	R_M	log *P*	log 1/MIC			LD_{50}
				S. aureus	*E. coli*	*C. albicans*	
Cis-**4a**	6	−0.18	0.215	3.737	2.533	3.131	250–350
Trans-**4a**	6	−0.25	0.350	3.436	2.232	4.039	200–300
Cis-**4b**	8	0.03	0.835	4.372	3.163	3.464	
Trans-**4b**	8	−0.14	0.935	4.671	3.464	4.367	
Cis-**4c**	10	0.0	1.496	4.791	4.095	4.189	300–400
Trans-**4c**	10	−0.02	1.778	5.383	4.093	4.790	300–400
Cis-**4d**	12	0.09	2.203	5.328	4.515	5.429	
Trans-**4d**	12	0.03	2.480	5.515	4.214	5.117	
Cis-**4e**	14	0.18	2.631	5.351	4.237	5.140	
Trans-**4e**	14	0.12	3.020	5.538	3.935	5.140	
Cis-**4f**	16	0.29	3.139	5.373	3.054	3.957	
Trans-**4f**	16	0.19	3.600	5.255	3.054	4.259	
Cis-**4g**	18	0.39	3.621	4.917	2.376	3.376	
Trans-**4g**	18	0.29	4.050	4.677	2.677	3.978	
Cis-**4h**	20	0.55		4.504	2.095	2.794	
Trans-**4h**	20	0.41		4.095	2.095	3.220	

[a] MIC minimal inhibitory concentrations in mol dm^{-3}.
[b] Number of atoms in alkyl chain.

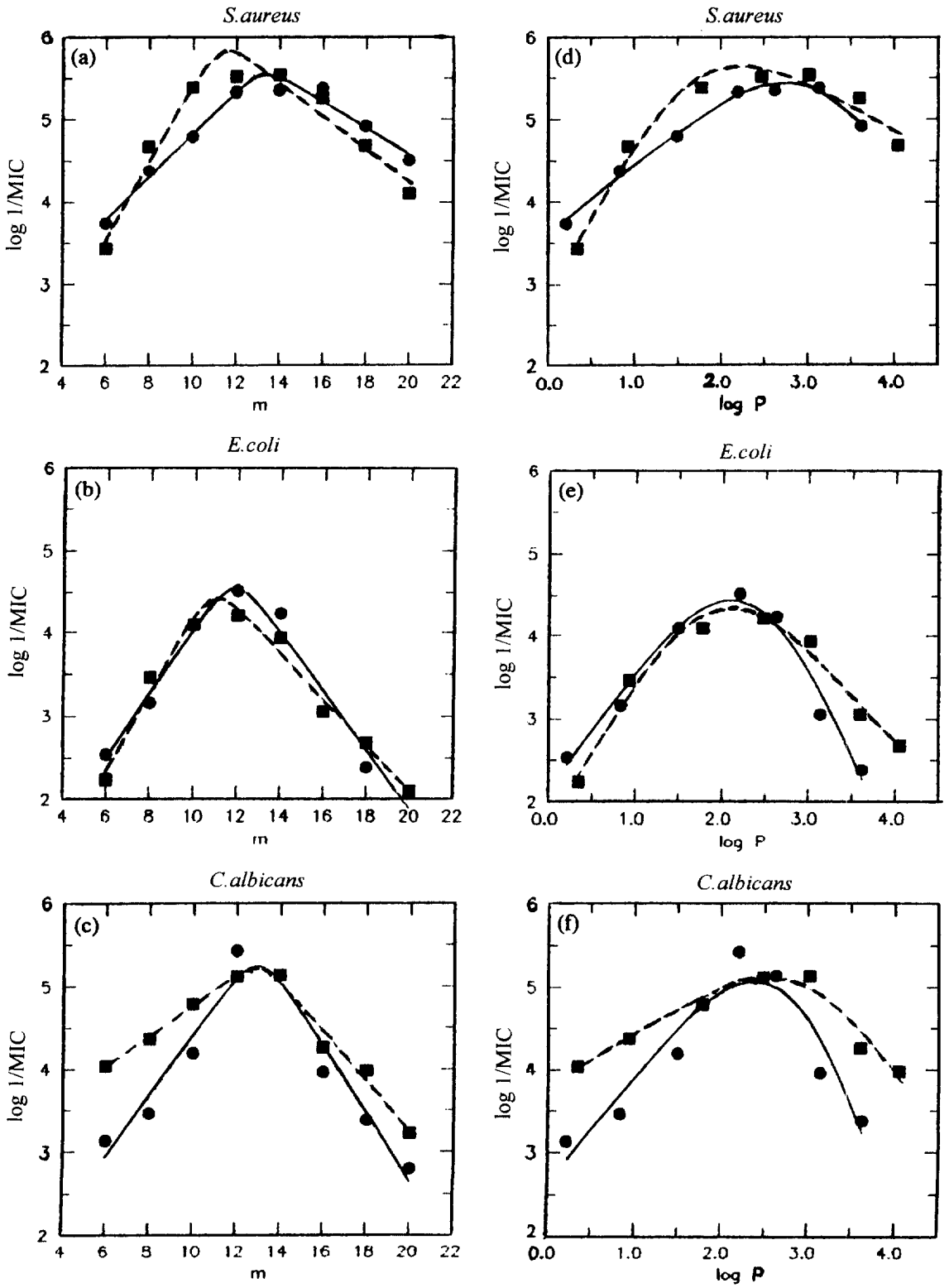


Fig. 3. The dependence log 1/MIC = $f(X)$; $X = m$ and log P for compounds **4a–h**. *Cis*, – • –; *trans*, – ■ –.

and (b)) at a lower number of carbon atoms than *cis* isomers. This difference is negligible for *C. albicans*. The difference of *cis* and *trans* isomers in antimicrobial activity results probably from the different lipophilicity of the *cis* and *trans* species (all *trans* isomers exibit higher values of P than *cis* ones).

In the case of the homologous series of *cis* and *trans* isomeric compounds, which differ by their spatial structure only, it is possible to suppose that the maximum of antimicrobial activity of the homologous series of *cis* and/or *trans* derivatives is achieved at equal lipophilicity (it means at identical values

of log P). This statement was confirmed in our investigations of *cis* and *trans* QAS (see Fig. 3). However, the results from *S. aureus* do not confirm this statement. As follows from Table 6, the prevailing number of *trans* isomers possess higher antimicrobial activity than the corresponding *cis* structures (*S. aureus* and *C. albicans* species). This phenomenon is not observed in experiments with *E. coli*. The reason for this different behaviour of compounds studied against various species of bacteria could be explained by the fact that both the lipophilicity and spatial structure are important characteristics of these compounds which regulate the bioavailability of the drug at the target site of action. The differences in physico-chemical properties and antimicrobial activity of diastereoisomers are caused by differences in spatial arrangement of two substituents in cyclohexane. In an isomer which is entitled as *cis* the benzoyloxy group is in the axial position and the *N,N*-dimethyl-*N*-alkylammoniummethyl group is in the equatorial position. In isomers entitled as *trans* both groups are in the equatorial position. One of the factors which influences the properties of compounds are intra- and intermolecular interactions. It is known that an isomer with a dominant intermolecular interaction has a higher melting point and better solubility in water (lower value of partition coefficient) than an isomer with predominating intramolecular interactions. Also in our compounds the stereoisomers with higher melting point (*cis* isomers) are more soluble in water and possess lower values of partition coefficient than the corresponding *trans* isomers. The same results were found for other diastereoisomers [12] with a similar structure to the compounds studied in this work.

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