Quaternary ammonium salts XVIII.* Preparation and relationship between structure, IR spectral characteristics, and antimicrobial activity of some new bis-quaternary isosters of 1,5-pentanediammonium dibromides

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Dedicated to Associate Professor DrPH. PhMr. J. Heger, in honour of his 60th birthday

Four series of bis-quaternary *N,N'*-bis(alkyldimethyl)-3-X-1,5-pentanediammonium dibromides (36 compounds) were prepared. The effect of alkyl chain length change, from 6 to 18 carbon atoms and of the isosteric exchange of methylene group for NCH_3, O, S upon characteristic vibrations in IR spectrum region and upon antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* was examined and described. The relationship of antimicrobial activity *vs.* R shows a nonlinear parabolic course; by isosteric exchange the effectiveness is not affected. The most effective substances are several times more active than the standard benzyldodecyldimethylammonium bromide.

Были получены четыре ряда бис-четвертичных дибромидов *N,N'*-бис(алкилдиметил)-3-X-1,5-пентандиаммония (36 соединений). Исследовалось и описано влияние изменения длины алкильной цепи (R) от 6 до 18 углеродных атомов и влияние изостерической замены метиленовой группы группой NCH_3, O или S на частоты характеристических колебаний в ИК-спектрах и на антимикробное действие по отношению к *Staphylococcus aureus, Escherichia coli* и *Candida albicans*. Зависимость антимикробной активности от R имела нелинейный параболический вид; изостерическая замена не влияла на действенность соединений. Наиболее эффективные соединения проявляли в несколько раз более высокую активность, чем стандартный бромид бензилдодецилдиметиламмония.

In spite of a relatively wide choice of disinfection agents nowadays the microorganisms represent hitherto a serious sanitary and economic problem.

*For Part *XVII* see Ref. [1].

The phenomenon of intrinsic resistance of some species as well as the ability of microorganisms to adapt for high concentrations of usual disinfectants stimulates the search for new antimicrobial compounds with higher effectiveness.

The surface-active organic ammonium salts (QUATs) belong to the group of reliable antimicrobial compounds and since the time of *Domagk* [2] who introduced in practice the well known disinfectant Zephirol (benzyldodecyldimethylammonium chloride) they have been widely used. These substances are, however, less effective upon Gram-negative bacteria and at the same time the adaptation and survival of the microorganisms also in concentrated solutions of mono-QUATs is known, as reported *e.g.* in [3, 4]. The possibility of QUATs antimicrobial activity improving has not been exhausted yet, therefore new compounds are searched for; these should have high disinfection activity.

One of the perspective groups from ammonium salts area are bis- and polyammonium salts, but only relatively few of them have been examined from the point of view of their antimicrobial effect. However, from accessible data [5—10] follows that in case of most active ones their effectiveness is higher than that of mono-QUATs, especially upon more resistant Gram-negative bacteria.

Nowadays the antimicrobial mode of action of the ammonium salts has been elucidated [11—13]. These compounds cause a generalized damage of cytoplasmic membrane so that the positively charged "head" of the molecule interacts with negatively charged membrane components followed by penetration of nonpolar tenside constituent of its hydrophobic part. The crucial first step at the membrane destruction is in this case the decrease of its electrical potential by Coulomb interactions. (This is also one of the reasons, why *e.g.* anionic and nonionic tensides of sodium dodecyl sulfate or polyethoxapropoxamers are not able to decrease the surface charge of the membrane and their too weak effect is caused by hydrophobic interactions only). Therefore, it may be assumed that increase of the number of positive centres in the molecule and at the same time also their hydrophobic parts may cause the activity increase and also a sooner start of action.

Following our previous investigation [7], in this work we examined isosteric derivatives of *N*,*N*′-bis(alkyldimethyl)-3-X-1,5-pentanediammonium dibromides

$$[R(CH_3)_2N^{\oplus}(CH_2)_2]_2X\ 2Br^{\ominus}$$

where X = CH_2, NCH_3, O or S and the linear alkyl chain (R) had 6 to 18 carbon atoms.

The surface-active and aggregation characteristics (critical micelle concentration (c.m.c.), maximum surface tension decrease at c.m.c. ($\gamma_{c.m.c.}$), concentration of surface saturation (Γ_{max}), surface area per molecule on the interphase boun-

dary (F), the standard Gibbs energy change of the micellization ($-\Delta G_m$), as well as the hydrophobicity index (I) and the equivalent chain length ($(N_{CH_2})_{eq}$)) have been reported in our previous paper [1].

The short-chain compounds of this type, where R varies from methyl to butyl are well known and were well examined, as they have been applied in clinical practice as ganglioplegica. There are considerably fewer data about the long--chain homologues, on the antimicrobial activity of decyl and dodecyl derivatives (X = CH_2) it was reported only in [7, 10] and on fungicidal activity of dodecyl and hexadecyl derivatives (X = S) upon *Gibberella fujikuroi spora* in papers [14, 15]. We have reported on the antimicrobial activity of some chosen alkyl derivatives (X = NCH_3) in [16].

Experimental

Infrared spectra were measured as a Nujol mull on KBr windows with a Specord IR-75 (Zeiss, Jena) apparatus. The accuracy of the wavenumbers readings was ± 1 cm^{-1}, the spectrophotometer calibration was performed using polystyrene foil.

The R_f values are average from five measurements and were gained on silica gel plates. The developing systems: X = S, V(acetone) : V(HCl) = 10 : 9, for all the other compounds V(acetone) : V(HCl) = 1 : 1, c(HCl) = 1 mol dm^{-3}. Detection: Dragendorf's reagent in Munier modification.

The prepared compounds are characterized in Tables 1 and 2, all melting points are uncorrected.

MIC — minimum inhibitory concentration — expressed as the lowest concentration of a compound, which is still effective at the microorganisms growth hindrance, was determined by dilution test method [8] upon strains delivered from the Czechoslovak State Collection of Type Cultures: *Staphylococcus aureus Oxford Mau 29/58, Escherichia coli E.c. 377/79, Candida albicans 45/54.* Results are presented in Table 1.

1,5-Dibromo-3-X-pentanes

1,5-Dibromo-3-thiapentane was prepared by reaction of PBr_3 in $CHCl_3$ with 3-thia--1,5-pentanediol (prepared from 2-chloroethanol and Na_2S according to [17], the yield was 87 %, b.p.(2.3 kPa) = 163 °C, n(D, 20 °C) = 1.5200; Ref. [17] reports yield 79—86 %, b.p.(2.7 kPa) = 164—166 °C) according to [18] in 92 % yield, b.p.(2.0 kPa) = 141 —143 °C, m.p. = 33 °C. Ref. [18] reports 78 % yield, b.p.(0.1 kPa) = 115.5 °C, m.p. = 31 —34 °C.

This compound is a very strong caustic and causes deep, poorly curable wounds! Decontamination of the skin should be carried out with 10 % aqueous $KMnO_4$ solution or 10 % aqueous $NaHCO_3$ solution and of the glass with 10 % aqueous NaOH or 5 % NH_4OH solution.

Table 1

N,N'-Bis(alkyldimethyl)-3-X-1,5-pentanediammonium dibromides and their antimicrobial activity

Compound	X	R	Formula[a]	M_r	Yield %	R_f	M.p. °C	MIC/(μg cm^{-3}) MIC/(μmol cm^{-3})		
								A	*B*	*C*
I	CH_2	Hexyl	$C_{21}H_{48}Br_2N_2$	488.96	89	0.45	161—162.5	5000	7000	8000
								10.225	14.316	16.361
II	CH_2	Octyl	$C_{25}H_{56}Br_2N_2$	544.56	97	0.52	217—219	30	500	400
								0.055	0.918	0.735
III	CH_2	Nonyl	$C_{27}H_{60}Br_2N_2$	572.61	92	0.54	224—226	9	100	80
								0.016	0.175	0.140
IV	CH_2	Decyl	$C_{29}H_{64}Br_2N_2$	600.67	92	0.50	226.5—228	3	20	15
								0.005	0.033	0.025
V	CH_2	Undecyl	$C_{31}H_{68}Br_2N_2$	628.72	83	0.46	225.5—227	2	15	7
								0.003	0.024	0.011
VI	CH_2	Dodecyl	$C_{33}H_{72}Br_2N_2$	656.78	95	0.40	231—233	3	20	4
								0.005	0.030	0.006
VII	CH_2	Tridecyl	$C_{35}H_{76}Br_2N_2$	684.83	86	0.33	229—230	6	30	7
								0.009	0.044	0.010
VIII	CH_2	Tetradecyl	$C_{37}H_{80}Br_2N_2$	712.88	95	0.25	227.5—229	40	100	15
								0.056	0.140	0.021
IX	CH_2	Hexadecyl	$C_{39}H_{84}Br_2N_2$	768.99	86	0.07	225.5—227	70	400	80
								0.091	0.520	0.104
X	NCH_3	Hexyl	$C_{21}H_{49}Br_2N_3$	503.46	74	0.69	155—160	1000	6000	10000
								1.986	11.917	19.862
XI	NCH_3	Heptyl	$C_{23}H_{53}Br_2N_3$	531.52	83	0.73	192—195	600	4000	3000
								1.129	7.526	5.644
XII	NCH_3	Octyl	$C_{25}H_{57}Br_2N_3$	559.57	78	0.76	217—218	60	700	400
								0.107	1.251	0.715
XIII	NCH_3	Nonyl	$C_{27}H_{61}Br_2N_3$	587.63	82	0.74	223—224	10	400	100
								0.017	0.681	0.170

Table 1 (Continued)

Compound	X	R	Formula[a]	M_r	Yield %	R_f	M.p. °C	MIC/(μg cm^{-3}) MIC/(μmol cm^{-3})		
								A	*B*	*C*
XIV	NCH_3	Decyl	$C_{29}H_{65}Br_2N_3$	615.68	74	0.70	226—227	3	60	50
								0.005	0.097	0.081
XV	NCH_3	Undecyl	$C_{31}H_{69}Br_2N_3$	643.74	83	0.62	228—229	2	15	20
								0.003	0.023	0.031
XVI	NCH_3	Dodecyl	$C_{33}H_{73}Br_2N_3$	671.79	76	0.45	229—230	2	25	8
								0.003	0.037	0.012
XVII	NCH_3	Tridecyl	$C_{35}H_{77}Br_2N_3$	699.84	76	0.30	225—226	7	50	9
								0.010	0.071	0.013
XVIII	NCH_3	Tetradecyl	$C_{37}H_{81}Br_2N_3$	727.90	75	0.15	228—229.5	30	100	20
								0.041	0.137	0.027
XIX	NCH_3	Pentadecyl	$C_{39}H_{85}Br_2N_3$	755.95	77	0.08	226.5—227	60	300	60
								0.079	0.395	0.079
XX	NCH_3	Hexadecyl	$C_{41}H_{89}Br_2N_3$	784.01	77	0.06	223.5—225	80	600	300
								0.102	0.765	0.383
XXI	O	Hexyl	$C_{20}H_{46}Br_2N_2O$	490.42	70	0.69	222—224	1000	1000	2000
								2.039	2.039	4.078
XXII	O	Octyl	$C_{24}H_{54}Br_2N_2O$	546.53	94	0.72	255—256	60	500	200
								0.110	0.915	0.366
XXIII	O	Nonyl	$C_{26}H_{58}Br_2N_2O$	574.58	76	0.70	256.5—259	10	90	100
								0.017	0.016	0.174
XXIV	O	Decyl	$C_{28}H_{62}Br_2N_2O$	602.63	88	0.48	260—262	5	50	30
								0.008	0.083	0.049
XXV	O	Undecyl	$C_{30}H_{66}Br_2N_2O$	630.69	78	0.38	256—258	4	20	9
								0.006	0.032	0.014
XXVI	O	Dodecyl	$C_{32}H_{70}Br_2N_2O$	658.75	94	0.32	257—258	3	20	4
								0.005	0.030	0.006
XXVII	O	Tridecyl	$C_{34}H_{74}Br_2N_2O$	686.80	80	0.30	255—256.5	7	30	6
								0.010	0.044	0.009

Table 1 (Continued)

Compound	X	R	Formula[a]	M_r	Yield %	R_f	M.p. °C	MIC/(μg cm^{-3}) MIC/(μmol cm^{-3}) *A*	*B*	*C*
XXVIII	O	Tetradecyl	$C_{36}H_{78}Br_2N_2O$	714.53	92	0.24	254—256	10 0.014	50 0.070	15 0.021
XXIX	O	Hexadecyl	$C_{40}H_{86}Br_2N_2O$	770.97	80	0.18	245—247	80 0.104	300 0.389	90 0.117
XXX	S	Hexyl	$C_{20}H_{46}Br_2N_2S$	506.48	84	0.55	158—161	1000 1.974	5000 9.872	6000 11.846
XXXI	S	Octyl	$C_{24}H_{54}Br_2N_2S$	562.59	80	0.59	220—221	40 0.071	400 0.711	60 0.107
XXXII	S	Decyl	$C_{28}H_{62}Br_2N_2S$	618.70	75	0.58	234—236	4 0.006	20 0.032	10 0.016
XXXIII	S	Dodecyl	$C_{32}H_{70}Br_2N_2S$	674.81	77	0.49	232—233.5	6 0.010	30 0.048	5 0.007
XXXIV	S	Tetradecyl	$C_{36}H_{78}Br_2N_2S$	730.92	97	0.35	225—226	10 0.014	80 0.109	8 0.011
XXXV	S	Hexadecyl	$C_{40}H_{86}Br_2N_2S$	787.03	89	0.16	214—216	40 0.051	2000 2.541	20 0.025
XXXVI	S	Octadecyl	$C_{44}H_{94}Br_2N_2S$	843.13	78	0.05	207—209	400 0.474	11000 13.047	80 0.095
Ajatin[b]	—	—	—	—	—	—	—	10 0.026	100 0.260	10 0.026

a) Analytical results of the elements C, H, N indicated were within ±0.2 % of the theoretical values. *b*) Benzyldodecyldimethylammonium bromide — commercially available.

MIC — minimum inhibitory concentration.

A — *Staphylococcus aureus*; *B* — *Escherichia coli*; *C* — *Candida albicans*.

1,5-Dibromo-3-oxapentane was prepared from 3-oxa-1,5-pentanediol by reaction with PBr_3 in pyridine solution according to [19], the yield was 91 %, b.p.(2.5 kPa) = 100 °C, n(D, 20 °C) = 1.5137. Ref. [20] reports b.p.(1.3 kPa) = 93—94 °C. The compound is carcinogenic!

N,N'-Bis(dimethyl)-3-X-1,5-pentanediamines

All tertiary diamines were prepared by Leuckart—Wallach's reductive methylation with formaldehyde and formic acid from primary amines according to [21]:

N,N'-Bis(dimethyl)-1,5-pentanediamine (X = CH_2) from 1,5-pentanediamine (Fluka) in 83 % yield, b.p.(1.7 kPa) = 100 °C, n(D, 20 °C) = 1.4334. Ref. [22] reports yield of 50—60 %, b.p. = 192—194 °C.

N,N'-Bis(dimethyl)-3-oxa-1,5-pentanediamine (X = O) from 3-oxa-1,5-pentanediamine (prepared by Gabriel synthesis according to [23—25] (diphthalimide yield = 75 %, m.p.(ethanol—water) = 156—158 °C)) in 44 % yield, b.p.(3.3 kPa) = 88—90 °C, n(D, 20 °C) = 1.4574. Ref. [26] reports b.p.(100 kPa) = 183—184 °C and yield of 65 %, b.p.(1.7 kPa) = 76 °C, n(D, 20 °C) = 1.4300. Ref. [27] reports yield of 52 %, b.p.(2.0 kPa) = 79—81 °C, n(D, 20 °C) = 1.4290.

N,N'-Bis(dimethyl)-3-methylaza-1,5-pentanediamine (X = NCH_3) from 3-aza-1,5--pentanediamine (diethylenetriamine) according to [6] in 85 % yield, b.p.(1.3 kPa) = = 82—83 °C, n(D, 20 °C) = 1.4426. Ref. [28] reports b.p.(1.6 kPa) = 85—86 °C.

N,N'-Bis(alkyldimethyl)-3-X-1,5-pentanediammonium dibromides I—XXXVI

Method A

To the mixture of dry methyl cyanide (30 cm^3) and 3-X-1,5-dibromopentane (0.05 mol), 0.12 mol of *N,N*-dimethylalkylamine was added (X = CH_2, O or S; the linear alkyl chain had 6, 8, 10, 12, 14, 16 carbon atoms if X = CH_2, 8, 10, 12, 14, 16 carbon atoms if X = O, and 6, 8, 10, 12, 14, 16, 18 carbon atoms if X = S).

Method B

To the mixture of dry methyl cyanide (30 cm^3) and *N,N'*-bis(dimethyl)-3-X-1,5--pentanediamine (0.05 mol), 0.12 mol of 1-bromoalkane was added (X = CH_2 or O). If X = S, the reaction mixture was warmed up for 12 h under reflux, if X = NCH_3 for 4 h. If X = CH_2 or O, the reaction mixture was left to stand for 24 h followed by 1 h warming up under reflux.

All compounds were further worked up by the same procedure: after having distilled the solvent off *in vacuo*, the drying of the product was completed by azeotropic distilla-

tion with toluene and crystallization from dry acetone, or from the mixture of dry acetone and dry ethanol. White, crystalline slightly hygroscopic compounds, soluble in water and in polar solvents, insoluble in nonpolar ones should be stored in vacuum desiccator.

Results and discussion

The preparation of ammonium salts was performed by reaction of corresponding bromo derivatives and tertiary amines in methyl cyanide. At the synthesis, we applied two methods: the even derivatives (X = CH_2, O or S) were prepared by reaction of 3-X-1,5-dibromopentane with accessible *N*,*N*-dimethylalkylamines with even number of carbon atoms in the chain; the odd homologues by reaction of *N*,*N'*-bis(dimethyl)-3-X-1,5-pentanediamines and 1-bromoalkanes with odd number of carbon atoms in the chain. In the case of X = NCH_3 all bis-QUATs were prepared from *N*,*N'*-bis(dimethyl)-3--methylaza-1,5-pentanediamine and 1-bromoalkanes, the starting 3-aza-1,5--pentanediamine being an accessible and nonexpensive compound. In contrast to usual methods of quaternization, where methanol or ethanol are used, we preferred methyl cyanide as a solvent. An advantage of this solvent is the fact that most of prepared bis-QUATs (except the short-chain derivatives C_6—C_8) are only very little soluble in it at room temperature and therefore the reaction equilibrium is very soon shifted in the direction of salt formation. In the case of derivatives with X = CH_2 or O the reaction takes place already at room temperature, the same is true also for X = NCH_3. The yields were in case of 3-methylaza isomers relatively low, therefore it was necessary to warm up the reaction mixture under reflux for 4 h. With the derivatives, where X = S it is surprising that the reaction at room temperature is very slow and also after 72 h standing the yield was only about 20 %. After 12 h warming up under reflux the yield increased to satisfactory level.

The products purity was besides elemental analysis and TLC proved also by reversed phase partition chromatography [29] and IR spectroscopy.

With regard to the substrates structure—presence of a long alkyl chain in the molecule — there were with all four types of compounds observed in IR spectra characteristic bands $\varrho(CH_2)$ belonging to vibration of aliphatic chain having at least 4 linearly bonded carbon atoms. This vibration occurs approximately at $\tilde{\nu} = 720\ cm^{-1}$ and with all compounds it was observed in this region ($\tilde{\nu} =$ = 713—721 cm^{-1}) (Table 2). Only when X = NCH_3 this vibration was shifted to values of $\tilde{\nu} = 707$—$715\ cm^{-1}$. The effect of chain length upon this vibration is relatively small, with 3-thia derivatives we observed a linear decrease of the $\varrho(CH_2)$ values beginning at the octyl homologue. Also in the case of isosters (X = O and NCH_3) the value of this vibration shows descending tendency,

Table 2

Infrared spectral data ($\tilde{\nu}$/cm^{-1}) of *N,N'*-bis(alkyldimethyl)-3-X-1,5-pentanediammonium dibromides

Compound	$\varrho(CH_2)$	Compound	$\delta(S—CH_2)$	$\Delta\delta(S—CH_2)$	$\varrho(CH_2)$
I	715	*XXX*	1230, 1243[a]	13	714
II	720	*XXXI*	1228, 1244[a]	16	721
III	718	*XXXII*	1225[a], 1243	18	719
IV	719	*XXXIII*	1222[a], 1234	12	718
V	719	*XXXIV*[b]	1220, 1228	8	717
VI	719	*XXXV*[b]	1218, 1226	8	717
VII	717	*XXXVI*	— 1226	—	716
VIII	717				
IX	719				

Compound	$\nu(N—CH_3)$	$\nu(C—N)$	$\varrho(CH_2)$	Compound	$\nu(C—O—C)_{as}$	$\varrho(CH_2)$
X	2785	1114	718	*XXI*	1145, 1129	721
XI	2786	1114	716	*XXII*	1146, 1127	722
XII	2787	1113	715	*XXIII*	1144, 1127	720
XIII	2789	1113	713	*XXIV*	1146, 1128	720
XIV	2788	1111	712	*XXV*	1143, 1128	719
XV	2791	1111	710	*XXVI*	1144, 1124	713
XVI	2792	1109	710	*XXVII*	1145, 1124	713
XVII	2793	1105	707	*XXVIII*	1144, 1124	713
XVIII	2793	1123	707	*XXIX*	1140, 1120	712
XIX	2793	1117	708			
XX	2793	1126	708			

a) More intensive band; *b*) both bands the same, low intensity.

beginning at $C_{10}—C_{12}$. With 1,5-pentanediammonium derivatives ($X = CH_2$) the change of alkyl chain length has no significant effect upon $\varrho(CH_2)$ vibrations. In the case of further absorption bands belonging at compounds with $X = NCH_3$ (*X—XX*) to stretching vibrations of C—H bond in NCH_3 group ($\nu(N—CH_3)$) which for this type of compounds (the molecule has a tertiary $N—CH_3$ group) occur in the region of $\tilde{\nu} = 2787$ to $2793\ cm^{-1}$, we observed beginning at decyl derivative the increase of these values, those starting from tridecyl derivative were stabilized at $\tilde{\nu} = 2793\ cm^{-1}$. Another characteristic vibration of this group of molecules ($\nu(C—N)$) showed no regular changes and occurred between $\tilde{\nu} = 1105—1126\ cm^{-1}$.

With 3-thia derivatives (*XXX—XXXVI*) we examined besides $\varrho(CH_2)$ also the characteristic "wagging" vibration of the $S—CH_2$ bond ($\delta(S—CH_2)_{wag}$: CH_2 group immediately adjacent to sulfur atom). This vibration in the region of

$\tilde{\nu} = 1250\ \mathrm{cm}^{-1}$ is the only absorption, which may indicate the presence of sulfide bond, because the band belonging to stretching vibration C—S in the region $\tilde{\nu} = 625$—$715\ \mathrm{cm}^{-1}$ is very weak and not suitable for identification purposes [30]. The mentioned bending vibration $\delta(\mathrm{S—CH_2})_{\mathrm{wag}}$ was relatively weak and could be registered only at high sample concentration. With investigated 3-thia bis-QUATs (Table 2) we observed doublet of bands corresponding to this vibration in the region of $\tilde{\nu} = 1218$—$1230\ \mathrm{cm}^{-1}$ and 1226—$1244\ \mathrm{cm}^{-1}$. Both these bands were affected by alkyl chain length and shifted to lower wavenumbers. At the same time we observed also changes in intensity of these bands and changes in their relative distance. From hexyl to decyl derivative (*XXX*—*XXXII*) the relative distance $\Delta\delta(\mathrm{S—CH_2})$ of the bands increases, at dodecyl derivative (*XXXIII*) a decrease begins and at octadecyl derivative (*XXXVI*) both bands coincide in one with $\tilde{\nu} = 1226\ \mathrm{cm}^{-1}$. The bands intensity decreases with chain lengthening.

The band at higher wavenumber ($\tilde{\nu} = 1226$—$1244\ \mathrm{cm}^{-1}$) is affected probably by molecule stereochemistry, because the wavenumber values decrease from dodecyl derivative; from tetradecyl homologue a stabilized course was observed. We assume, it is the case — similarly as in other cases of the characteristic vibrations of groups affected by the chain length — of hydrocarbon chains interaction with polar ammonium groups proceeding by mechanism we have reported in [31]. The second band ($\tilde{\nu} = 1218$—$1230\ \mathrm{cm}^{-1}$) is affected by electronic effects in molecule, this may be concluded from the linear decrease, which shows no linearity break (in contrast to all previous cases of the characteristics affected by aliphatic chain length change).

The derivatives with oxygen in position 3 of the joining bridge showed characteristic ether band $\nu(\mathrm{C—O—C})_{\mathrm{as}}$ in the region of $\tilde{\nu} = 1120$—$1146\ \mathrm{cm}^{-1}$ (Table 2). The band, which at aliphatic ethers occurs at about $\tilde{\nu} = 1110\ \mathrm{cm}^{-1}$ [32] was in case of examined ammonium salts shifted to higher wavenumbers and splitted to distinctly resolvable doublet of similar intensity. The splitting is similar as in the case of 3-thia derivatives and could be explained by the change of electron arrangement in C—O—C bond. However, with regard to significantly higher electronegativity of oxygen in comparison with sulfur and considerably lower polarizability of the oxygen than that of the sulfur the change of the alkyl chain length does not cause so outstanding changes as in the case of thia isomers. The shift of bands to higher wavenumbers is caused by the presence of strong $-I$ (inductive) effect of two ammonium groups (similarly there was observed a shift of $\nu(\mathrm{C—O—C})_{\mathrm{as}}$ at conjugated ethers of the vinyl type up to $\tilde{\nu} = 1200\ \mathrm{cm}^{-1}$, or at aromatic ethers to $\tilde{\nu} = 1250\ \mathrm{cm}^{-1}$, in consequence of mesomeric effects), as well as by the possibility of formation of different conformers with diverse internal energy (splitting of the bands).

The evaluation of antimicrobial activity tests (Table 1) showed that the relationship MIC $= f(R)$ has a nonlinear, parabolic course, similarly, as we reported in [5]. The described bis-QUATs reach the maximum activity in the region of 10—12 carbon atoms in the alkyl chain, regardless of microbial strain and isosteric exchange. All examined compounds with comparable alkyl chains (*e.g.* dodecyl derivatives but also others) show approximately the same activity. In comparison with classical commercial disinfectant Ajatin (benzyldodecyldimethylammonium bromide), the most effective from examined bis-QUATs are several times more active upon all the applied microorganisms.

The isosteric exchange of one atom in the joining bridge in principle has no effect upon antimicrobial activity (Table 1); this is affected neither by the change of molecule stereochemistry due to the change of heteroatom or group size. That means, such molecule modification does not affect its mode of action which is with all four types the same and the rate of which depends almost upon structural changes immediately affecting the ammonium group.

We came to similar conclusions with these compounds also at quantification of the relationships structure—physical characteristics—antimicrobial activity by methods of QSAR analysis [33]. The results indicate that the antimicrobial mode of action for all examined compounds is the same, independent of the isosteric change and the investigated characteristics (log $(1/\{\text{MIC}\}) = f(R)$ and log $(1/\{\text{MIC}\}) = f(\log(\{\text{c.m.c.}\}))$ expressed in regression equations at significance level of 99 to 99.9 % may be for individual strains of microorganisms generalized in the form of only one correlation equation.

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