# SYNTHESIS, IR SPECTRA, AND ANTIMICROBIAL ACTIVITY OF SOME BIS-AMMONIUM SALTS OF N,N'-BIS(2-DIMETHYLAMINOETHYL)METHYLAMINE*

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Preparation of 2-bromoethyl alkanoates and alkenoates, N,N'-bis(2-dimethylaminoethyl)methylamine (pentamethyldiethylenetriamine) and some of its bis-ammonium salts is described. IR spectra of the esters and ammonium salts have been interpreted. Antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* for the bis-ammonium salts has been determined.

N,N-Bis(2-aminoethyl)amine (diethylenetriamine, DETA) belongs to the most wide-spread and applied polyamines. Also some of its derivatives are very important, *viz. e.g.* Pendiomid (N,N'-bis(ethyldimethyl)-3-methylaza-1,5-pentanediammonium dibromide) which is used in clinical practice as a very efficient ganglioblocking agent with negligible side effects[1] and replaced almost completely such agents as *e.g.* hexamethonium. Although the mono- as well as the bis-ammonium salts which contain at least one long chain in their molecules are known to possess – besides very good surface activity – also a significant disinfection effect[2,3], the long-chain bis-ammonium salts of DETA have been paid little attention so far[4,5], in spite of DETA being a cheap and easily accessible raw material. Therefore, the present paper deals with preparation and investigation of antimicrobial activity of some bis-ammonium salts of DETA whose molecules contain long alkyl chains attached to the ammonium nitrogen atoms by means of ester groups. Such compounds are classified as so called soft antimicrobial agents[6], which means that their high antimicrobial activity is maintained and, at the same time, it is presumed that undesirable side effects are suppressed and/or their possible metabolites represent biologically inactive or little active units. We have investigated N,N'-bis(acyloxyethyl)-N,N,N',N',3-pentamethyl-3-aza-1,5-pentanediammonium dibromides.

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## EXPERIMENTAL

### 2-Bromoethyl Alkanoates and Alkenoates (Table I, Nos 1—10)

These compounds were prepared by reactions of the respective acids with 2-bromoethanol (prepared[7] from 1,2-ethanediol and $PBr_3$) in benzene medium in the presence of sulphuric acid[8,9].

### N,N-Bis(2-dimethylaminoethyl)methylamine

The compound was prepared by methylation of DETA according to refs[10,11] (15 mol formic acid and 10 mol 36% aqueous formaldehyde per 1 mol DETA). The minimum reaction time is 8 h. Yield 85%, b.p. 82—83°C/1·3 kPa; $n_D^{20}$ = 1·4425 (ref.[1] gives b.p. 85—86°C/1·6 kPa).

### Bis-ammonium Salts (Table II, Nos 11—20)

N,N'-Bis(acyloxyethyl)-N,N,N',N',3-pentamethyl-3-aza-1,5-pentanediammonium dibromides were prepared by reaction of 0·05 mol N,N-bis(2-dimethylaminoethyl)methylamine with 0·1 mol respective 2-bromoethyl ester in 30 ml acetonitrile at room temperature (24 h). The solvent was distilled off *in vacuo*, the residue was dried by azeotropic distillation with benzene or toluene, and recrystallized from a 100 : 1 acetone–methanol mixture until constant melting point.

### Measurements

The IR spectra were measured with an IR-75 spectrometer (Zeiss, Jena), all the liquid compounds being measured in original state as a film on KBr windows. The bis-ammonium salts were measured in Nujol mulls suspensions on KBr windows, in KBr pellets, and as solutions in dichloromethane and chloroform. The apparatus was calibrated by means of a polystyrene film the wavenumber readout accuracy was $\pm 1\ cm^{-1}$. The spectral characteristics are given in Tables III and IV. Purity of all the products was checked by elemental analysis, that of the bis-ammonium salts also by TLC on Silufol with acetone–1M-HCl (1 : 1) as eluent[12] or on cellulose plates (Merck) with chloroform–methanol–water (75 : 22 : 3) as eluent[13]. In both cases the spots were made visible with the Draggendorf reagent in the Munier modification[14]. The melting points are uncorrected.

The antimicrobial activity of the bis-ammonium salts was determined against microorganisms *Staphylococcus aureus* Mau 29/58, *Escherichia coli* 355/7, and *Candida albicans* 45/53 by the dilution test[3] method. Table V gives the values of the minimum inhibition concentrations (MIC). MIC is defined as the lowest amount of the substance which inhibits the microorganisms in the medium.

## RESULTS AND DISCUSSION

One of the starting components for preparation of the bis-ammonium salts of N,N'-bis(acyloxyethyl)-N,N,N',N',3-pentamethyl-3-aza-1,5-pentanediamonium dibromides was the N,N-bis(2-dimethylaminoethyl)methylamine which was synthetized in 85% yield by permethylation of DETA. For high yields it is important to carry out the reaction at 100—110°C for at least 8 h, because the methylation consists of three steps. The methylation at the two terminal nitrogen atoms is almost equally fast ($k'_1 = 5{\cdot}66\,.\,10^{-2}$ and $k'_2 = 2{\cdot}03\,.\,10^{-2}\,\mathrm{dm^3\,mol^{-1}\,min^{-1}}$), but that at the central nitrogen atom is markedly affected by both electrostatic and steric effects, and the reaction rate decreases by as much as two orders of magnitude ($k'_3 = 8{\cdot}83\,.\,.\,10^{-4}\,\mathrm{dm^3\,mol^{-1}\,min^{-1}}$)[15]. Besides that, the nucleophilicity of the central nitrogen atom is decreased, too, which can be seen *e.g.* from the $pK_A$ values of the individual nitrogen atoms in DETA molecule ($pK_{A1} = 9{\cdot}35$, $pK_{A2} = 8{\cdot}12$, $pK_{A3} = 2{\cdot}73$ at 20°C)[11,16].

TABLE I

Properties of 2-bromoethyl alkanoates and alkenoates $RCO_2CH_2CH_2Br$

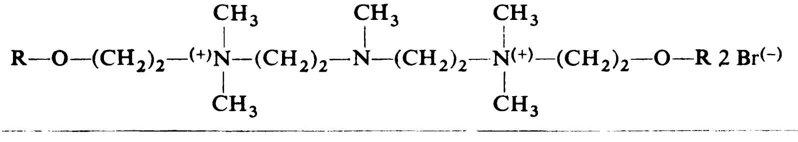
| Compound<br>R | Formula<br>(mol.wt.) | B.p., °C/kPa<br>$n_D^{20}$ | Calculated/Found |  | Yield<br>% |
|---|---|---|---|---|---|
|  |  |  | % C | % H |  |
| 1<br>Pentyl | $C_8H_{15}BrO_2$<br>(223·1) | 107/2·00<br>1·4551 | 43·07<br>43·15 | 6·78<br>6·58 | 78[a] |
| 2<br>Hexyl | $C_9H_{17}BrO_2$<br>(237·2) | 123—124/2·40<br>1·4548 | 45·58<br>45·71 | 7·23<br>7·12 | 72 |
| 3<br>Heptyl | $C_{10}H_{19}BrO_2$<br>(251·2) | 137/1·90<br>1·4555 | 47·82<br>47·98 | 7·62<br>7·85 | 72 |
| 4<br>Octyl | $C_{11}H_{21}BrO_2$<br>(265·2) | 150—151/2·40<br>1·4565 | 49·82<br>50·06 | 7·98<br>8·11 | 70 |
| 5<br>Nonyl | $C_{12}H_{23}BrO_2$<br>(279·2) | 162/1·60<br>1·4570 | 51·62<br>51·69 | 8·30<br>8·37 | 82 |
| 6<br>Decyl | $C_{13}H_{25}BrO_2$<br>(293·3) | 174/1·60<br>1·4595 | 53·25<br>53·36 | 8·59<br>8·65 | 71 |
| 7<br>9-Decenyl | $C_{13}H_{23}BrO_2$<br>(291·2) | 131/0·09<br>1·4681 | 53·62<br>53·73 | 7·96<br>8·10 | 70 |
| 8<br>Undecyl | $C_{14}H_{27}BrO_2$<br>(307·3) | 123/0·01<br>m.p. 25°C | 54·72<br>54·92 | 8·86<br>8·99 | 83[b] |
| 9<br>Tridecyl | $C_{16}H_{31}BrO_2$<br>(335·3) | 164/0·05<br>m.p. 30—32°C | 57·31<br>57·55 | 9·32<br>9·58 | 69[c] |
| 10<br>*cis*-8-Heptadecenyl | $C_{20}H_{37}BrO_2$<br>(389·4) | 190/0·04<br>1·4710 | 61·68<br>61·88 | 9·58<br>9·73 | 71[d] |

[a] Ref.[9] gives 74%, b.p. 116—118°C/2·7 kPa, $n_D^{20} = 1{\cdot}4505$; [b] ref.[9] gives 84%, b.p. 114—119°C/0·07 kPa, $n_D^{20} = 1{\cdot}4564$; [c] ref.[9] gives 59%, b.p. 157—160°C/0·02 kPa, $n_D^{20} = 1{\cdot}4539$; [d] ref.[9] gives 74%, b.p. 185—186°C/0·08 kPa, $n_D^{20} = 1{\cdot}4669$.

With regard to preparation of 2-bromoethanol it must be mentioned that the distillation of the product must be carried out in inert atmosphere, because in the presence of air the distillation residue can undergo self-ignition after the distillation is finished.

TABLE II

Properties of N,N'-bis(acyloxyethyl)-N,N,N',N'-3-pentamethyl-3-aza-1,5-pentanediammonium dibromides



$$R{-}O{-}(CH_2)_2{-}{}^{(+)}\underset{CH_3}{\overset{CH_3}{N}}{-}(CH_2)_2{-}\overset{CH_3}{N}{-}(CH_2)_2{-}\underset{CH_3}{\overset{CH_3}{N}}{}^{(+)}{-}(CH_2)_2{-}O{-}R\ 2\,Br^{(-)}$$

| Compound R | Formula (m.wt.) | M.p., °C Yield, % | Calculated/Found | | | $R_F$ $a/b$ |
|---|---|---|---|---|---|---|
| | | | %C | %H | %N | |
| 11 | $C_{25}H_{53}Br_2N_3O_4$ | 191–192 | 48·47 | 8·62 | 6·78 | 0·79 |
| Hexanoyl | (619·5) | 60 | 48·21 | 8·81 | 6·90 | 0·40 |
| 12 | $C_{27}H_{57}Br_2N_3O_4$ | 194–196 | 50·08 | 8·87 | 6·49 | 0·79 |
| Heptanoyl | (647·6) | 60 | 49·81 | 9·03 | 6·29 | 0·46 |
| 13 | $C_{29}H_{61}Br_2N_3O_4$ | 204–206 | 51·55 | 9·10 | 6·22 | 0·78 |
| Octanoyl | (675·7) | 60 | 51·53 | 9·25 | 6·38 | 0·51 |
| 14 | $C_{31}H_{65}Br_2N_3O_4$ | 207–208 | 52·91 | 9·33 | 5·97 | 0·76 |
| Nonanoyl | (703·7) | 60 | 52·74 | 9·40 | 6·01 | 0·54 |
| 15 | $C_{33}H_{69}Br_2N_3O_4$ | 209–211 | 54·17 | 9·50 | 5·74 | 0·73 |
| Decanoyl | (731·8) | 61 | 53·92 | 9·32 | 5·99 | 0·57 |
| 16 | $C_{35}H_{73}Br_2N_3O_4$ | 212–213 | 55·33 | 9·68 | 5·53 | 0·68 |
| Undecanoyl | (759·8) | 61 | 55·26 | 9·26 | 5·70 | 0·61 |
| 17 | $C_{35}H_{69}Br_2N_3O_4$ | 203–204 | 55·62 | 9·20 | 5·56 | 0·75 |
| 10-Undecenoyl | (755·8) | 60 | 55·51 | 9·21 | 5·76 | 0·58 |
| 18 | $C_{37}H_{77}Br_2N_3O_4$ | 197–199 | 56·41 | 9·85 | 5·33 | 0·59 |
| Dodecanoyl | (787·9) | 62 | 56·20 | 10·00 | 5·35 | 0·62 |
| 19 | $C_{41}H_{85}Br_2N_3O_4$ | 203–204 | 58·35 | 9·91 | 4·98 | 0·00 |
| Tetradecanoyl | (844·0) | 59 | 58·57 | 9·79 | 4·86 | 0·72 |
| 20 | $C_{49}H_{97}Br_2N_3O_4$ | 196–198 | 61·28 | 10·48 | 4·41 | 0·00 |
| *cis*-9-Octadecenoyl | (952·2) | 44 | 61·05 | 10·66 | 4·22 | 0·75 |

[a] Silufol; [b] cellulose.

For the preparation of the final bis-ammonium salts the currently used method of heating of the reaction mixture at the boiling point of the solvent[19] failed, because the resulting ammonium salt (whose yields were about 70%) was always contaminated with elimination products which cannot be separated by usual purification procedures. It is, therefore, important to carry out this $S_N2$ reaction at room temperature (at lower temperatures the reaction practically ceases). The tris-ammonium salts derived from N,N-bis(2-dimethylaminoethyl)methylamine (the preparation is described: by the reaction of this amine with various types of halogen derivatives) cannot be prepared even in autoclave. The bis-ammonium salts only are formed, being contaminated by the elimination reaction products. The data given in literature[1,17,18] on the tris-ammonium salts of DETA do not obviously characterize the compounds with the presumed structure.

The IR spectra were interpreted according to refs[20,21]. We found that the stretching vibrations $\nu(C{=}O)$ of 2-bromoethyl esters are not substantially changed with the length of the chain and are in the range of 1 743 – 1 740 $cm^{-1}$. The compounds containing multiple bonds in their chains (No 7 and 10) form an exception, their vibrations being shifted to lower values of wave number (1 729 and 1 737 $cm^{-1}$, resp.). Except for the first three compounds (Nos 1 – 3), the asymmetric vibrations $\nu(C{-}O{-}C)$ of all compounds are in the region below 1 160 $cm^{-1}$, and position of the absorption bands shows linear dependence on the chain length (Table III)

TABLE III

Infrared spectral characteristics of 2-bromoethyl esters of alkane and alkene carboxylic acids (wave numbers in $cm^{-1}$)

| Compound | $\nu(C{=}O)$ | $\nu(C{-}O{-}C)$ | $\varrho(CH_2)$ | $\nu(C{-}Br)$ |
|---|---|---|---|---|
| 1 | 1 741 | 1 161 | 732 | 581 |
| 2 | 1 741 | 1 160 | 729 | 577 |
| 3 | 1 742 | 1 160 | 725 | 574 |
| 4 | 1 741 | 1 159 | 723 | 572 |
| 5 | 1 740 | 1 158 | 722 | 571 |
| 6 | 1 740 | 1 157 | 721 | 570 |
| 7[a] | 1 729 | 1 152 | 718 | 568 |
| 8 | 1 742 | 1 156 | 720 | 571 |
| 9 | 1 743 | 1 155 | 719 | 570 |
| 10[b] | 1 737 | 1 153 | 718 | 569 |

[a] 10-Undecenoic acid derivative $\nu({=}CH)$ 3 059, $\nu(C{=}C)$ 1 634, $\delta$ $({=}CH_2)$ 904; [b] *cis*-9-octadecenoic acid derivative $\nu({=}CH)$ 2 987, $\nu(C{=}C)$ 1 648.

(the difference between the compounds 1 and 9 is $\Delta\nu(C—O—C) = 6\,cm^{-1}$). Similar regular changes were also observed in case of the deformation (rocking) vibrations $\varrho(CH_2)$ ($\Delta\varrho(CH_2) = 13\,cm^{-1}$) and stretching vibrations $\nu(C—Br)$ ($\Delta\nu(C—Br) = 11\,cm^{-1}$) wherefrom it can be concluded that the probable reason consists in mutual influence of the aliphatic chain and the functional group by the mechanism given in ref.[22]. In case of the final bis-ammonium salts we also investigated the vibrations due to $\nu(C{=}O)$, $\nu(C—O—C)$, $\varrho(CH_2)$, and $\nu(N—CH_3)$. Both $\nu(C{=}O)$ and $\nu(C—O—C)$ were found to have a shape of doublet. In case of $\nu(C{=}O)$ the difference of the absorption maxima of the doublet gradually decreased with increasing chain length until with the compounds 19 and 20 (the derivatives of tetra-

TABLE IV

Infrared spectral characteristics of N,N′-bis(acyloxyethyl)-N,N,N′,N′-3-pentamethyl-3-aza-1,5-pentanediammonium dibromides (wave numbers in $cm^{-1}$)

| Compound | $\nu(C{=}O)$[a] | $\Delta\nu(C{=}O)$ | $\nu(C—O—C)$ | $\varrho(CH_2)$ | $\nu(N—CH_3)$ |
|---|---|---|---|---|---|
| 11 | 1 741<br>1 723 | 18 | 1 152[b]<br>1 140 | 715 | 2 757 |
| 12 | 1 742<br>1 723 | 19 | 1 153<br>1 139[b] | 712 | 2 756 |
| 13 | 1 742<br>1 723 | 19 | 1 154<br>1 139[b] | 711 | 2 757 |
| 14 | 1 741<br>1 723 | 18 | 1 155<br>1 138[b] | 711 | 2 753 |
| 15 | 1 739<br>1 723 | 16 | 1 156<br>1 138[b] | 710 | 2 751 |
| 16 | 1 738<br>1 730 | 8 | 1 158[b]<br>1 139 | 710 | 2 751 |
| 17[c] | 1 736<br>1 728 | 8 | 1 160[b]<br>1 142 | 710 | 2 751 |
| 18 | 1 734<br>1 729 | 5 | 1 156<br>1 138[b] | 711 | 2 754 |
| 19 | 1 729 | — | 1 158[b]<br>1 141 | 710 | 2 751 |
| 20[d] | 1 728 | — | 1 140 | 704 | 2 749 |

[a] Sharp doublet, second member of the doublet was in all cases more intense; [b] slightly more intense; [b] 10-undecenoic acid derivative $\nu(=CH)$ 3 027, $\nu(C{=}C)$ 1 627, $\delta(=CH_2)$ 912; [d] *cis*-9-octadecenoic acid derivative $\nu(=CH)$ 2 994, $\nu(C{=}C)$ 1 607.

decanoic and *cis*-9-octadecenoic acids) we observed a single band only. In the case of $\nu$(C—O—C) such a regular decrease in the differences $\Delta\nu$(C—O—C) was not observed. The shape of spectrum was the same for the Nujol mull and KBr pellets in the region of about 1 700 as well as about 1 150 cm$^{-1}$. If solutions (dichloromethane, chloroform) of the compounds were measured, the doublet only appeared

TABLE V

Antimicrobial activity (MIC[a]) of N,N'-bis(acyloxyethyl)-N,N,N',N'-3-pentamethyl-3-aza-1,5-pentanediammonium dibromides

| Compound | *S. aureus* | *E. coli* | *C. albicans* |
|---|---|---|---|
| 11 | >1 000<br>(>1·6141) | >1 000<br>(>1·6141) | >1 000<br>(>1·6141) |
| 12 | 300<br>(0·4633) | 900<br>(1·3898) | 400<br>(0·6167) |
| 13 | 80<br>(0·1184) | 800<br>(1·1840) | 300<br>(0·4440) |
| 14 | 10<br>(0·0142) | 60<br>(0·0853) | 100<br>(0·1421) |
| 15 | 7<br>(0·0096) | 40<br>(0·0547) | 30<br>(0·0410) |
| 16 | 10<br>(0·0132) | 50<br>(0·0658) | 20<br>(0·0263) |
| 17[b] | 20<br>(0·0265) | 60<br>(0·0794) | 50<br>(0·0662) |
| 18 | 20<br>(0·0254) | 200<br>(0·2538) | 20<br>(0·0254) |
| 19 | 200<br>(0·2370) | >1 000<br>(>1·1849) | 200<br>(0·2370) |
| 20[c] | 200<br>(0·2100) | >1 000<br>(>1·0502) | >1 000<br>(>1·0502) |
| Ajatin[d] | 10<br>(0·0260) | 100<br>(0·2600) | 10<br>(0·0260) |
| Septonex[e] | 2<br>(0·0047) | 20<br>(0·0470) | 0·8<br>(0·0019) |

[a] MIC minimal inhibition concentration in μg cm$^{-3}$ (mmol dm$^{-3}$); [b] 10-undecenoic acid derivative; [c] *cis*-9-octadecenoic acid derivative; [d] benzyldodecyldimethylammonium bromide; [e] 2-((ethoxycarbonyl)pentadecyl)trimethylammonium bromide.

as a shoulder at the main band. The stretching vibration $\nu(N—CH_3)$ served as analytical band, and the disappearance of $\nu(C—Br)$ in spectra of the final bis-ammonium salts also had diagnostical value.

The antimicrobial activity of the bis-ammonium salts was determined by means of the dilution test and was found to increase with increasing chain length of the carboxylic acid, reaching its maximum value with the compounds 15 and 16 (derivatives of decanoic and undecanoic acids), wherefrom it again decreases. The terminal double bond in compound 17 does not affect substantially the activity (the activity is slightly lowered with respect to *C. albicans*), but the increase of the chain length above 12 carbon atoms is accompanied by complete loss of antimicrobial activity. Obviously this phenomenon is closely connected – besides other effects – also with the ability of such compounds to form aggregates in aqueous media, which alters significantly not only their physico-chemical properties but also their biological effects[23]. Comparison with standards (the currently used disinfection agents of the type of organic ammonium salts, Ajatin and Septonex) showed that the most efficient of the compounds investigated have higher effects than Ajatin on all tested types of microorganisms, the compound 15 being comparable with Septonex (the latter being more efficient towards *Candida albicans* only).

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