Synthesis, Lipophilicity Studies and Antibacterial Properties of Some Novel Quaternary Ammonium Salts

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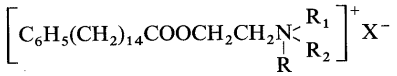
The synthesis of some novel quaternary ammonium salts, derivatives of 15-phenyl-decapentanoic acid, is described. Their lipophilicity was estimated applying the Hansch–Leo fragmental procedure and measured by means of reversed phase thin layer chromatography. All compounds were tested for their antibacterial activitiy against Gram positive and gram negative microorganisms. The less lipophilic compounds showed weak activity, mainly against gram positive microorganisms.

Keywords quaternary ammonium salt; 15-phenyl-pentadecanoic acid; antibacterial activity; lipophilicity; thin layer chromatography; hydrophobic fragmental constant

From the beginning of this century until now, considerable attention has been given by medicinal chemists to the design and synthesis of quaternary ammonium salts due to the antibacterial activity characteristic of such compounds.[1-3]

The present work describes the synthesis of some novel quaternary ammonium salts which are derivatives of 15-phenyl-pentadecanoic acid and belong to the general type I.



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All compounds have been tested against gram positive and gram negative bacteria. In addition, their lipophilicity has been investigated, since the role of that property in antibacterial activity has been established since the early quantitative structure–activity relationship (QSAR) studies.[4,5]

Chemistry The synthesis of the products of type I was achieved, as shown in Chart 1. 15-Phenyl-pentadecanoic acid (**7**) was synthesized according to Soffer *et al.*[6] Thus, direct esterification of 1,12-dodecane-dicarboxylic acid (**1**) was followed by partial saponification to obtain the monoethylester (**3**), which, upon treatment with thionyl chloride gave the 14-chloroketo-tetradecacarboxylic acid ethylester (**4**). The latter was allowed to react with cadmiumdibenzyl to yield ester (**5**), which, upon saponification, gave the corresponding carboxylic acid (**6**). After a Wolf–Kishner reduction, modified by Huang–Minlon,[7] the acid (**7**) was obtained. The chloride of the latter (**8**) was esterified by heating with suitable aminoalcohols in benzene and in the presence of pyridine to the corresponding esters of type (**9**). The target molecules I(a—g) were obtained upon reaction of the esters (**9**) with a variety of alkyl halides, and they are presented in Table I.

Lipophilicity Studies The octanol/water partition coefficients of the compounds were calculated using the Leo–Hansch fragmental procedure[8] and are included in Table II. No reliable prediction was possible for the morpholino-derivative Ig, since the influence of the charged nitrogen participating in a ring structure could not be accounted for. The high values of Table II do not permit direct measurement of the partition coeffients by the conventional shaking flask method.[9] Therefore, reversed phase thin layer chromatography was applied for a further study of lipophilicity.[10] *Rmo* values (Table II) were obtained through linear extrapolation of *Rm* values determined at different acetone/water mixtures, to which a fixed concentration of KCl was added in order to suppress silanophilic interactions of eventually uncovered silanol groups.[11] According to both sets of lipophilicity data, the compounds can be classified into two distinct groups. The first group includes compounds Ia—Ic with log $P > 8.8$ and *Rmo* values around 4. It seems

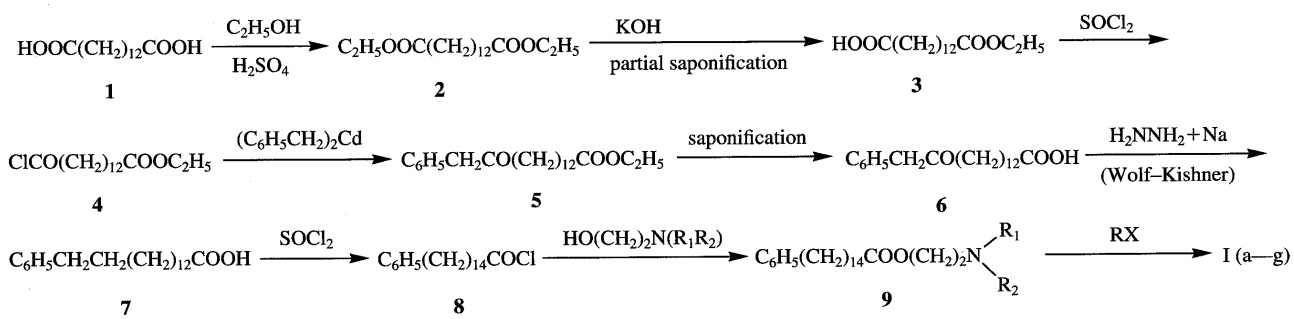
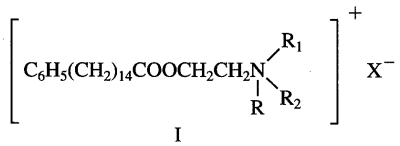


Chart 1

TABLE I. Quaternary Ammonium Salts of Dialkylaminoalkylesters of 15-Phenyl-pentadecanoic Acid



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Compound No.	$N(R_1)(R_1)$	R	X	Molecular formula	mp (°C)	Analysis (%) Calcd (Found)		
						C	H	N
Ia	$-N(CH_3)(CH_3)$	$-(CH_2)_9CH_3$	Br	$C_{35}H_{64}BrNO_2$	128—130	68.82 (69.03	10.56 10.66	2.29 2.68)
Ib	$-N(CH_3)(CH_3)$	$-(CH_2)_{11}CH_3$	Br	$C_{37}H_{68}BrNO_2$	100—102	69.56 (69.68	10.73 11.01	2.19 2.50)
Ic	$-N(CH_3)(CH_3)$	$-(CH_2)_{13}CH_3$	Br	$C_{39}H_{72}BrNO_2$	67—68	70.24 (70.40	10.88 11.02	2.10 2.50)
Id	$-N(C_2H_5)(C_2H_5)$	$-CH_3$	Br	$C_{28}H_{50}BrNO_2$	104—106	60.00 (60.08	8.57 8.53	2.51 2.30)
Ie	$-N(C_2H_5)(C_2H_5)$	$-C_2H_5$	I	$C_{29}H_{52}INO_2$	168—170	60.41 (60.31	9.03 8.92	2.43 2.40)
If	$-N(C_2H_5)(C_2H_5)$	$-C_6H_5$	Cl	$C_{33}H_{52}ClNO_2$	120—121	79.78 (79.68	9.82 9.80	2.69 2.90)
Ig	$-N\langle O \rangle$	$-CH_3$	I	$C_{28}H_{48}INO_3$	178—180	58.30 (58.21	8.28 8.00	2.90 2.70)

TABLE II. Calculated log *P* Values and Extrapolated *Rmo* Data

Compound	log *P* (Calcd)	*Rmo*
Ia	8.83	4.00 (±0.35)
Ib	9.61	3.79 (±0.20)
Ic	10.39	4.04 (±0.47)
Id	6.06	2.03 (±0.23)
Ie	6.12	2.27 (±0.16)
If	6.05	2.46 (±0.28)

TABLE III. MIC for Compounds Id—Ig (mg/l)

Strain	Compound			
	Id	Ie	If	Ig
Cocci				
Staphylococcus aureus (ATCC 14775)	128	128	128	128
Staphylococcus aureus (WT)	256	256	128	128
Staphylococcus epidermidis (ATCC 35547)	512	512	128	128
Rods				
Escherichia coli (ATCC 35218)	512	512	512	256
Escherichia coli (WP)	512	512	128	128
Enterobacter cloacae (ATCC 43091)	>512	>512	>512	>512
Enterobacter cloacae (HIP)	>512	>512	>512	>512
Pseudomonas aeruginosa (ATCC 27588)	>512	>512	>512	>512
Pseudomonas aeruginosa (WP)	>512	>512	>512	512

that for such high lipophilicity levels, discrimination is not possible in reversed phase TLC, so compounds Ia—Ic possess, within statistical error, the same *Rmo* value. To the second group belong isolipophilic compounds with log *P* values around 6 and *Rmo* values around 2.

Results and Discussion

All compounds were tested for their antibacterial activity against gram positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and gram negative microorganisms (*Eschrichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*).[12] Compounds of the first group, Ia—Ic, were found to be inactive against all bacterial species, most probably because of their extremely high lipophilicity. Compounds of the second group Id—Ig showed weak activity against *Staphylococcus aureus* (MIC (minimal inhibitory concentration) 128 mg/l, Table III), while compounds If and Ig exhibited weak activity also against *Staphylococcus epidermidis* and *Escherichia coli* (MIC 128 mg/l, Table III). It should be noticed that the log *P* values of compounds Id—If correspond to the optimum $\log P_0 = 6$, generally proposed for antibacterial activity against gram positive microorganisms. However, for cationic compounds lower log *P* values are usually reported.[4,5] Our results provide further evidence that lower lipophilicity may be favorable for antibacterial activity in the case of quaternary ammonium salts.

Experimental

Chemistry Melting points have been determined on a Buchi capillary melting point apparatus and are uncorrected. IR spectra have been recorded on a Perkin-Elmer 883 spectrophotometer. Analyses were carried out at Service Central de Microanalyse of C.N.R.S., Vernaison, France.

1,12-Dodecane-dicarboxylic Acid Diethylester (2) Compound **2** was prepared by heating 1 mol of 1,12-dodecane-dicarboxylic acid with 4 mol of EtOH in benzene in the presence of conc. H_2SO_4.

1,12-Dodecane-dicarboxylic Acid Monoethylester (3) A solution of 62 g (0.13 mol) KOH in 600 ml abs. EtOH was added under vigorous stirring to a solution of 24.2 g (0.1 mol) of the diethylester (**2**) in 100 ml abs. EtOH. Stirring was continued for 1 h after the addition was completed. After concentration *in vacuo* and treatment with Et_2O and H_2O, the aqueous phase was acidified with an excess of HCl and extracted with Et_2O. The ethereal solutions were collected and washed with H_2O saturated with NaCl. The solvent was evaporated and the residual semi-solid product was eluted with benzene through neutral alumina. (85%) mp 35 °C. *Anal.* Calcd for $C_{16}H_{30}O_4$: C, 76.66; H, 10.00. Found: C, 76.48; H, 9.66.

14-Chloroketo-tetradecacarboxylic Acid Ethylester (4) A solution of **3** in benzene was allowed to react with an excess of $SOCl_2$ in the presence of $CaCl_2$. The mixture was left overnight at room temperature. After filtration of $CaCl_2$, the solvent was evaporated and the residue was used without further purification.

14-Keto-15-phenyl-tetradecacarboxylic Acid Ethyl Ester (5) A solution of 1.85 g (0.06 mol) benzyl bromide in 50 ml anhydr. Et_2O was slowly added to a vigorously stirred suspension of 1.47 g (0.06 mol) Mg in 20 ml anhydr. Et_2O under N_2. Stirring was continued until all Mg had reacted. Then, a solution of 5.95 g (0.32 mol) $CdCl_2$ in 50 ml Et_2O was added over a period of 1 h and the mixture was refluxed for 1 h. After removal of the solvent, the residue was stirred for 45 min with 80 ml anhydr. benzene. Benzene was evaporated and a solution containing 11.3 g (0.48 mol) of **4** in 20 ml benzene was added. The mixture was stirred at ambient temperature for 4 h and then treated with an excess of HCl. The separated benzene layer was washed with $NaHCO_3$, dried (Na_2SO_4) and evaporated. The residue, a viscous liquid, was distilled at 52 °C (0.03 mmHg). (53%) mp 32 °C. *Anal.* Calcd for $C_{23}H_{36}O_3$: C, 76.68; H, 10.00. Found: C, 76.35; H, 9.88.

14-Keto-15-phenyl-tetradecacarboxylic Acid (6) 8.2 g (0.20 mol) of ester (**5**) was heated for 4 h with an excess for KOH in EtOH. After concentration of the solvent, the residue was diluted with water and neutralized (pH = 7.0) with AcOH. The aqueous solution was washed with Et_2O and acidified with dil. HCl. The precipitate (**6**) was extracted with Et_2O and recrystallized from MeOH/petroleum ether. (80%) mp 178 °C. *Anal.* Calcd for $C_{21}H_{32}O_3$: C, 75.92; H, 9.63. Found: C, 75.58; H, 9.35.

15-Phenyl-pentadecanoic Acid (7) A mixture of 7.0 g (0.2 mol) of **6** and 5 ml of 85% hydrazine was added to a solution of 2.5 g (0.11 mol) Na in 70 ml diethyleneglycol. After reflux for 1 h, the temperature was raised to 200 °C; the mixture was stirred at that temperature for 3 h, cooled to ambient temperature, acidified with HCl and extracted with benzene. The benzene layer was washed with water, dried (Na_2SO_4) and evaporated. The residue was recrystallized from a mixture of benzene/pentane (82%) mp 77 °C. *Anal.* Calcd for $C_{21}H_{34}O_2$: C, 79.25; H, 10.68. Found: C, 79.00; H, 10.40.

15-Phenyl-pentadecanoyl-chloride (8) 10.8 g (0.3 mol) $SOCl_2$ was slowly added to a solution of 3.3 g (0.1 mol) of **7** in 50 ml benzene and refluxed for 3 h. The yellowish solid obtained after evaporation of the solvent and removal of the excess of $SOCl_2$ was not further purified. (85%) mp 38 °C.

Dialkylamino-alkylesters of 15-Phenyl-pentadecanoic Acid (9) 0.3 mol of the appropriate dialkylamino ethanol in pyridine was added to a solution of 0.1 mol of the chloride (**8**) in 50 ml benzene. The mixture was refluxed under stirring for 3 h and was allowed to cool to room temperature. The precipitate was filtered off, the filtrate was washed with water until a neutral reaction was obtained, and dried (Na_2SO_4). The solvent was evaporated. The crude product was purified by distillation.

According to that procedure, the following compounds were prepared in yields between 55—65%: 15-Phenyl-pentadecanoic acid 1-dimethylamino-ethylester; bp 87— 90 °C (15 mmHg). IR (liquid film): 1730 cm^{-1} (C=O). 15-Phenyl-pentadecanoic acid 1-diethylamino-ethylester; bp 110—115 °C (15 mmHg). IR (liquid film): 1744 cm^{-1} (C=O). 15-Phenyl-pentadecanoic acid 1-morpholino-ethylester; bp 102—105 °C (15 mmHg). IR (liquid film): 1744 cm^{-1} (C=O).

Quaternary Ammonium Salts Ia—Ig 0.4 mol of the appropriate alkyl halide was added to a solution of 0.1 mol of the dialkylaminoalkylester (**9**) in anhydr. Me_2CO and refluxed for several hours. The mixture was cooled and the residue was recrystallized from Me_2CO/Et_2O. All compounds were obtained in almost quantitative yield. Elemental analyses and physical constants of the compounds are presented in Table I.

Chromatography Silica gel plates (Merck F_{254}) were developed overnight with 5% paraffin oil in petroleum ether (bp 40—60 °C) and dried at 40 °C before use. Five different Me_2CO/H_2O mixtures containing 0.05 M KCl were used as eluents, the proportion of Me_2CO ranging from 50 to 75%. Spots were detected under iodine vapors. *Rm* values were calculated according to equation $Rm = \log(1/Rf - 1)$.

Antibacterial Activity The antibacterial activity was tested against two strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Staphylococcus aureus* and one strain of *Staphylococcous epidermidis*. The bacterial strains were either wild type clinical isolates (WT) or from the American Type Culture Collection (ATCC).

MIC were determined in Mueller–Hindon broth by a standard microdilution method.[12] Bacterial suspensions (5×10^5 CFU/ml) were incubated for 18 h at 37 °C in the presence of different concentrations of each compound. MIC was the last dilution at which no bacterial growth was evident.

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