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Antibacterial activity of fluoroalkylated allyl- and diallyl-ammonium chloride oligomers

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Abstract

A series of fluoroalkylated allyl- and diallyl-ammonium chloride oligomers were prepared using fluoroalkanoyl peroxides as key intermediates, and their antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* were studied. Antibacterial activity was found to be sensitive to the structure of these oligomers. Fluoroalkylated allyl-type co-oligomers containing carboxy, sulfo and trimethylsilyl segments were inactive; however, the allyl- or diallyl-type homo- and co-oligomers were in general active. Of these, the perfluoro-1-methyl-2-oxapentylated allylammonium chloride–diallylammonium chloride co-oligomer was found to be the most active against both *S. aureus* and *E. coli*. Furthermore, the fluoroalkylated oligomers possessing antibacterial activity were able to reduce the surface tension of water to around 10 mN m^{-1}. Therefore, these fluoroalkylated oligomers are new attractive functional materials possessing not only unique properties imparted by fluorine but also antibacterial activity. © 1997 Elserier Science S.A.

Keywords: Fluoroalkylated oligomers; Antibacterial activity; Allylammonium chloride; Diallylammonium chloride; Surface tension

1. Introduction

Recently, there has been intensive research interest in polymeric drugs due to their promising advantages over low molecular weight drugs [1]. For example, polymeric drugs are known to have superior properties such as a high local density of the active groups in the vicinity of the polymer chains and film-forming properties that set them apart from low molecular weight drugs. In particular, polycationic biocides possess charge density and excellent processability, and have found remarkable utility in hygiene and in biomedical applications. In fact, polycationic biocides possessing quaternary ammonium and phosphonium segments were reported to exhibit outstandingly high antibacterial activities, and have been widely used for the treatment of various infections [2].

Also, fluoroalkylated compounds have been receiving increasing attention owing to their various unique properties which cannot be achieved by the corresponding non-fluoroalkylated ones [3]. From such a viewpoint, it is of considerable interest to develop these materials to provide novel fluoralkylated cationic biocides. However, the exploration of fluoroalkylated polymeric biocides has hitherto been very limited, since the direct introduction of fluoroalkyl groups into organic molecules is not easy, but these fluoroalkylated compounds have been the subject of considerable research of both a fundamental and applied nature. In our continuing effort to design and develop new fluoroalkylated materials by carbon–carbon bond formation using fluoroalkanoyl peroxides [$R_FC(=O)OO(O=)CR_F$] as key intermediates [4], we reported on the synthesis of fluoroalkylated allyl- and diallyl-ammonium chloride oligomers with fluoroalkanoyl peroxides [5]. In this paper, we would like to report on the antibacterial activity of these fluoroalkylated allyl- and diallyl-ammonium chloride oligomers.

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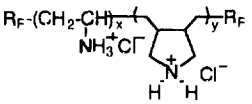
2. Results and discussion

A series of fluoroalkylated allyl- and diallyl-ammonium chloride oligomers were prepared according to our previously reported method (as shown in Scheme 1) [5].

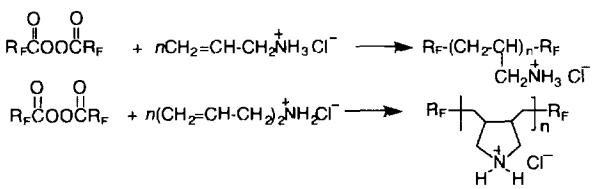
Similarly, we prepared a series of fluoroalkylated allyl- (or diallyl-) ammonium chloride co-oligomers as in Scheme 2.

In addition, a fluoroalkylated allylammonium chloride–diallylammonium chloride co-oligomer



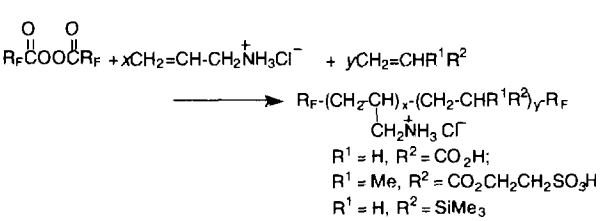
was prepared under similar conditions. We investigated the antibacterial activity of these fluoroalkylated oligomers against *Staphylococcus aureus* by the viable cell counting method. About 10^7–10^8 cells ml^{-1} of *S. aureus* were exposed to 100 $\mu g\ ml^{-1}$ (or 1000 $\mu g\ ml^{-1}$) of the oligomers in saline, and Table 1 shows the colony-forming units versus exposure for these oligomers against *S. aureus*.

As shown in Table 1, fluoroalkylated allylammonium chloride–acrylic acid co-oligomers and fluoroalkylated allylammonium chloride-2-(methacryloxy)ethane sulfonic acid co-oligomers were inactive. In general, it is well known that bacterial cell surfaces are negatively charged, and adsorption onto the bacterial cell surface is strongly affected by the effective positive charge density of the cationic species [6]. Therefore, this result clearly indicates that the cationic segments in these fluoroalkylated co-oligomers are not able to interact tightly with the negatively charged bacterial cell owing to the presence of carboxy or sulfonic segments in the co-oligomers. However, it was found that fluoroalkylated allyl- and diallyl-ammonium chloride oligomers exhibit bacterial activity against *S. aureus*, and in particular, longer perfluoro-oxaalkylated diallylammonium chloride oligomers were more active (10–10^2 colony forming units levels). Fluoroalkylated allylammonium chloride co-oligomer containing trimethylsilyl segments (Run 27) was found to be not so



$R_F = C_3F_7$, $CF(CF_3)[OCF_2CF(CF_3)]_m$-$OC_3F_7$; m = 0, 1, 2

Scheme 1.



$R_F = C_3F_7$, $CF(CF_3)[OCF_2CF(CF_3)]_mOC_3F_7$; m = 0, 1

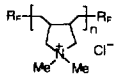
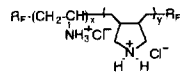
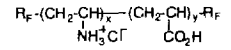
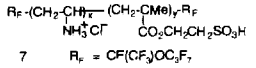
Scheme 2.

Table 1
Antibacterial activity of fluoroalkylated oligomers against *Staphylococcus aureus* [a]

Run	Oligomer	$\overline{Mn}$ (x : y)[b]	*Staphylococcus aureus*
	None		8.7×10^7 cfu/ml[c]
	R_F-$(CH_2$-$CH)_x$-R_F (NH$_3^+$ Cl$^-$)		
1	$R_F = CF(CF_3)OC_3F_7$	1990	1.8×10^4
2	$R_F = CF(CF_3)OC_3F_7$	2640	1.5×10^4
3	$R_F = CF(CF_3)OC_3F_7$	3200	1.2×10^4
4	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	2220	1.3×10^3
5	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	2530	1.5×10^3
	R_F-(pyrrolidinium)-R_F Cl$^-$		
6	$R_F = CF(CF_3)OC_3F_7$	1320	4.3×10^3
7	$R_F = CF(CF_3)OC_3F_7$	1470	4.0×10^3
8	$R_F = CF(CF_3)OC_3F_7$	1500	3.7×10^3
9	$R_F = CF(CF_3)OC_3F_7$	1550	5.6×10^3
10	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	1420	1.8×10^3 (<10)[d, e]
11	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	1430	2.5×10^3
12	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	2330	3.2×10^2 (<10)[d, e]
13	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	3500	1.1×10^4 (<10)[d, e]
14	$R_F = CF(CF_3)OCF_2CF(CF_3)OCF_2CF(CF_3)OC_3F_7$, 1840		<10[d, g]
15	$R_F = CF(CF_3)OCF_2CF(CF_3)OCF_2CF(CF_3)OC_3F_7$, 2350		<10[d, g]
16	$R_F = CF(CF_3)OCF_2CF(CF_3)OCF_2CF(CF_3)OC_3F_7$, 2600		<10[d, g]
17	$R_F = CF(CF_3)OCF_2CF(CF_3)OCF_2CF(CF_3)OC_3F_7$, 3120		<10[d, g]
	R_F-$(CH_2$-$CH)_x$-$(CH_2$-$CH)_y$-R_F (CO_2H; $CH_2NH_3^+$ Cl$^-$)		
18	$R_F = CF(CF_3)OC_3F_7$	2530(30 : 70)	3.3×10^7[d, f]
19	$R_F = CF(CF_3)OC_3F_7$	2900(40 : 60)	2.2×10^8[d, g]
20	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	1680(70 : 30)	1.4×10^7[d, f]
21	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	3220(75 : 25)	1.9×10^7[d, g]
22	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	6050(37 : 63)	2.5×10^8[d, g]
23	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	6200(23 : 77)	3.0×10^8[d, g]
	R_F-$(CH_2$-$CH)_x$-$(CH_2$-$CMe)_y$-R_F ($CO_2CH_2CH_2SO_3H$; $CH_2NH_3^+$ Cl$^-$)		
24	$R_F = C_3F_7$	9850(76 : 24)	1.9×10^8[d, e]
25	$R_F = CF(CF_3)OC_3F_7$	12100(77 : 23)	2.5×10^7[d, e]
26	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	12200(56 : 44)	1.6×10^7[d, e]
	R_F-$(CH_2$-$CH)_x$-$(CH_2$-$CH)_y$-R_F ($SiMe_3$; $CH_2NH_3^+$ Cl$^-$)		
27	$R_F = CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	1910(87 : 13)	2.1×10^5[d, g]
	Hibitane[h]		<10

[a] Concentration of each oligomer is 100 $\mu g/ml$.
[b] $\overline{Mn}$ and x:y indicate the number-average molecular weight of oligomer determined by GPC analyses and co-oligomerization of oligomer determined by ^1H NMR, respectively.
[c] cfu indicates colony forming units.
[d] Concentration of oligomer is 1000 $\mu g/ml$.
[e] cfu in the absence of oligomer is 3.4×10^8.
[f] cfu in the absence of oligomer is 2.5×10^8.
[g] cfu in the absence of oligomer is 4.6×10^8.
[h] Hibitane: 1,1′-hexamethylenebis[5-(4-chlorophenyl)biguanide]diglu-conate.

Table 2
Minimal inhibitory concetration (MIC) of fluoroalkylated oligomers with *Straphylococcus aureus* and *Escherichia coli*

Run	Oligomer	$\overline{Mn}$ (x : y)	MIC(μg/ml) *Staphylococcus aureus*	MIC(μg/ml) *Escherichia coli*
1	 R_F = $CF(CF_3)OC_3F_7$	11300	>500	>500
2	= $CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	3970	500	125
3	= $CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	7280	500	500
4	 R_F = $CF(CF_3)OC_3F_7$	2770 (22 : 78)	125	60
5	 R_F = $CF(CF_3)OC_3F_7$	2530 (41 : 59)	>500	>50
6	= $CF(CF_3)OCF_2CF(CF_3)OC_3F_7$	3220(18 : 82)	>500	>500
7	 R_F = $CF(CF_3)OC_3F_7$	12100(77 : 23)	>500	>500

active against *S. aureus*. Of these, perfluoro-1,4-dimethyl-2,5-dioxaoctylated diallylammonium chloride oligomers (Run 12; $\overline{Mn}$ = 2330) was the most active, with a 3.2×10^2 cfu. In addition, this co-oligomer was also active against *Pseudomonas aeruginosa* (< 10 colony forming units levels at 1000 μg ml^{-1} of oligomer). These values were similar to that of Hibitane, which has been considered to be a potent low molecular weight biocide.

Fluoroalkylated diallylammonium-type oligomers showed promise as polymeric antibacterial materials. Therefore, fluoroalkylated diallylammonium chloride oligomers were tested in detail for antibacterial activity against *S. aureus* and *Escherichia coli* by the viable cell counting method, and the minimum inhibitory concentration (MIC) for each compound tested is shown in Table 2. Fluoroalkylated allylammonium chloride–acrylic acid [or 2-(methacryloxy)ethane sulfonic acid] co-oligomers were also tested, for comparison.

As shown in Table 2, perfluoro-1-methyl-2-oxapentylated dimethyldiallylammonium chloride oligomer (Run 1) was inactive; however, longer perfluoro-oxaalkylated dimethyldiallylammonium chloride oligomers (Runs 2 and 3) have an MIC of 500 μg ml^{-1} against *S. aureus*, and have MICs of 125 and 500 μg ml^{-1} against *E. coli*, respectively. More interestingly, a perfluoro-oxaalkylated allylammonium chloride–diallylammonium chloride co-oligomer (Run 4) was found to be significantly more active than the other oligomers, and has MICs of 125 μg ml^{-1} and 60 μg ml^{-1} against *S. aureus* and *E. coli*, respectively. On the other hand, fluoroalkylated co-oligomers containing carboxy or sulfonic segments were inactive against *S. aureus* or *E. coli*.

Hitherto, the development of antibacterial cationic materials possessing fluoroalkyl segments have been limited since the fluoroalkyl group should weaken the cationic properties due to the strong electron-withdrawing property of fluorine atom. However, since our present fluoroalkylated allyl- and diallyl-ammonium chloride oligomers have only two-fluoroalkylated end-caps, only the ammonium segments near to the fluoroalkyl units in the oligomers are affected by the strong inductive effect of the fluoroalkyl groups. Hence, most ammonium segments in these oligomers should interact strongly with negatively charged bacterial cell surfaces.

Surface tensions of aqueous solutions of some of the fluoroalkylated allyl- and diallyl-ammonium chloride oligomers mentioned in Table 1 and 2 were measured with the Wilhelmy plate method at 25 °C, and the results are shown in Fig. 1.

Interestingly, as shown in Fig. 1, there is some correlation between the surface tensions of the aqueous solutions and the antibacterial activity of fluoroalkylated oligomers. Fluoroalkylated oligomers possessing higher antibacterial activity (Runs 4, 2 and 3 in Table 2) showed a clear break-point resembling a CMC (critical micelle concentration) in aqueous solutions and were able to reduce the surface tension of water effectively to around 10 mN m^{-1} levels. These values for the surface tensions of water are almost the same level as that achieved by the usual low molecular weight fluorinated surfactant. On the other hand, in fluoroalkylated oligomers, as the degree of the reduction in the surface tension of water decreased, the antibacterial activity is likely to decrease. For example, fluoroalkylated oligomers (Runs 27 and 5 in Table 1) showed no antibacterial activity or a clear break point in the surface tension measurements of water, and were not able to reduce the surface tension of water more effectively than the corresponding active compounds as shown in Fig. 1. We have previously demonstrated that the formation of such a clear break point in the surface tension measurements in these oligomers strongly suggests the formation of intra- or inter-molecular aggregates in aqueous solution [5]. Thus, it can be considered that the intra- or inter-molecular aggregations of these fluoroalkylated allyl- or diallyl-ammonium chloride oligomers interact strongly with the bacterial cell to exhibit the higher activity.

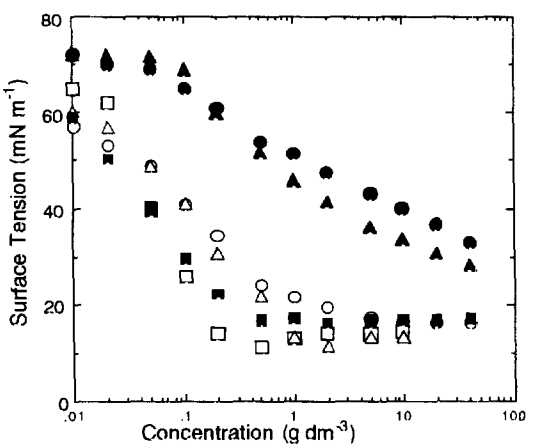


Fig. 1. Surface tensions of aqueous solutions of fluoroalkylated oligomers: ○, Run 4 in Table 2; ●, Run 27 in Table 1 (see Ref. [[5]b]); □, Run 2 in Table 2 (see Ref. [[5]b]); △, Run 3 in Table 2; ▲, Run 5 in Table 1; ■, Run 12 in Table 1.

In this way, it was verified that our present fluoroalkylated oligomers can exhibit effectively not only the unique properties imparted by fluorine as well as the usual low molecular weight fluorinated surfactant, but also antibacterial activity, although these compounds are high molecular mass materials containing only two fluoroalkylated end-groups in one oligomeric molecule. Hence, our oligomers are expected to be widely applicable in various fields as new attractive fluorinated functional materials possessing antibacterial activity.

3. Experimental details

3.1. Synthesis of a series of fluoroalkylated allyl- and diallyl-ammonium chloride homo- and co-oligomers

A series of fluoroalkylated allyl- and diallyl-ammonium chloride oligomers were prepared by the reactions from the corresponding allyl- or diallyl-ammonium chloride and fluoroalknaoyl peroxides according to our previously reported method [5].

3.2. Surface tension measurements

The surface tensions of aqueous solutions of fluoroalkylated oligomers were measured at 25 °C using a Wilhelmy-type surface tensiometer(ST-1, Shimadzu Co.) with a glass plate [7].

3.3. Antibacterial assessment

Bacterial activity was evaluated by the viable cell counting method according to our previously reported method [8].

References

[1] L.G. Donaruma, O. Vogel (eds.), Polymeric Drugs, Academic, New York, 1978.
[2] (a) A. Rembaum, J. Polym. Sci. Appl. Polym. Symp. 22 (1973) 299; (b) T. Ikeda, H. Yamaguchi, S. Tazuke, J. Bioact. Comp. Polym. 5 (1990) 31; (c) A. Kanazawa, T. Ikeda, T. Endo, J. Poly. Sci. A: Polym. Chem. 31 (1993) 3031.
[3] (a) R.E. Banks, B.E. Smart, J.C. Tatlow (eds.), Organofluorine Chemistry, Plenum, New York, 1994; (b) J.F. Liebman, A. Greenberg, W.R. Dolbier, Jr., Fluorine-Containing Molecules, VCH, Weinheim, 1988.
[4] H. Sawada, Chem. Rev. 96 (1996) 1779.
[5] (a) H. Sawada, K. Tanba, M. Oue, T. Kawase, Y. Hayakawa, M. Mitani, Y. Minoshima, M. Nishida, Y. Moriya, Polymer 36 (1995) 2103; (b) H. Sawada, K. Tanba, T. Kawase, M. Baba, Y. Hayakawa, J. Jpn. Oil Chem. Soc. 46 (1997) 191.
[6] A. Katchalsky, Biophys. J. 4 (1964) 9.
[7] H. Sawada, K. Tanba, N. Itoh, C. Hosoi, M. Oue, M. Baba, T. Kawase, M. Mitani, H. Nakajima, J. Fluorine Chem. 77 (1996) 51.
[8] H. Sawada, S. Katayama, M. Oue, T. Kawase, Y. Hayakawa, M. Baba, T. Tomita, M. Mitani, J. Jpn. Oil Chem. Soc. 45 (1996) 161.