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Synthesis and Antimicrobial Characteristics of 4,4′-(α,ω-Polymethylenedithio)bis(1-alkylpyridinium iodide)s

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Bis-quaternary ammonium compounds (bis-QACs), 4,4′-(α,ω-polymethylenedithio)bis(1-alkylpyridinium iodide)s (4DTBP-m,n), which have 3 to 10 carbon atoms in the connecting methylene chain (m) and 8 to 18 carbon atoms of the *N*-alkyl chain (n), were synthesized. 4DTBP-6,12 exhibited a wide antimicrobial spectrum against gram-positive and gram-negative bacteria and fungi. The activity was stronger than those of *N*-dodecylpyridinium iodide (P-12), benzyldodecyldimethylammonium chloride and 2-(4-thiazolyl)benzimidazole. The bactericidal activities of 4DTBP-m,n were scarcely affected by the lengths of the alkyl chain and methylene chain. The bis-QAC that showed the highest activity was 4DTBP-6,8 (minimum inhibitory concentration (MIC) = 1.6 μM, minimum bactericidal concentration (MBC) = 2.6 μM), and its activity was about 10 times that of *N*-hexadecylpyridinium iodide (P-16), which was the most active in the P-n series. In addition, 4DTBP-6,12 showed a high bactericidal activity in the ranges of pH 5 to 8.5 and 10 to 40 °C, in contrast to mono-QACs. The bis-QACs synthesized in this study have excellent bactericidal properties.

Key words bis-quaternary ammonium compound; *N*-alkyl-2-alkylthiopyridinium salt; bactericidal activity; *N*-alkyl-4-alkylthiopyridinium salt; bacteriostatic activity

Quaternary ammonium compounds (QACs) have been used widely as disinfectants in the food and textile industries, and in hospitals. Some of them have potent bactericidal and fungicidal activities. The QACs can be classified by the number of quaternary nitrogen atoms in the molecule, that is, mono-QAC, bis-QAC and polymeric QAC. The synthesis and the antimicrobial characteristics of various series of mono-QACs,[1–4] especially the pyridinium salt derivatives,[5–8] have been reported. The activities of mono-QACs are generally influenced by structural features, such as *N*-alkyl chain length[5] and the kind of substituent groups and their positions on the pyridine ring.[9] Electron-releasing groups such as amino, methyl and propyl groups markedly enhance the bactericidal activities of mono-QACs, while electron-attracting groups such as carboxyl and carbamoyl groups reduce them. Quantitative structure–activity relationship analysis indicated that the antibacterial activity of mono-QACs depends on the molecular hydrophobicity,[10] the critical micelle concentration,[11] and the acidic dissociation constant (pK_a) of the corresponding pyridine.[9] Further, the bacteriolastic activity, which was found by Kourai *et al.*,[12] was closely correlated to the mechanism of bactericidal activity.

On the other hand, there is relatively little information about the antimicrobial characteristics of bis-QACs. The synthesis and the antimicrobial activities of bis-QACs have been reported by Devinsky *et al.*[11,13] and Pavlikova-Moricka *et al.*,[14] while Kourai *et al.*[15] also reported that *N*,*N*′-dialkyl-γ,γ′-dipyridinium diiodides (PP-n,n) exhibited stronger antibacterial activity than mono-QAC. In addition, the antimicrobial characteristics of PP-n,n, with two similar alkyl chains, were comparable to those of QACs with two different alkyl chains. These reports show that bis-QACs can possess strong antimicrobial activities. The synthesis and the antimicrobial[16–18] or anti-tumor[19] effects of polymeric QACs have also been reported.

In this study we synthesized new bis-QACs and investigated the effect of changes in the chemical structure on the antimicrobial activity. The bis-QACs, 4,4′-(α,ω-polymethylenedithio)bis(1-alkylpyridinium iodide)s (4DTBP-m,n), have two active moieties for bactericidal action and two long alkyl chains in the molecule. The antimicrobial activities of 4DTBP-m,n were measured and compared with those of *N*-alkylpyridinium iodides (P-n), which are mono-QACs with the simplest structures.

Chemistry

The bis-QACs listed in Table 1 were synthesized from the corresponding polymethylene bromide (trimethylene bromide, tetramethylene bromide, hexamethylene bromide, octamethylene bromide and decamethylene bromide), *n*-alkyl iodide (hexyl iodide, octyl iodide, decyl iodide, dodecyl iodide, tetradecyl iodide, hexadecyl iodide and octadecyl iodide) and 4-mercaptopyridine (Chart 1). *N*-Alkylpyridinium iodides (P-n) were synthesized as described earlier.[5] Benzyldodecyldimethylammonium chloride (10% (w/v) benzalkonium chloride (BAC) solution) was purchased from Takeda Pharmaceutical Co., Ltd. (Osaka) and 2-(4-thiazolyl)benzimidazole (TBZ) was obtained from San-ai Oil Co., Ltd. (Tokyo) (Fig. 1).

Results and Discussion

Antimicrobial Activity The new bis-QAC (4DTBP-6,12) was tested for antimicrobial activity against gram-negative bacteria (10 strains), gram-positive bacteria (9 strains) and fungi (7 strains). A typical mono-QAC (P-12), which has the pyridinium moiety of 4DTBP-6,12 and has no substituents on the pyridine ring, and BAC, which is a QAC commercially available as a disinfectant, were also measured for comparison. Table 2 shows their minimum inhibitory concentrations (MICs) against various bacteria.

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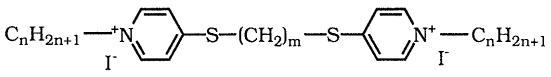
Table 1. Physical Properties of 4,4′-(α,ω-Polymethylenedithio)bis(1-alkylpyridinium iodide)s (4DTBP-m,n)



Compd.	Yield (%)	mp (°C)	Formula	Elemental analysis (%) Calcd (found)		
				C	H	N
4DTBP-3,8	80.5	125—128	$C_{29}H_{48}I_1N_2S_2$	46.90	6.51	3.77
				(46.81	6.25	3.51)
4DTBP-3,10	81.2	159—161	$C_{33}H_{56}I_2N_2S_2$	49.62	7.07	3.51
				(49.43	6.94	3.78)
4DTBP-3,12	80.3	169—170	$C_{37}H_{64}I_2N_2S_2$	51.99	7.55	3.28
				(51.87	7.29	2.99)
4DTBP-3,14	82.1	174—175	$C_{41}H_{72}I_2N_2S_2$	54.06	7.97	3.08
				(53.78	7.77	2.93)
4DTBP-3,16	83.4	166—169	$C_{45}H_{80}I_2N_2S_2$	55.89	8.34	2.90
				(55.80	8.04	2.98)
4DTBP-3,18	85.0	149—151	$C_{49}H_{88}I_2N_2S_2$	57.52	8.67	2.74
				(57.25	8.33	2.71)
4DTBP-4,8	80.0	147—149	$C_{30}H_{50}I_2N_2S_2$	47.62	6.66	3.70
				(47.37	6.53	4.00)
4DTBP-4,10	81.2	149—151	$C_{34}H_{58}I_2N_2S_2$	50.24	7.19	3.45
				(49.95	6.96	3.40)
4DTBP-4,12	80.7	168—171	$C_{38}H_{66}I_2N_2S_2$	52.53	7.66	3.22
				(52.34	7.38	3.01)
4DTBP-4,14	80.5	165—168	$C_{42}H_{74}I_2N_2S_2$	54.54	8.06	3.03
				(54.31	8.00	3.02)
4DTBP-4,16	83.2	175—178	$C_{46}H_{82}I_2N_2S_2$	56.31	8.42	2.86
				(56.08	8.21	3.12)
4DTBP-4,18	84.2	165—168	$C_{50}H_{90}I_2N_2S_2$	57.90	8.75	2.70
				(57.65	8.50	2.66)
4DTBP-6,8	88.0	102—103	$C_{32}H_{54}I_2N_2S_2$	48.98	6.94	3.57
				(48.93	6.80	3.57)
4DTBP-6,10	87.2	121—122	$C_{36}H_{62}I_2N_2S_2$	51.42	7.43	3.33
				(51.54	7.28	3.39)
4DTBP-6,12	87.3	136—137	$C_{40}H_{70}I_2N_2S_2$	53.56	7.87	3.12
				(53.47	7.63	3.09)
4DTBP-6,14	86.5	135—136	$C_{44}H_{78}I_2N_2S_2$	55.45	8.25	2.94
				(55.15	7.96	2.74)
4DTBP-6,16	86.8	140—141	$C_{48}H_{86}I_2N_2S_2$	57.13	8.59	2.78
				(57.06	8.41	2.77)
4DTBP-6,18	87.0	138—139	$C_{52}H_{94}I_2N_2S_2$	58.63	8.89	2.63
				(58.38	8.97	2.40)
4DTBP-8,8	84.3	87—89	$C_{34}H_{58}I_2N_2S_2$	50.24	7.19	3.45
				(49.96	7.07	3.74)
4DTBP-8,10	83.2	122—123	$C_{38}H_{66}I_2N_2S_2$	53.53	7.66	3.22
				(52.32	7.57	3.09)
4DTBP-8,12	86.5	132—133	$C_{42}H_{74}I_2N_2S_2$	54.54	8.06	3.03
				(54.31	7.85	2.73)
4DTBP-8,14	84.6	137—138	$C_{46}H_{82}I_2N_2S_2$	56.31	8.42	2.86
				(56.01	8.27	3.11)
4DTBP-8,16	83.5	148—149	$C_{50}H_{90}I_2N_2S_2$	57.90	8.75	2.70
				(57.68	8.55	2.65)
4DTBP-8,18	84.0	155—157	$C_{54}H_{98}I_2N_2S_2$	59.32	9.03	2.56
				(59.15	8.73	2.49)
4DTBP-10,8	82.1	119—120	$C_{36}H_{62}I_2N_2S_2$	51.42	7.43	3.33
				(51.12	7.17	3.18)
4DTBP-10,10	82.6	128—129	$C_{40}H_{70}I_2N_2S_2$	53.56	7.87	3.12
				(53.43	7.84	3.36)
4DTBP-10,12	84.6	134—136	$C_{44}H_{78}I_2N_2S_2$	55.45	8.25	2.94
				(55.15	8.10	3.22)
4DTBP-10,14	85.3	142—144	$C_{48}H_{86}I_2N_2S_2$	57.13	8.59	2.78
				(56.86	8.33	2.56)
4DTBP-10,16	85.6	148—149	$C_{52}H_{94}I_2N_2S_2$	58.63	8.89	2.63
				(58.65	8.65	2.54)
4DTBP-10,18	85.6	152—154	$C_{54}H_{102}I_2N_2S_2$	60.00	9.17	2.50
				(59.76	8.95	2.39)

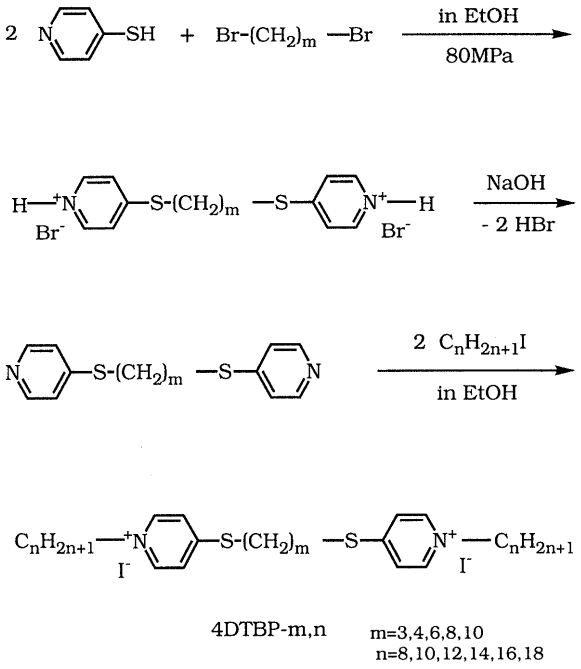


Chart 1

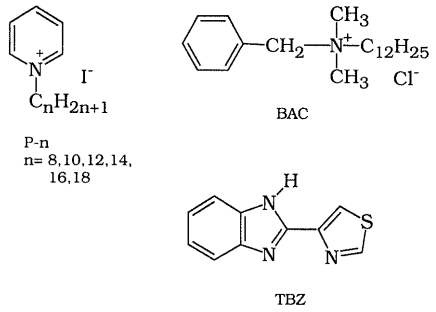


Fig. 1. Chemical Structures of *N*-Alkylpyridinium Iodide (P-n), Benzyldodecyldimethylammonium Chloride (BAC) and 2-(4-Thiazolyl)benzimidazole (TBZ)

4DTBP-6,12 exhibited a wide antibacterial spectrum and a strong bacteriostatic activity (log MIC^{-1}) against all bacteria tested in this study. The activity was much higher than those of P-12 and BAC, especially against gram-negative bacteria. In general, mono-QACs such as P-12 and BAC are more effective against gram-positive bacteria than against gram-negative bacteria.[7] As the cell surfaces of gram-positive bacteria are more hydrophobic than those of gram-negative bacteria,[20] it is thought that gram-positive bacteria have a higher susceptibility to compounds which interact with the cell surfaces. Bis-QAC (4DTBP-6,12), however, showed high bacteriostatic activity against both gram-negative and gram-positive bacteria. Other bis-QACs synthesized in this study also exhibited wide antibacterial spectra against the bacteria listed in Table 2 (data not shown). This may imply that the activities of 4DTBP-m,n are independent of the hydrophobicity of the bacterial cell surface.

Table 3 shows the MICs against fungi of 4DTBP-6,12, P-12 and TBZ, which is one of the most widely used antifungal agents. Though QACs are generally not effective against fungi,[18] 4DTBP-6,12 exhibited a wide spectrum and a high antifungal activity in addition to its

Table 2. Minimum Inhibitory Concentrations (MICs) of 4DTBP-6,12, P-12, and BAC against Gram-negative and Gram-positive Bacteria

Strain	MIC (μM)[a]		
	4DTBP-6,12[b]	P-12[c]	BAC[d]
Pseudomonas aeruginosa ATCC 27583	10.0	256	53.7
Pseudomonas aeruginosa ATCC 10145	8.0	164	53.7
Pseudomonas aeruginosa IFO 3080	10.0	205	67.1
Klebsiella pneumoniae ATCC 4352	4.1	21.0	10.5
Klebsiella pneumoniae ACTT 13883	3.3	51.2	64.0
Proteus rettgeri NIH 96	4.1	100	53.7
Proteus vulgaris ATCC 13315	1.7	32.8	16.4
Proteus mirabilis IFO 3849	8.0	500	205
Escherichia coli K12 OUT 8401	2.6	26.2	10.5
Escherichia coli K12 W3110	5.1	64.0	21.0
Bacillus subtilis IFO 3134	2.1	8.4	4.2
Bacillus subtilis ATCC 6633	2.1	16.4	6.6
Bacillus cereus IFO 3001	3.3	12.8	6.6
Bacillus megaterium IFO 3003	3.3	16.4	6.6
Micrococcus luteus IFO 12708	2.1	16.0	6.7
Micrococcus lysodeikticus NCTC 2665	1.1	2.7	2.8
Staphylococcus aureus IFO 12732	1.1	4.2	5.4
Staphylococcus epidermidis ATCC 12228	2.1	6.6	6.6
Staphylococcus aureus JC1 (MRSA)	1.3	50.0	13.1

a) MICs were measured by a broth dilution method using nutrient broth at 37 °C for 24 h. *b*) 4,4′-(1,6-Hexamethylenedithio)bis(1-dodecylpyridinium iodide). *c*) Dodecylpyridinium iodide. *d*) Benzyldodecyldimethylammonium chloride.

Table 3. Minimum Inhibitory Concentrations (MICs) of 4DTBP-6,12, P-12, and TBZ against Fungi

Strain	MIC (μM)[a]		
	4DTBP-6,12[b]	P-12[c]	TBZ[d]
Aspergillus terreus IFO 6346	8.4	131	156
Penicillium funiculosum IFO 6345	3.4	15.6	9.7
Chaetomium globosum IFO 6347	2.8	27.5	311
Cladosporium cladosporioides IFO 6348	8.4	27.5	9.7
Aureobasidium pullulans IFO 6353	16.4	67.1	38.8
Gliocladium virens IFO 6355	8.4	83.9	156
Rhizopus stolonifer IFO 4781	20.5	256	311

a) MICs were measured by a broth dilution method using Sabouraud broth at 28 °C for 7 d. *b*) 4,4′-(1,6-Hexamethylenedithio)bis(1-dodecylpyridinium iodide). *c*) Dodecylpyridinium iodide. *d*) 2-(4-Thiazolyl)benzimidazole.

antibacterial activity (Table 2). The activity (MIC = 2.8 – 20.5 μM) was much higher than those of P-12 and TBZ.

Effect of Alkyl Chain Length on Antibacterial Activity The antibacterial activities of QACs are generally influenced by the length of the alkyl group attached to the pyridinium nitrogen atom.[5] As can be seen in Fig. 2, the bacteriostatic activity of P-n was also affected by the length of the alkyl chain and was maximum with C16. In the case of 4DTBP-m,n, their activities increased when the molecules had shorter alkyl chains. On the other hand, the length of the chain connecting the two symmetrical quaternary ammonium moieties scarcely affected the activities. As the QACs tested in this study contain hydrophobic alkyl chains, it is plausible that there is a hydrophobic interaction between the molecule and medium components in the MIC measurement system. The bacteriostatic activities of QACs with long alkyl chains, such as P-18, could be inhibited by such an interaction. In the case of bis-QACs, which have two

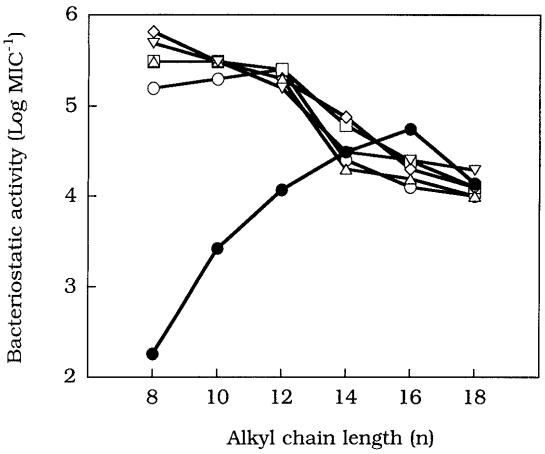


Fig. 2. Effect of Alkyl Chain Length (n) on the Bacteriostatic Activity of 4DTBP-m,n and P-n against Stationary-Phase Cells of *E. coli* K12 W3110

MICs were measured by a broth dilution method with nutrient broth at 37 °C for 24 h. Bacteriostatic activity was defined as the logarithm of the reciprocal of the molar concentration. ○, 4DTBP-3,n; □, 4DTBP-4,n; ◇, 4DTBP-6,n; △, 4DTBP-8,n; ▽, 4DTBP-10,n; ●, P-n.

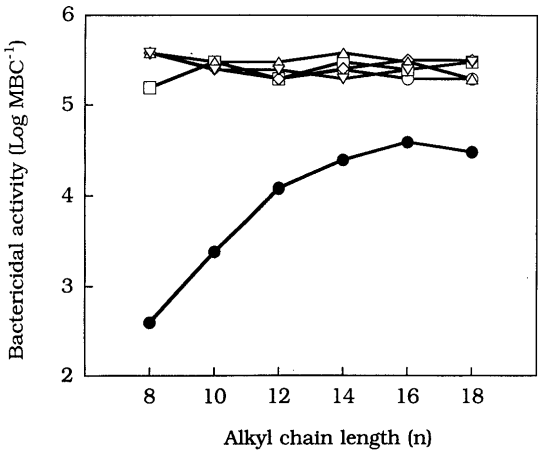


Fig. 3. Effect of Alkyl Chain Length (n) on the Bactericidal Activity of 4DTBP-m,n and P-n against Exponential-Phase Cells of *E. coli* K12 W3110

MBCs were measured by a dilution method at 30 °C for 30 min. Bactericidal activity was defined as the logarithm of the reciprocal of the molar concentration. ○, 4DTBP-3,n; □, 4DTBP-4,n; ◇, 4DTBP-6,n; △, 4DTBP-8,n; ▽, 4DTBP-10,n; ●, P-n.

hydrophobic alkyl chains, this could explain why the activities increased with shorter alkyl chain length. The bis-QAC that showed the strongest activity was 4DTBP-6,8 (MIC = 1.6 μM). It is possible that a hydrophobic association between 4DTBP-m,n (n = 14 – 18) and medium components caused a decrease in the effective concentration of these compounds in the measurement system.

Further, the effect of alkyl chain length of 4DTBP-m,n and P-n on the activity was investigated in terms of minimum bactericidal concentration (MBC) in the absence of medium components. The bactericidal activities (log MBC^{-1}), as well as the bacteriostatic activities, of P-n were remarkably influenced by the alkyl chain length, as shown in Fig. 3. The activities of 4DTBP-m,n, however, were not influenced by the alkyl group or the methylene group length. This result supports the idea that the lowering of the bacteriostatic activity of the tested bis-

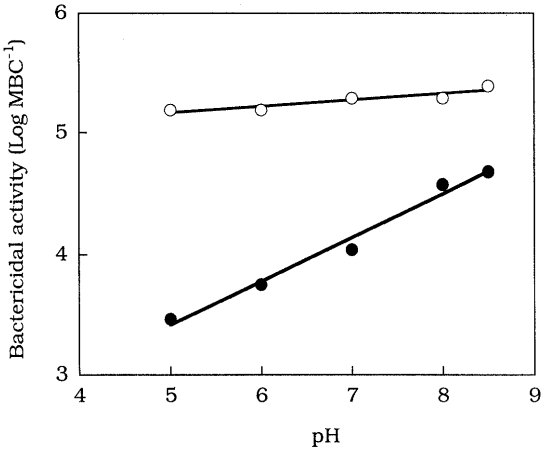


Fig. 4. Effect of pH on the Bactericidal Activity of 4DTBP-6,12 (○) and P-12 (●) against Exponential-Phase Cells of *E. coli* K12 W3110

MBCs were measured at 30 °C for 30 min using 0.05 M phosphate buffer.

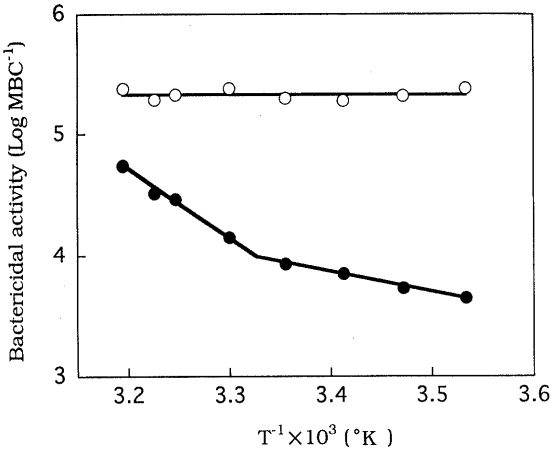


Fig. 5. Effect of Temperature on the Bactericidal Activity of 4DTBP-6,12 (○) and P-12 (●) against Exponential-Phase Cells of *E. coli* K12 W3110

QACs was caused by hydrophobic interaction with the medium components. Though the bactericidal activity of P-18 was lower than that of P-16, the difference was small, compared with that in the bacteriostatic activities (Fig. 2). 4DTBP-6,8 (MBC = 2.6 μM) was again the most potent of all and the activity was about 10 times that of P-16.

Effect of pH on Bactericidal Activity The MBCs of 4DTBP-6,12 and P-12 were measured using 0.05 M phosphate buffer (pH: 5, 6, 7, 8 and 8.5). As the bactericidal activities of QACs are influenced by salts such as NaCl and KCl in solution, we experimentally determined that the concentration of phosphate in the buffer had a negligible influence on the activity. As shown in Fig. 4, 4DTBP-6,12 maintained high activity in the pH range from 5 to 8.5. On the other hand, the activity of P-12 was strongly affected by the pH of the test solution, and it was significantly enhanced with a rise of pH. It is well-known that QACs such as P-12 are more effective in alkaline solution than in acidic solution.[8] This is one of the disadvantages of QACs. However, 4DTBP-6,12 showed a low dependence of the bactericidal activity on pH, possibly due to its dimeric structure.

Effect of Temperature on Bactericidal Activity Generally, an increase in temperature tends to enhance the bactericidal activities of QACs, because the temperature is closely related to the fluidity of the bacterial cell membrane and most QACs interact with the membrane to exhibit bactericidal action. To investigate the interaction between the bacterial membrane and the bis-QACs synthesized in this study, the bactericidal activities of 4DTBP-6,12 and P-12 were measured at various temperatures. The following results should not have been influenced by bacterial growth since the MBCs were measured in a suspension without nutrient substances for 30 min. The plot of log MBC^{-1} against the reciprocal of the absolute temperature (T^{-1}) for P-12 gave two straight lines, as can be seen in Fig. 5. The temperature at the intersection of two lines seems to be the phase transition temperature of the bacterial cell membrane. On the other hand, the activity of 4DTBP-6,12 was not affected by temperature. This suggests that the activity of 4DTBP-6,12 is not dependent on the membrane fluidity. In addition, we have previously reported that the bactericidal activity of QACs can be regarded as a rate of the bactericidal action.[21] Thus, Fig. 5 is a quasi Arrhenius plot and each slope shows the apparent activation energy of bactericidal action. Both slopes for P-12 are very large, which indicates that the activation energies of P-12 are large, whereas the slope of 4DTBP-6,12 is very small. It is concluded that 4DTBP-6,12 has a different bactericidal mechanism from the common QACs such as P-12.

Experimental

Melting points were measured with a melting point apparatus (Mitamura Riken Kogyo Inc.) and are uncorrected. Elemental analyses were done with a Yanagimoto NT-5 elemental analysis apparatus. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured with a JEOL NM-EX400 spectrometer using tetramethylsilane (TMS) as an internal standard.

4,4′-(α,ω-Polymethylenedithio)bis(1-alkylpyridinium iodide)s (4DTBP-m,n) A solution of 4-mercaptopyridine (1.0 mol) and polymethylene bromide (0.5 mol) in ethyl alcohol was refluxed for 4 h. The solvent was removed by evaporation, and the residue was recrystallized from ethyl alcohol–water (9 : 1) to give 4,4′-(α,ω-polymethylenedithio)bispyridine hydrobromides. This product was made strongly basic to litmus with 0.1 N aqueous NaOH and extracted with hot benzene (50 °C). Evaporation of the benzene gave 4,4′-(α,ω-polymethylenedithio)bispyridine. A mixture of the bispyridine (0.1 mol), *n*-alkyl iodide (0.2 mol) and ethyl alcohol (100 ml) was refluxed for 48 h. The solvent was removed by evaporation, and the residue was recrystallized twice from ethyl acetate–ethyl alcohol to give the title compounds.

Analytical and physical data for 4DTBP-m,n are summarized in Table 1 and ^{1}H-NMR data for the series of 4DTBP-m,8 and 4DTBP-6,n are summarized in Table 4.

Minimum Inhibitory Concentration (MIC) The MICs against bacteria and molds were measured by a broth dilution method.[7] A nutrient broth (Bacto beef extract 0.3% (w/v), Bacto peptone 0.5% (w/v), Difco Laboratories, Detroit, MI, U.S.A.) and a Sabouraud broth (Nissui Pharmaceutical Co., Ltd., Tokyo) were used for the antibacterial and antifungal tests. Ten ml of the broth containing 10 mg of each tested compound was diluted stepwise with the broth. The bacterium was preincubated in L-broth (Bacto tryptone 1% (w/v), yeast extract 0.5% (w/v), NaCl 0.5% (w/v), pH 7.2) for 18 h at 37 °C. A 1 ml aliquot of the culture was inoculated into 500 ml of nutrient broth, then 2 ml portions of the inoculated broth were pipetted into sterilized test tubes each containing 2 ml of diluted QAC. The mixture was incubated at 37 °C for 24 h, and the MICs were determined by visual inspection. Molds were incubated on Sabouraud-agar plates for 7 d at 28 °C. Spore suspensions were prepared by adding sterile physiological saline (10 ml) containing 0.2% (v/v) polyoxyethylene sorbitan monooleate (Tween-80, Nacalai

Table 4. ^{1}H-NMR Data for 4DTBP-m,8 and 4DTBP-6,n

Compd.	^{1}H-NMR (CD_3OD) δ
4DTBP-3,8	0.88 (6H t, J=6.8 Hz), 1.29—1.38 (20H, m), 1.99 (4H, m), 2.27 (2H, m), 3.56 (4H, t, J=7.3 Hz), 4.54 (4H, t, J=7.6 Hz), 7.98 (4H, d, J=6.8 Hz), 8.75 (4H, d, J=6.8 Hz)
4DTBP-4,8	0.89 (6H, t, J=6.8 Hz), 1.29—1.38 (20H, m), 1.97 (4H, m), 2.03 (4H, m), 3.40 (4H, t, J=7.2 Hz), 4.51 (4H, t, J=7.6 Hz), 7.94 (4H, d, J=7.3 Hz), 8.69 (4H, d, J=6.8 Hz)
4DTBP-6,8	0.89 (6H, t, J=6.8 Hz), 1.29—1.38 (20H, m), 1.62 (4H, m), 1.83 (4H, m), 1.97 (4H, m), 3.32 (4H, t, J=7.3 Hz), 4.50 (4H, t, J=7.6 Hz), 7.90 (4H, d, J=6.8 Hz), 8.68 (4H, d, J=7.3 Hz)
4DTBP-8.8	0.89 (6H, t, J=6.8 Hz), 1.30—1.38 (20H, m), 1.42 (4H, m), 1.55 (4H, m), 1.81 (4H, m), 1.98 (4H, m), 3.29 (4H, t, J=7.3 Hz), 4.49 (4H, t, J=7.6 Hz), 7.88 (4H, d, J=7.3 Hz), 8.66 (4H, d, J=7.3 Hz)
4DTBP-10,8	0.89 (6H, t, J=7.1 Hz), 1.30—1.38 (28H, m), 1.53 (4H, m), 1.79 (4H, m), 1.97 (4H, m), 3.28 (4H, t, J=7.3 Hz), 4.48 (4H, t, J=7.6 Hz), 7.86 (4H, d, J=6.8 Hz), 8.65 (4H, d, J=6.8 Hz)
4DTBP-6,10	0.89 (6H, t, J=6.8 Hz), 1.29—1.38 (28H, m), 1.62 (4H, m), 1.83 (4H, m), 1.97 (4H, m), 3.32 (4H, t, J=7.1 Hz), 4.49 (4H, t, J=7.3 Hz), 7.89 (4H, d, J=6.3 Hz), 8.67 (4H, d, J=6.4 Hz)
4DTBP-6,12	0.89 (6H, t, J=6.8 Hz), 1.28—1.37 (36H, m), 1.61 (4H, m), 1.83 (4H, m), 1.96 (4H, m), 3.30 (4H, t, J=7.6 Hz), 4.44 (4H, t, J=7.3 Hz), 7.85 (4H, d, J=7.3 Hz), 8.59 (4H, d, J=7.3 Hz)
4DTBP-6,14	0.89 (6H, t, J=6.8 Hz), 1.28—1.37 (44H, m), 1.61 (4H, m), 1.83 (4H, m), 1.95 (4H, m), 3.31 (4H, t, J=7.6 Hz), 4.43 (4H, t, J=7.3 Hz), 7.85 (4H, d, J=7.3 Hz), 8.58 (4H, d, J=7.3 Hz)
4DTBP-6,16	0.89 (6H, t, J=6.8 Hz), 1.28—1.37 (52H, m), 1.60 (4H, m), 1.83 (4H, m), 1.95 (4H, m), 3.31 (4H, t, J=7.6 Hz), 4.43 (4H, t, J=7.6 Hz), 7.84 (4H, d, J=7.3 Hz), 8.58 (4H, d, J=7.3 Hz)
4DTBP-6,18	0.90 (6H, t, J=6.6 Hz), 1.28—1.37 (60H, m), 1.61 (4H, m), 1.83 (4H, m), 1.96 (4H, m), 3.27 (4H, t, J=7.6 Hz), 4.43 (4H, t, J=7.6 Hz), 7.84 (4H, d, J=6.4 Hz), 8.58 (4H, d, J=5.9 Hz)

Tesque Inc., Kyoto) to the plates. The spore suspension was diluted 1000-fold with Sabouraud broth. Two-ml portions of the diluted spore suspension were mixed with 2 ml portions of diluted QACs and incubated at 20 °C for 48 h. The MICs against molds were determined.

Minimum Bactericidal Concentration (MBC) The cell suspension (1 ml) of *E. coli* K12 preincubated in L-broth was inoculated into 100 ml of nutrient broth. After incubation for 2 h at 37 °C, the exponentially growing cells were harvested by centrifugation at 5000 × ***g*** for 10 min at 2 °C, washed and suspended in sterilized ice-cooled water. The cell concentration of the suspension was adjusted to 1×10^6 cells per ml with ice-cooled water. One ml of an aqueous solution containing 1 mg of the tested QAC was diluted stepwise with sterilized water. Aliquots of 0.5 ml of the diluted solutions were mixed with 0.5 ml of cell suspension, and the mixtures were incubated in a water bath shaker for 30 min at 37 °C. Then 0.1 ml aliquots of mixtures were taken out and inoculated into 2 ml of nutrient broth containing 1% (v/v) Tween 80. After the incubation at 37 °C for 24 h, MBC was determined by visual inspection.

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