Synthesis and Biological Properties of Gemini Quaternary Ammonium Compounds, 5,5′-[2,2′-(α,ω-Polymethylnedicarbonyldioxy)diethyl]bis-(3-alkyl-4-methylthiazolium iodide) and 5,5′-[2,2′-(*p*-Phenylenedicarbonyldioxy)diethyl]bis(3-alkyl-4-methylthiazolium bromide)

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We synthesized gemini quaternary ammonium compounds (gemini QACs) having two thiazolium moieties in a molecule, 5,5′-[2,2′-(α,ω-polymethylnedicarbonyldioxy)diethyl]bis(3-alkyl-4-methylthiazolium iodide) (5DEBT-m,n), on which the carbon number of the methylene chain linking the two thiazoles (m) is 2, 6 or 8 and that of the alkyl group (n) is 8, 10, 12, 14 or 16. 5,5′-[2,2′-(*p*-Phenylenedicarbonyldioxy)diethyl]bis(3-alkyl-4-methylthiazolium bromide) (5DEBT-P,n) was then synthesized, which is composed of a *p*-phenylene as the methylene spacer. For five gemini QAC series, in addition to the previously described 5DEBT-4,n to the four new compound series, their antimicrobial activities were determined. 5DEBT-m,10 and -P,10 exhibited a wide and strong bacteriostatic activity against gram-negative and -positive bacteria, fungi and then yeast in comparison with *N*-tetradecyl-5-(2-hydroxyethyl)-4-methylthiazolium iodide as a mono-QAC. The bactericidal activity of the 5DEBT series against *Escherichia coli* IFO 12713 and *Staphylococcus aureus* IFO 12732 was investigated on the basis of the effects of their alkyl chain length and their molecular hydrophobicity. It was found that the effect of theses factors on their activity significantly changes by the difference between the gram-negative and -positive bacteria. Although against the gram-negative bacterium, the change in the activity due to extension of the alkyl group for each compound affected the kind of methylene spacer, against the gram-positive bacterium, it was almost equal in spite of the methylene spacer. This result could be responsible for the bactericidal mechanism of the gemini QACs being influenced by the diversity of the steric structure participating in the methylene chain length and by the bacterium cell surface hydrophobicity.

Key words gemini quaternary ammonium compound; *N*-alkylthiazolium salt; antimicrobial activity; bactericidal characteristic; molecular hydrophobicity

Thiazole derivatives, which are representative by sulfurol, 4-methyl-5-hydroxyethyl-thiazole, are well known as perfumes of food additives in the food industry and have been widely utilized as antibiotics in the medical industry. One of the commercial antimicrobial agents, 2-(4-thiazolyl)benzimidazole (TBZ), based on the thiazole skeleton has fungicidal activity, which is used for the antimicrobial treatment of products such as foods, textiles, papers and synthetic resins. However, this compound shows a weak antibacterial activity.[1] The *N*-alkyl-5-(2-hydroxyethyl)-4-methylthiazolium iodide (T-n,n: alkyl chain length), quaternized the sulfurol with long alkyl groups, successfully acquired a wide antimicrobial activity for both bacteria and fungi, and was a more effective fungicide than TBZ.[2] In recent years, 4-methylthiazolium betaine also has been reported to show a significant antibacterial activity under hyperosmotic conditions.[3]

Gemini quaternary ammonium compounds (gemini QACs) are linking two molecules of tertiary amines-quaternarized compounds (mono-QACs), which are famous as significant antimicrobial agents in clinical applications, food production and health care, with hydrocarbon chains. The gemini QACs possess a unique structure, a greater surface activity and greater antimicrobial potency than conventional mono-QACs. Chemists have studied their physicochemical properties[4—6] and greater effective antibacterial potency than the corresponding mono-QACs.[7—9]

We have also described the syntheses of gemini QACs, which have two cationic pyridiniums, and discussed their antimicrobial activities and characteristics.[10—15] It has been proved that the antimicrobial potency of their gemini QACs shows a wider and more effective spectrum than that of mono-QACs against both gram-negative and gram-positive bacteria and fungi. 4,4′-(α,ω-Polymethylenedithio)bis(1-alkylpyridinium iodide) (4DTBP-m,n, m: methylene chain length, 3, 4, 6, 8 or 10; n: 8, 10, 12, 14, 16 or 18) as the gemini QACs was studied in great detail. The antimicrobial activity was not only wider and stronger than that of the usual mono-QACs, *N*-dodecylpyridinium iodide and benzyldimethyldodecylammonium chloride (BAC), but also independent of pH, temperature and molecular hydrophobicity, the characteristics of which were different from those of mono-QACs.[10,11] Sumitomo *et al.* revealed the leakage of magnesium ion stabilizing the outer membrane of bacterial cells and that of ATP from the cells, and then the respiratory inhibition before bacteriolastic action by treatment of 4DTBP-6,8 toward *Escherichia coli* (*E. coli*) IFO 12713. This result suggested that gemini QACs conform to a specific bactericidal mechanism unlike mono-QACs.[16] The structural feature of 4DTBP-m,8, which affected the length of the methylene chain, was examined on the basis of the transitions of the conformer and solvation free energy during their holding processes.[17]

In this study, we focused on 5,5′-[2,2′-(1,4-tetramethylnedicarbonyldioxy)diethyl]bis(3-alkyl-4-methylthiazolium

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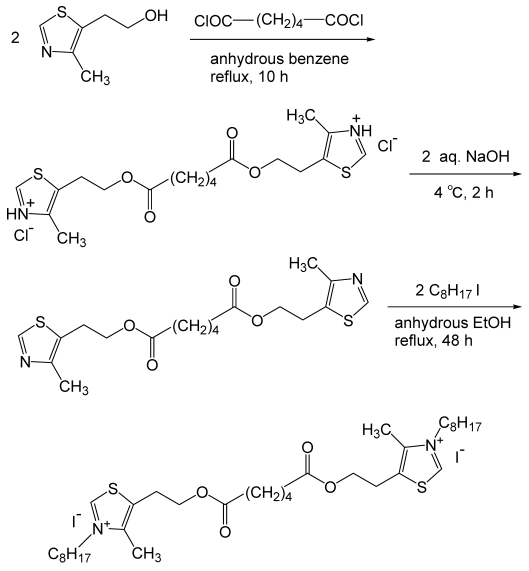

5DEBT-4,8

Chart 1

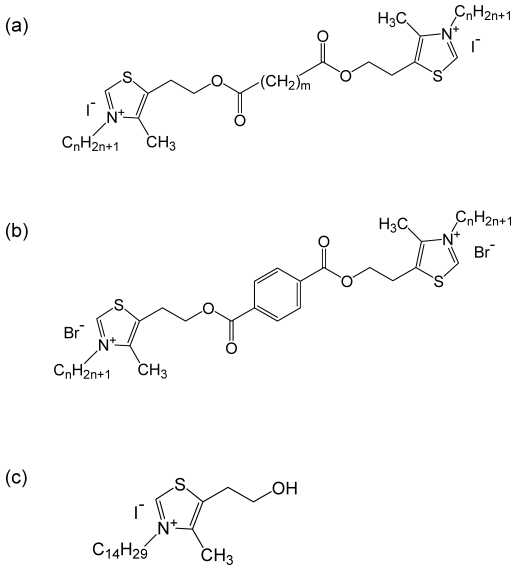

Fig. 1. Chemical Structures of Two Gemini QACs and One Comparative Mono-QAC: (a) 5,5′-[2,2′-(α,ω-Polymethylnedicarbonyldioxy)diethyl]bis-(3-alkyl-4-methylthiazolium iodide) (5DEBT-m,n); (b) 5,5′-[2,2′-(*p*-Phenylenedicarbonyldioxy)diethyl]bis(3-alkyl-4-methylthiazolium bromide) (5DEBT-P,n); (c) *N*-Tetradecyl-5-(2-hydroxyethyl)-4-methylthiazolium Iodide (T-14) as a Mono-QAC

Abbreviations, m and n, indicate 2, 4, 6 or 8 as the carbon number of the spacer chain, and 8, 10, 12, 14 or 16 as that of the alkyl chain.

iodide) (5DEBT-4,n, n: 8, 10, 12, 14 or 16) as the gemini QAC described in the previous paper, in which the cationic moiety is a thiazole ring,[18] because the structure of the methylene chain linking the two thiazoles can be altered to other methylene structures similar to that of 4DTBP-m,n. Moreover, the investigation of the biological properties of the systematic synthesized 5DEBT series could be useful for the study of the structural activity relationship (SAR) for a typical gemini QAC possessing a thiazole skeleton similar to the SAR study of 4DTBP-m,n as the gemini QACs of the pyridinium type. The compound, 5DEBT-4,n has already been confirmed to have a strong antimicrobial activity for a wide range of microbes beside T-n, TBZ and BAC [18] and the relationship between the cytotoxicity and the antibacterial activity was studied along with a global minimum analysis and the determination of the solvation free energy.[19] Four novel gemini QAC series, 5DEBT-m,n (m: 2, 6 or 8; n: 8, 10, 12, 14 or 16) and 5,5′-[2,2′-(*p*-phenylenedicarbonyldioxy)diethyl]bis(3-alkyl-4-methylthiazolium bromide) (5DEBT-P,n, n: 8, 10, 12, 14 or 16), which possess spacer structures such as ethylene, hexamethylene, octamethylene or *p*-phenylene, were then synthesized. Using the five gemini QAC series, 5DEBT-2,n, 5DEBT-6,n, 5DEBT-8,n and 5DEBT-P,n, in addition to the previously described 5DEBT-4,n to the new four series, we evaluated the antimicrobial activity against bacteria, fungi and yeast, compared to *N*-tetradecyl-5-(2-hydroxyethyl)-4-methylthiazolium iodide (T-14) as a monomeric compound prior to dimerization of the thiazole ring. Furthermore, the bactericidal activities of the five 5DEBT series were measured for the alkyl chain length ranging from 8 to 16 against *E. coli* IFO 12713 and *Staphylococcus aureus* (*Staph. aureus*) IFO 12732, which are representative as both gram-negative and -positive bacteria, discussing how the effect of their alkyl chain length on their activities changes between the strains and with the length of the methylene chain or a phenylene, and establishing the relationship between their activities for the two strains and their molecular hydrophobicities.

Results and Discussion

Syntheses of Gemini QAC Derivatives All the analytical and physical data confirmed that all compounds synthesized are 5DEBT-m,n (m: 2, 4, 6 or 8; n: 8, 10, 12, 14 or 16) and 5DEBT-P,n (n: 8, 10, 12, 14 or 16) as the purposed gemini QAC. Elemental analyses, similar to their theoretical values, and the melting points of these compounds suggested high purity compounds, and the ^{1}H-NMR spectra for these compounds were consistent with their desired structure.

These gemini QACs series, which are shown in Fig. 1, were employed for the evaluation of the biological activities, and a comparative biocide for the antimicrobial test is T-14[18] possessing one thiazolium moiety as a mono-QAC corresponding to the gemini QACs.

Antimicrobial Activity In a previous report, 5DEBT-4,12 have been proved to have a higher antimicrobial activity and a wider antimicrobial spectrum than T-12 by the comparison of the minimum inhibitory concentration (MIC) for the same alkyl chain length.[18] So the MICs of the 5DEBT series were measured for the alkyl chain carbon number of 10 against various microbes, gram-negative bacteria (5 strains) and -positive bacteria (5 strains), fungi (6 strains) and then yeast (1 strain) in this study. Because the highest MIC of the previous 5DEBT-4,n had been observed for the alkyl chain length (n=10).[18] The results for 5DEBT-m,10 (m: 2, 4, 6 or 8) and 5DEBT-P,10 are summarized in Tables 1 and 2. A mono-QAC, T-14, which is comprised of an *N*-alkylthiazolium salt as a pre-dimerization compound of 5DEBT-m,n and possesses the highest bacteriostatic activity among the alkyl chain lengths from 8 to 16,[18] was also measured for comparison. In general, mono-QACs have a more effective activity against gram-positive bacteria than against gram-

Table 1. MIC Spectra of 5DEBT-m,10 (m; 2, 4, 6 or 8) and 5DEBT-P,10 as a Gemini QAC, and T-14 as a Mono-QAC against Bacteria

	MIC (μM)[a]					
	m[b]				-P,10	Mono-QAC
	2	4	6	8		T-14
Ps. aeruginosa ATCC 27583[c]	13±0.0	13±0.0	13±0.0	17±7.2	13±0.0	50±0.0
Kl. pneumoniae ATCC 4352[d]	1.0±0.45	2.6±0.91	2.1±0.91	2.1±0.91	1.6±0.0	8.3±3.6
Pr. mirabilis IFO 3849[e]	17±7.2	15±9.6	4.2±1.8	3.1±0.0	5.2±0.18	50±0.0
E. coli IFO 12713[f]	1.6±0.0	1.6±0.0	1.6±0.0	1.6±0.0	1.6±0.0	25±0.0
Ser. marcescens ATCC 13880[g]	7.3±4.8	6.3±0.0	4.2±1.8	3.1±0.0	6.3±0.0	17±7.2
M. luteus IFO 12708[h]	1.0±0.45	0.78±0.0	0.65±0.23	0.65±0.23	0.78±0.0	5.2±1.8
B. subtilis ATCC 6633[i]	0.78±0.0	0.78±0.0	1.0±0.45	0.91±0.60	1.0±0.45	1.8±1.2
B. cereus IFO 3001[j]	1.6±0.0	1.6±0.0	1.3±0.45	1.6±0.0	1.3±0.45	6.3±0.0
Staph. aureus IFO 12732[k]	0.46±0.3	0.33±0.11	0.29±0.17	0.23±0.15	0.33±0.11	1.0±0.45
Staph. aureus COL 1 (MRSA)	0.78±0.0	1.0±0.45	1.0±0.45	1.6±0.0	1.3±0.45	2.6±0.91

a) All experiments were performed at least in triplicate, and each value represents the mean±S.D. ($n \geqq 3$). *b*) Carbon number of methylene group of 5DEBT-m,n. *c*) *Pseudomonas aeruginosa* ATCC 27583. *d*) *Klebsiella pneumoniae* ATCC 4352. *e*) *Proteus mirabilis* IFO 3849. *f*) *Escherichia coli* IFO 12713. *g*) *Serratia marcescens* ATCC 13880. *h*) *Micrococcus luteus* IFO 12708. *i*) *Bacillus subtilis* ATCC 6633. *j*) *Bacillus cereus* IFO 3001. *k*) *Staphylococcus aureus* IFO 12732.

Table 2. MIC Spectra of 5DEBT-m,10 (m; 2, 4, 6 or 8) and 5DEBT-P,10 as a Gemini QAC, and T-14 as a Mono-QAC against Fungi and Yeast

	MIC (μM)[a]					
	m[b]				-P,10	Mono-QAC
	2	4	6	8		T-14
A. niger IFO 6342[c]	100±0.0	50±0.0	33±7.2	25±0.0	50±0.0	67±29
A. terreus IFO 6346[d]	6.3±0.0	8.3±3.6	25±0.0	13±0.0	13±0.0	33±14
Cha. globosum IFO 6347[e]	13±0.0	6.3±0.0	13±0.0	25±0.0	4.2±1.8	67±29
Pe. funiculosum IFO 6345[f]	2.1±0.91	2.6±0.91	2.1±0.91	5.2±1.8	5.2±1.8	29±19
Tr. mentagrophytes NBRC 2412[g]	10±3.6	8.3±3.6	17±7.2	25±0.0	4.2±1.8	25±0.0
Rhi. stolonifer IFO 4781[h]	100±0.0	25±0.0	25±0.0	21±7.2	25±0.0	50±0.0
Sacc. cerevisiae NBRC 10217[i]	6.3±0.0	6.3±0.0	6.3±0.0	13±0.0	8.3±3.6	4.2±1.8

a) All experiments were performed at least in triplicate, and each value represents the mean±S.D. ($n \geqq 3$). *b*) Carbon number of methylene group of 5DEBT-m,n. *c*) *Aspergillus niger* IFO 6342. *d*) *Aspergillus terreus* IFO 6346. *e*) *Chaetomium globosum* IFO 6347. *f*) *Penicillium funiculosum* IFO 6345. *g*) *Trichophyton mentagrophytes* NBRC 2412. *h*) *Rhizopus stolonifer* IFO 4781. *i*) *Saccharomyces cerevisiae* NBRC 10217.

negative bacteria.[18,20] As shown in Table 1, T-14 as the mono-QAC inhibited the growth of gram-positive bacteria at a lower concentration than the MICs against gram-negative bacteria. In previous studies, gemini QACs, however, showed a high activity regardless of the bacteria species.[10,13,18] All the 5DEBT series exhibited a wide antibacterial spectra and a strong bacteriostatic activity against all tested bacteria similar to the previously reported gemini QACs. Their MICs against gram-negative bacteria in particular were lower than the MICs of T-14 against the bacteria. Also compared with the kind of methylene chain, such as the length of the methylene chain and aromatic hydrocarbon for their tested biocides, the activity was almost equal and hardly influenced by the chain length.

Table 2 lists the MICs of the 5DEBT series and T-14 against fungi and yeast. The 5DEBT series proved to have a stronger antifungal and anti-yeast fungal activities and a wider antifungal spectrum than T-14, except for the activities of 5DEBT-2,10 against *A. niger* IFO 6342 and *Rhi. stolonifer* IFO 4781, which were lower than those of T-14. The short methylene chain as the spacer chain for 5DEBT-m,n decreased the activity against *A. niger* IFO 6342 and *Rhi. stolonifer* IFO 4781 as 5DEBT-2,10 showed MICs of 100 μM against them. For the other 5DEBT-m,n, the methylene chain length, which is longer than ethylene, including a phenylene structure, had little effect on the activity against their fungi.

Accordingly, these results led to the conclusion that 5DEBT-m,10 and -P,10, except for the spacer structure linking the two thiazole rings being ethylene, display a stronger antimicrobial activity and broader antimicrobial spectrum than the mono-QAC, T-14, and a significant activity was scarcely observed by possessing the methylene structure longer than ethylene or a phenylene group.

Effect of Alkyl Chain and Spacer Chain Lengths on Antibacterial Activity The minimum bactericidal concentration (MBC) of the five gemini QAC series, 5DEBT-2,n, -4,n, -6,n, -8,n and -P,n (n: 8, 10, 12, 14 or 16), was measured, and the bactericidal activity (Log MBC^{-1}) was investigated for the effect of the alkyl chain and spacer chain lengths. Figure 2 shows the bactericidal activities of the five gemini QACs based on their alkyl chain lengths against *E. coli* IFO 12713 [Fig. 2a] and *Staph. aureus* IFO 12732 [Fig. 2b].

The alkyl chain and methylene chain lengths for 4DTBP-m,n (m: 3, 4, 6, 8 or 10; n: 8, 10, 12, 14, 16 or 18), which was synthesized in our laboratories, hardly influenced their bactericidal activity against *E. coli* IFO 12713.[10] However, it was found for the 5DEBT series in this research that the effect of the alkyl chain length on their activity significantly changed for the compounds having different methylene spacers such as ethylene, tetramethylene, hexamethylene, octa-

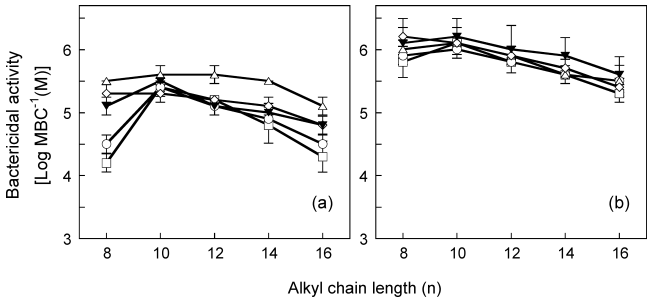

Fig. 2. Effect of Alkyl Chain Length (n) on the Bactericidal Activity (Log MBC^{-1}) of 5DEBT-m,n and 5DEBT-P,n against *E. coli* IFO 12713 (a) and *Staph. aureus* IFO 12732 (b)

Symbols: □, 5DEBT-2,n; ○, 5DEBT-4,n; △, 5DEBT-6,n; ◇, 5DEBT-8,n; ▼, 5DEBT-P,n. Value is the mean and the error bar represents the standard deviation obtained from three independent experiments.

methylene and phenylene [Fig. 2a]. For both 5DEBT-6,n and -8,n, their activities were almost constant in the range of n=8 to 14 and decreased for n=16. The activities of 5DEBT-2,n, -4,n and -P,n increased in the range of n=8 to 10, and their compounds had the highest activity at n=10. The activities of the three gemini QACs were observed to gradually decrease as the alkyl chain length became longer from n=12 to 16. Therefore, these 5DEBT-m,n having a short methylene spacer (m: 2 and 4) were found to have the tendency that the activity increased and decreased due to the alkyl chain length stretching between n=8 and 16. The tendency of the activity for 5DEBT-P,n linking with a phenylene group, the spacer structure of which is presumed to be rigid structure, was also identified for these compounds. It was considered that the change in the alkyl chain length is slightly reflected in the activity of 5DEBT-m,n, which has a long methylene spacer, that is, m=6 and 8. Although 5DEBT-m,n, which is composed of a short methylene spacer, that is, m=2, 4 and phenylene, significantly changed its activity by an increase in its alkyl chain length, compared with the gemini-QAC having the long methylene spacer. Lengthening the methylene chain from 4 to 6 affected the transition of the activity for the alkyl chain length in the range of 8 to 16, suggesting a transformation point due to the steric structure which might exist between the methylene chain lengths. The length of the methylene chain for the gemini QACs has been reported to contribute to the occupational area per molecule at the air-water interface[21] and to the diversity in the steric structure which is understood from an increase in the conformer by extension of its methylene chain based on a global minimum analysis for 4DTBP-m,n as the gemini QAC using CONFLEX.[17] Also, an increase in the conformer for 5DEBT-m,n, presumably due to extending its methylene chain, possibly affects the action of the bactericide on the bacterium cells, which results in a decreased dependence on the alkyl chain for the bactericidal activity, that is, it implies an alternation in the role of the alkyl chain on the bactericidal mechanism.

We then examined the effect of the alkyl chain length on the bactericidal activity of the 5DEBT series against *Staph. aureus* IFO 12732 [Fig. 2b]. All the compounds except 5DEBT-2,n indicated the high activity for the short alkyl chain (n: 8 and 10) and the activity then gradually decreased with an alkyl chain longer than the *n*-decyl group. On the other hand, the plots of 5DEBT-2,n resulted in the highest activity at 10 alkyl length. However, the activity at each alkyl chain length for all the compounds was almost equal, which were independent of the methylene spacer structure beside the relation between the effect of the alkyl chain on the activity against *E. coli* IFO 12713 and the structure of the spacer chain. This reason suggested the influence on the bacterium cell surface hydrophobicity which is caused by a difference in the cell membrane structure between the strains. Gram-positive bacteria generally have a higher hydrophobicity than gram-negative bacteria. The susceptibility of gemini QACs against gram-positive bacteria is higher than that of against gram-negative bacteria.[11] It is presumed that gemini QACs as a hydrophobic molecule possessing two long alkyl chains disrupt the bacterium cell membrane by a hydrophobic interaction between the long alkyl chain and the cell surface, captured to the bacterium cell with a more hydrophobic surface. Therefore, the inactivation of other bactericidal factors contributing to the bactericidal mechanism of gemini QACs, such as the steric structure in connection with the methylene chain length, might be responsible for the acceleration of the hydrophobic interaction against more hydrophobic bacteria, *i.e.*, gram-positive bacteria.

Effect of Molecular Hydrophobicity on Antibacterial Activity The relationships between the molecular hydrophobicities and the bactericidal activities of 5DEBT-m,n and -P,n were investigated. In Figs. 3a and b, the activity against *E. coli* IFO 12713 and *Staph. aureus* IFO 12732 are plotted as a function of the hydrophobicity (R_M) of their gemini QACs calculated from *Rf* obtained by TLC.

A parabolic curve generated by the plot of the bactericidal activity of 5DEBT-2,n, which is significantly dependent on the molecular hydrophobicity, proved that the activity has a connection with the hydrophobicity [Fig. 3a]. Plots of the mono-QACs, T-n and *N*-alkylpyridinium iodide, were also parabolic like those of 5DEBT-2,n.[11,18] On the contrary, the activity of 5DEBT-6,n and -8,n has a linear relationship against their hydrophobicity, that is, the activity was almost equal regardless of their hydrophobicities. No correlation with the hydrophobicity was then observed for the activity of 5DEBT-4,n and -P,n, indicating that their activity is not attributable to the hydrophobicity similar to 5DEBT-6,n and -8,n. Consequently, it was found that the length of the methylene chain affects the relationship between the activity and hydrophobicity; when 5DEBT-m,n has a short methylene spacer (m: 2), the activity is significantly dependent on the hydrophobicity, the plot of which follows a parabolic curve; when the compound has a long methylene spacer (m: 6 and 8), the hydrophobicity scarcely changes the activity, the plot of which is a straight line. The extension of alkyl group of 5DEBT-m,n mainly increases its hydrophobicity,[11,15] so that the independence of the activity on the hydrophobicity might suggest a bactericidal action which is different from the action caused by the long alkyl group relating to the hydrophobicity. The investigation into the specific bactericidal mechanism of 4DTBP-6,8 as a gemini QAC was already carried out and confirmed the leakage of magnesium ion stabilizing the outer membrane of the bacterial cell as the first stage, the leakage of ATP, and then the respiratory inhibition before the bacterioclastic action.[16] Therefore, as mentioned in the previous section, 5DEBT-m,n having a long methylene spacer, which possibly forms more steric structures, could easily

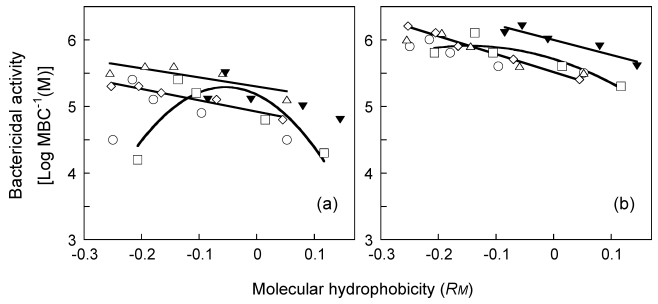

Fig. 3. Relation between Molecular Hydrophobicity (R_M) and the Bactericidal Activity (Log MBC^{-1}) of 5DEBT-m,n and 5DEBT-P,n against *E. coli* IFO 12713 (a) and *Staph. aureus* IFO 12732 (b)

Symbols: □, 5DEBT-2,n; ○, 5DEBT-4,n; △, 5DEBT-6,n; ◇, 5DEBT-8,n; ▼, 5DEBT-P,n. Value is the mean from three independent experiments.

form a more optimum structure on the bactericidal action against bacterium cell surfaces so that such steric-structural properties as the distance between the ammonium cations or the distribution of two alkyl groups are superior to the properties of 5DEBT-m,n having a short one. Moreover, it is suggested that the 5DEBT-m,n with a long methylene spacer conforms to a specific bactericidal action for the gemini QAC, which is independent of the molecular hydrophobicity, rather than the typical action for mono-QACs which is influenced by the hydrophobicity, and causes bacterium cellular death.

The activity of the 5DEBT series against *Staph. aureus* IFO 12732 was discussed on the basis of the effect of their hydrophobicity [Fig. 3b]. The plots of 5DEBT-2,n possessing the shortest methylene spacer had a gentle parabolic curve, but those of the other compounds showed a straight line. However, whatever the length of methylene spacer the compound has, the correlation was almost the same, compared with the correlation for the *E. coli* IFO 12713. Furthermore, their activity gradually decreased by an increase in their hydrophobicity, suggesting that the activity of all the compounds was easily influenced by their hydrophobicity. Consequently, this hydrophobic dependence of the activity in spite of the methylene spacer structure implies that these compounds disrupt the bacterium cell membrane by the hydrophobic interaction between the alkyl chain and the cell surface, the bactericidal mechanism of which may be ascribed to the fact that the cell surface hydrophobicity of *Staph. aureus* IFO 12732 is higher than *E. coli* IFO 12713.

Conclusion

Four novel gemini QAC series, 5DEBT-m,n (m: 2, 6 or 8; n: 8, 10, 12, 14 or 16) and 5DEBT-P,n (n: 8, 10, 12, 14 or 16) were synthesized and investigated for their antimicrobial activity against bacteria, fungi and yeast, in addition to the previously described 5DEBT-4,n to the four compound series, compared with T-14 as a mono-QAC having the thiazole ring. 5DEBT-m,10 (m: 4, 6 and 8) and -P,10 showed a stronger antimicrobial activity and broader antimicrobial spectrum than T-14, and a significant activity change was hardly observed due to the methylene structure becoming longer than ethylene or having a phenylene group. The MBC measurement of the 5DEBT series against *E. coli* IFO 12713 and *Staph. aureus* IFO 12732 revealed that the effects of the alkyl chain length and the hydrophobicity on their activity significantly changed due to the difference between the gram-negative and -positive bacteria. This result suggested that the bactericidal mechanism of the gemini QACs could be responsible for the diversity of the steric structure participating in the methylene chain length and for the bacterium cell surface hydrophobicity.

Experimental

General Procedures Chemicals used in the synthesis of the QACs and materials used in the biological experiments were reagent grade commercial materials and used without further purification. The melting points of the synthesized compounds were determined using a trace amounts melting point apparatus (Mitamura Riken Kogyo Inc., Japan). The elemental analysis was done using a Yanagimoto MT-5 elemental analysis apparatus (Japan). The ^{1}H-NMR spectrum (400 MHz) was recorded on a JOEL NMR spectrometer (JEM-EX 400, JOEL, Japan), the chemical shifts of which are given from tetramethylsilane as the internal standard and $CDCl_3$. The purity of the compounds was examined by TLC plates (Merck silica gel 60 F_{254} and Merck RP-18 F_{254S} plates; thickness 0.25 mm, Merck Japan Ltd., Japan). A comparative biocide for the antimicrobial test, T-14 possessing one thiazolium moiety as a mono-QAC was synthesized in accordance with a previous report.[2)]

5,5′-[2,2′-(α,ω-Polymethylnedicarbonyldioxy)diethyl]bis(3-alkyl-4-methylthiazolium iodide) (5DEBT-m,n) and 5,5′-[2,2′-(*p*-Phenylenedicarbonyldioxy)diethyl]bis(3-alkyl-4-methylthiazolium bromide) (5DEBT-P,n) All the gemini QACs, 5DEBT-m,n and 5DEBT-P,n, were synthesized according to the procedure of 5DEBT-4,n described by Maeda *et al.*[18)] The synthetic procedure of 5DEBT-4,8 is summarized in Chart 1. The abbreviations, m and n, indicate the carbon number of the spacer chain (m=2, 4, 6 or 8) and alkyl chain (n=8, 10, 12, 14 or 16), respectively, and the spacer abbreviation, m=P, denotes the *p*-phenylene group. Reagents treated with 5-(2-hydroxyethyl)-4-methylthiazole were converted by the spacer chain of the desired gemini QAC derivatives, which are succinyl chloride for the synthesis of 5DEBT-2,n, suberoyl chloride for that of 5DEBT-6,n, sebacoyl chloride for that of 5DEBT-8,n and terephthaloyl chloride for that of 5DEBT-P,n. To produce a theoretical 5 g yield, the series of 5DEBT-m,n were quaternized with an *n*-alkyl iodide and the quaternization of 5DEBT-P,n was carried out with an *n*-alkyl bromide. The 5DEBT-m,n and 5DEBT-P,n were obtained as a yellow or a white crystal, respectively.

5DEBT-2,8 Yield; 1.23 g, 24.5 %. mp 169—172 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (6H, t, J=6.8 Hz), 1.27—1.36 (16H, m), 1.42—1.44 (4H, m), 1.95 (4H, quintet, J=7.6 Hz), 2.59 (6H, s), 2.60 (4H, s), 3.36 (4H, t, J=5.6 Hz), 4.43 (4H, t, J=5.6 Hz), 4.63 (4H, t, J=7.8 Hz), 10.84 (2H, s). *Anal.* Calcd for $C_{32}H_{54}N_2O_4S_2I_2$: C, 45.29; H, 6.41; N, 3.30. Found: C, 45.13; H, 6.23; N, 3.34.

5DEBT-2,10 Yield; 1.33 g, 26.5%. mp 172—175 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0 Hz), 1.23—1.37 (24H, m), 1.40—1.47 (4H, m), 1.94 (4H, quintet, J=7.7 Hz), 2.58 (6H, s), 2.60 (4H, s), 3.35 (4H, t, J=5.5 Hz), 4.43 (4H, t, J=5.6 Hz), 4.63 (4H, t, J=7.8 Hz), 10.85 (2H, s). *Anal.* Calcd for $C_{36}H_{62}N_2O_4S_2I_2$: C, 47.79; H, 6.91; N, 3.10. Found: C, 47.65; H, 6.65; N, 3.11.

5DEBT-2,12 Yield; 1.22 g, 24.4%. mp 174—177 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25—1.35 (32H, m), 1.40—1.46 (4H, m), 1.94 (4H, quintet, J=7.6 Hz), 2.58 (6H, s), 2.60 (4H, s), 3.35 (4H, t, J=5.5 Hz), 4.43 (4H, t, J=5.6 Hz), 4.62 (4H, t, J=7.8 Hz), 10.86 (2H, s). *Anal.* Calcd for $C_{40}H_{70}N_2O_4S_2I_2$: C, 50.00; H, 7.34; N, 2.92. Found: C, 49.82; H, 7.08; N, 2.91.

5DEBT-2,14 Yield; 1.28 g, 25.6%. mp 180—182 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25—1.35 (40H, m), 1.40—1.46 (4H, m), 1.94 (4H, quintet, J=7.7 Hz), 2.58 (6H, s), 2.60 (4H, s), 3.36 (4H, t, J=5.5 Hz), 4.43 (4H, t, J=5.5 Hz), 4.63 (4H, t, J=7.8 Hz), 10.84 (2H, s). *Anal.* Calcd for $C_{44}H_{78}N_2O_4S_2I_2$: C, 51.96; H, 7.73; N, 2.75. Found: C, 51.66; H, 7.51; N, 2.84.

5DEBT-2,16 Yield; 1.18 g, 23.5%. mp 182—185 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25—1.35 (48H, m), 1.40—1.46 (4H, m), 1.94 (4H, quintet, J=7.7 Hz), 2.57 (6H, s), 2.60 (4H, s), 3.35 (4H, t, J=5.5 Hz), 4.43 (4H, t, J=5.6 Hz), 4.62 (4H, t, J=7.8 Hz), 10.85 (2H, s). *Anal.* Calcd for $C_{48}H_{86}N_2O_4S_2I_2$: C, 53.72; H, 8.08; N, 2.63. Found: C, 53.90; H, 7.86; N, 2.66.

5DEBT-6,8 Yield; 1.24 g, 24.7%. mp 119—122 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (6H, t, J=7.0 Hz), 1.27—1.31 (16H, m), 1.34—1.37 (4H, m), 1.41—1.49 (4H, m), 1.61—1.65 (4H, m), 1.96 (4H, quintet, J=7.6 Hz), 2.38 (4H, t, J=7.4 Hz), 2.61 (6H, s), 3.33 (4H, t, J=5.7 Hz), 4.36 (4H, t, J=5.7 Hz),

4.67 (4H, t, J=7.8 Hz), 10.86 (2H, s). *Anal.* Calcd for $C_{36}H_{62}N_2O_4S_2I_2$: C, 47.79; H, 6.91; N, 3.10. Found: C, 47.55; H, 6.65; N, 3.19.

5DEBT-6,10 Yield; 1.23 g, 24.5%. mp 121—125 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0 Hz), 1.26—1.29 (24H, m), 1.35 (4H, m), 1.41—1.48 (4H, m), 1.61—1.65 (4H, m), 1.95 (4H, quintet, J=7.6 Hz), 2.38 (4H, t, J=7.3 Hz), 2.61 (6H, s), 3.33 (4H, t, J=5.7 Hz), 4.36 (4H, t, J=5.7 Hz), 4.67 (4H, t, J=7.8 Hz), 10.85 (2H, s). *Anal.* Calcd for $C_{40}H_{70}N_2O_4S_2I_2$: C, 50.00; H, 7.34; N, 2.92. Found: C, 49.74; H, 7.12; N, 3.05.

5DEBT-6,12 Yield; 1.13 g, 22.6%. mp 130—134 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0 Hz), 1.25—1.32 (32H, m), 1.35—1.37 (4H, m), 1.41—1.47 (4H, m), 1.61—1.65 (4H, m), 1.95 (4H, quintet, J=7.7 Hz), 2.39 (4H, t, J=7.4 Hz), 2.60 (6H, s), 3.32 (4H, t, J=5.7 Hz), 4.36 (4H, t, J=5.7 Hz), 4.66 (4H, t, J=7.8 Hz), 10.86 (2H, s). *Anal.* Calcd for $C_{44}H_{78}N_2O_4S_2I_2$: C, 51.96; H, 7.73; N, 2.75. Found: C, 51.67; H, 7.48; N, 2.82.

5DEBT-6,14 Yield; 1.05 g, 21.0%. mp 139—143 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25—1.32 (40H, m), 1.34—1.36 (4H, m), 1.41—1.47 (4H, m), 1.62—1.70 (4H, m), 1.95 (4H, quintet, J=7.7 Hz), 2.39 (4H, t, J=7.4 Hz), 2.59 (6H, s), 3.30 (4H, t, J=5.6 Hz), 4.36 (4H, t, J=5.7 Hz), 4.66 (4H, t, J=7.8 Hz), 10.86 (2H, s). *Anal.* Calcd for $C_{48}H_{86}N_2O_4S_2I_2$: C, 53.72; H, 8.08; N, 2.63. Found: C, 53.61; H, 7.97; N, 2.67.

5DEBT-6,16 Yield; 1.43 g, 28.5%. mp 143—146 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25—1.32 (48H, m), 1.35—1.37 (4H, m), 1.41—1.45 (4H, m), 1.62—1.65 (4H, m), 1.95 (4H, quintet, J=7.6 Hz), 2.39 (4H, t, J=7.4 Hz), 2.60 (6H, s), 3.31 (4H, t, J=5.6 Hz), 4.36 (4H, t, J=5.9 Hz), 4.66 (4H, t, J=7.7 Hz), 10.87 (2H, s). *Anal.* Calcd for $C_{48}H_{86}N_2O_4S_2I_2$: C, 55.31; H, 8.39; N, 2.48. Found: C, 55.03; H, 8.33; N, 2.51.

5DEBT-8,8 Yield; 1.31 g, 26.1%. mp 101—103 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (6H, t, J=6.8 Hz), 1.27—1.36 (24H, m), 1.41—1.47 (4H, m), 1.60—1.63 (4H, m), 1.96 (4H, quintet, J=7.7 Hz), 2.36 (4H, t, J=7.4 Hz), 2.61 (6H, s), 3.32 (4H, t, J=5.7 Hz), 4.34 (4H, t, J=5.9 Hz), 4.69 (4H, t, J=7.8 Hz), 10.92 (2H, s). *Anal.* Calcd for $C_{38}H_{66}N_2O_4S_2I_2$: C, 48.93; H, 7.13; N, 3.00. Found: C, 48.68; H, 6.92; N, 3.10.

5DEBT-8,10 Yield; 1.04 g, 20.8%. mp 104—107 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.7 Hz), 1.26 (24H, m), 1.32 (8H, m), 1.45 (4H, m), 1.62 (4H, m), 1.95 (4H, m), 2.36 (4H, t, J=7.4 Hz), 2.60 (6H, s), 3.30 (4H, t, J=5.6 Hz), 4.34 (4H, t, J=5.7 Hz), 4.68 (4H, t, J=7.8 Hz), 10.93 (2H, s). *Anal.* Calcd for $C_{42}H_{74}N_2O_4S_2I_2$: C, 51.01; H, 7.54; N, 2.83. Found: C, 51.03; H, 7.31; N, 2.96.

5DEBT-8,12 Yield; 1.28 g, 25.5%. mp 110—114 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25 (32H, m), 1.30—1.32 (8H, m), 1.41—1.45 (4H, m), 1.60—1.62 (4H, m), 1.95 (4H, quintet, J=7.7 Hz), 2.36 (4H, t, J=7.4 Hz), 2.60 (6H, s), 3.30 (4H, t, J=5.7 Hz), 4.34 (4H, t, J=5.9 Hz), 4.68 (4H, t, J=7.7 Hz), 10.90 (2H, s). *Anal.* Calcd for $C_{46}H_{82}N_2O_4S_2I_2$: C, 52.87; H, 7.91; N, 2.68. Found: C, 52.72; H, 7.68; N, 2.53.

5DEBT-8,14 Yield; 1.34 g, 26.7%. mp 112—113 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25 (40H, m), 1.30—1.33 (8H, m), 1.41—1.45 (4H, m), 1.62—1.65 (4H, m), 1.95 (4H, quintet, J=7.6 Hz), 2.37 (4H, t, J=7.4 Hz), 2.58 (6H, s), 3.27 (4H, t, J=5.7 Hz), 4.34 (4H, t, J=5.9 Hz), 4.69 (4H, t, J=7.8 Hz), 10.96 (2H, s). *Anal.* Calcd for $C_{50}H_{90}N_2O_4S_2I_2$: C, 54.54; H, 8.24; N, 2.54. Found: C, 54.37; H, 8.09; N, 2.31.

5DEBT-8,16 Yield; 1.18 g, 23.5%. mp 114—116 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25 (48H, m), 1.29—1.32 (8H, m), 1.41—1.45 (4H, m), 1.58—1.62 (4H, m), 1.95 (4H, quintet, J=7.7 Hz), 2.36 (4H, t, J=7.6 Hz), 2.59 (6H, s), 3.30 (4H, t, J=5.9 Hz), 4.34 (4H, t, J=6.1 Hz), 4.68 (4H, t, J=7.7 Hz), 10.91 (2H, s). *Anal.* Calcd for $C_{50}H_{90}N_2O_4S_2I_2$: C, 56.04; H, 8.54; N, 2.42. Found: C, 55.87; H, 8.30; N, 2.50.

5DEBT-P,8 Yield; 1.18 g, 23.5%. mp 209—212 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.86 (6H, t, J=6.8 Hz), 1.24—1.33 (16H, m), 1.38—1.46 (4H, m), 1.93 (4H, quintet, J=7.6 Hz), 2.68 (6H, s), 3.50 (4H, t, J=5.6 Hz), 4.60 (4H, t, J=5.6 Hz), 4.65 (4H, t, J=7.7 Hz), 8.05 (4H, s), 11.02 (2H, s). *Anal.* Calcd for $C_{36}H_{54}N_2O_4S_2Br_2$: C, 53.86; H, 6.78; N, 3.49. Found: C, 53.58; H, 6.51; N, 3.59.

5DEBT-P,10 Yield; 1.18 g, 23.5%. mp 212—215 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (6H, t, J=7.0 Hz), 1.24—1.33 (24H, m), 1.38—1.44 (4H, m), 1.93 (4H, quintet, J=7.6 Hz), 2.68 (6H, s), 3.49 (4H, t, J=5.5 Hz), 4.60 (4H, t, J=5.6 Hz), 4.65 (4H, t, J=7.7 Hz), 8.05 (4H, s), 11.00 (2H, s). *Anal.* Calcd for $C_{40}H_{62}N_2O_4S_2Br_2$: C, 55.94; H, 7.28; N, 3.26. Found: C, 55.79; H, 7.11; N, 3.38.

5DEBT-P,12 Yield; 1.18 g, 23.5%. mp 220—223 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (6H, t, J=7.0 Hz), 1.24—1.31 (32H, m), 1.38—1.42 (4H, m), 1.93 (4H, quintet, J=7.7 Hz), 2.67 (6H, s), 3.48 (4H, t, J=5.5 Hz), 4.59 (4H, t, J=5.6 Hz), 4.64 (4H, t, J=7.7 Hz), 8.05 (4H, s), 11.00 (2H, s). *Anal.* Calcd for $C_{44}H_{70}N_2O_4S_2Br_2$: C, 57.76; H, 7.71; N, 3.06. Found: C, 57.51; H, 7.46; N, 3.21.

5DEBT-P,14 Yield; 1.18 g, 23.5%. mp 218—219 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.24—1.33 (40H, m), 1.39—1.42 (4H, m), 1.93 (4H, quintet, J=7.7 Hz), 2.66 (6H, s), 3.47 (4H, t, J=5.5 Hz), 4.59 (4H, t, J=5.6 Hz), 4.64 (4H, t, J=7.7 Hz), 8.06 (4H, s), 11.00 (2H, s). *Anal.* Calcd for $C_{48}H_{78}N_2O_4S_2Br_2$: C, 59.37; H, 8.10; N, 2.89. Found: C, 59.11; H, 7.92; N, 3.00.

5DEBT-P,16 Yield; 1.18 g, 23.5%. mp 224—226 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.24—1.31 (48H, m), 1.39—1.42 (4H, m), 1.93 (4H, quintet, J=7.7 Hz), 2.66 (6H, s), 3.46 (4H, t, J=5.5 Hz), 4.59 (4H, t, J=5.6 Hz), 4.64 (4H, t, J=7.7 Hz), 8.07 (4H, s), 11.02 (2H, s). *Anal.* Calcd for $C_{52}H_{86}N_2O_4S_2Br_2$: C, 60.80; H, 8.44; N, 2.72. Found: C, 60.55; H, 8.14; N, 2.73.

Element analyses, melting points and ^{1}H-NMR spectra of the synthesized 5DEBT-4,n were consistent with the analytical and physical data described in a previous report.[18]

5DEBT-4,8 Yield; 0.715 g, 14.3%. mp 122—124 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.27—1.36 (16H, m), 1.41—1.48 (4H, m), 1.65—1.68 (4H, m), 1.95 (4H, quintet, J=7.7 Hz), 2.43 (4H, t, J=6.2 Hz), 2.59 (6H, s), 3.31 (4H, t, J=5.6 Hz), 4.37 (4H, t, J=5.7 Hz), 4.62 (4H, t, J=7.8 Hz), 10.79 (2H, s). *Anal.* Calcd for $C_{34}H_{58}N_2O_4S_2I_2$: C, 46.58; H, 6.67; N, 3.20. Found: C, 46.38; H, 6.41; N, 3.10.

5DEBT-4,10 Yield; 0.910 g, 18.2%. mp 134—137 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.26—1.36 (24H, m), 1.41—1.46 (4H, m), 1.65—1.68 (4H, m), 1.95 (4H, quintet, J=7.7 Hz), 2.43 (4H, t, J=6.2 Hz), 2.59 (6H, s), 3.31 (4H, t, J=5.6 Hz), 4.37 (4H, t, J=5.6 Hz), 4.62 (4H, t, J=7.8 Hz), 10.78 (2H, s). *Anal.* Calcd for $C_{38}H_{66}N_2O_4S_2I_2$: C, 48.93; H, 7.13; N, 3.00. Found: C, 48.65; H, 6.94; N, 2.75.

5DEBT-4,12 Yield; 1.12 g, 22.4%. mp 136—139 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0 Hz), 1.26—1.36 (32H, m), 1.41—1.46 (4H, m), 1.65—1.68 (4H, m), 1.95 (4H, quintet, J=7.7 Hz), 2.43 (4H, t, J=6.5 Hz), 2.60 (6H, s), 3.32 (4H, t, J=5.7 Hz), 4.37 (4H, t, J=5.7 Hz), 4.62 (4H, t, J=7.8 Hz), 10.78 (2H, s). *Anal.* Calcd for $C_{42}H_{74}N_2O_4S_2I_2$: C, 51.01; H, 7.54; N, 2.83. Found: C, 50.76; H, 7.28; N, 2.59.

5DEBT-4,14 Yield; 1.19 g, 23.7%. mp 142—146 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25—1.36 (40H, m), 1.41—1.48 (4H, m), 1.65—1.68 (4H, m), 1.95 (4H, quintet, J=7.7 Hz), 2.44 (4H, t, J=6.2 Hz), 2.60 (6H, s), 3.31 (4H, t, J=5.6 Hz), 4.37 (4H, t, J=5.6 Hz), 4.61 (4H, t, J=7.8 Hz), 10.79 (2H, s). *Anal.* Calcd for $C_{46}H_{82}N_2O_4S_2I_2$: C, 52.87; H, 7.91; N, 2.68. Found: C, 52.64; H, 7.66; N, 2.48.

5DEBT-4,16 Yield; 1.06 g, 21.1%. mp 152—155 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8 Hz), 1.25—1.36 (48H, m), 1.44—1.45 (4H, m), 1.66—1.69 (4H, m), 1.96 (4H, t, J=6.8 Hz), 2.42—2.44 (4H, m), 2.59 (6H, s), 3.32 (4H, t, J=5.6 Hz), 4.37 (4H, t, J=5.7 Hz), 4.58 (4H, t, J=7.8 Hz), 10.63 (2H, s). *Anal.* Calcd for $C_{50}H_{90}N_2O_4S_2I_2$: C, 54.54; H, 8.24; N, 2.54. Found: C, 54.26; H, 8.01; N, 2.27.

Strains The antimicrobial tests were performed using bacteria, fungi and yeast which were stored in our laboratory. The microorganisms included *Pseudomonas aeruginosa* (*Ps. aeruginosa*) American Type Culture Collection (ATCC) 27583, *Klebsiella pneumoniae* (*Kl. pneumoniae*) ATCC 4352, *Proteus mirabilis* (*Pr. mirabilis*) Institute for Fermentation, Osaka, Japan (IFO) 3849, *Escherichia coli* (*E. coli*) IFO 12713, *Serratia marcescens* (*Ser. marcescens*) ATCC 13880, *Micrococcus luteus* (*M. luteus*) IFO 12708, *Bacillus subtilis* (*B. subtilis*) ATCC 6633, *B. cereus* IFO 3001, *Staphylococcus aureus* (*Staph. aureus*) IFO 12732, *Staph. aureus* COL 1 (MRSA), *Aspergillus niger* (*A. niger*) IFO 6342, *A. terreus* IFO 6346, *Chaetomium globosum* (*Cha. globosum*) IFO 6347, *Penicillium funiculosum* (*Pe. funiculosum*) IFO 6345, *Trichophyton mentagrophytes* (*Tr. mentagrophytes*) National Institute of Technology and Evaluation-Biological Resource Center (NBRC) 2412, *Rhizopus stolonifer* (*Rhi. stolonifer*) IFO 4781 and *Saccharomyces cerevisiae* (*Sacc. cerevisiae*) NBRC 10217.

Antimicrobial Activity A tube standard 2-fold dilution method determined the antimicrobial activities, MBC against exponential-phase cells and MIC against stationary-phase cells. For the MBC and MIC measurements, the bacteria, cultivated and prepared by the previously described method,[10] were diluted to 2×10^6 cells/ml using sterilized ion-exchanged water and to 2×10^5 cells/ml with nutrient broth (Difco Laboratories, Detroit, MI, U.S.A.), respectively. Cultivated and collected by the previous method,[10] the fungi (conidia) were adjusted to 2×10^4 cells/ml with Sabouraud broth [polypepton 1% (w/v), glucose 4% (w/v)].[15] The yeast was prepared at 2×10^4 cells/ml with the Sabouraud broth following the cultivation and the collection by the same method as the preparation of the bacteria suspension.

Hydrophobicity The flow rate of the gemini QACs, *Rf* value, deter-

mined by partition chromatography using reversed phase TLC plate (Merck RP-18 F_{254S} plate; thickness 0.25 mm, Merck Japan Ltd., Japan) in an acetonitrile–ethanol–water (10 : 9 : 1, v/v) solvent system, and the value was used to calculate the molecular hydrophobicity, R_M value.[15)]

Statistical Analysis When the relationships between bactericidal activities and hydrophobicities were discussed, the regression analysis was processed using the computer programs, Kaleida Graph Version 3.52.

References

1) Nakajima T., Kourai H., *Bokin Bobai*, **24**, 767—769 (1996) (in Japanese).
2) Kourai H., Kume M., Takechi H., Ohtsuru Y., Ohizumi M., Shibasaki I., *Bokin Bobai*, **14**, 435—442 (1986) (in Japanese).
3) Peddie B. A., Wood J. E., Lever M., Happer D. A. R., Zwart F., Chambers S. T., *Antonie van Leeuwenhoek*, **83**, 175—181 (2003).
4) Menger F. M., Littau C. A., *J. Am. Chem. Soc.*, **115**, 10083—10090 (1993).
5) Rosen M. J., Liu L., *J. Am. Oil Chem. Soc.*, **73**, 885—890 (1996).
6) Rosen M. J., Mathias J. H., Davenport L., *Langmuir*, **15**, 7340—7346 (1999).
7) Devínsky F., Lacko I., Mlynarčík D., Račanský V., Krasnec Ĺ., *Tenside Deterg.*, **22**, 10—15 (1985).
8) Diz M., Manresa A., Pinazo A., Erra P., Infante M. R., *J. Chem. Soc. Perkin Trans. 2*, **1994**, 1871—1876 (1994).
9) Menger F. M., Keiper J. S., *Angew. Chem., Int. Ed. Engl.*, **39**, 1906—1920 (2000).
10) Okazaki K., Maeda T., Nagamune H., Manabe Y., Kourai H., *Chem. Pharm. Bull.*, **45**, 1970—1974 (1997).
11) Maeda T., Okazaki K., Nagamune H., Manabe Y., Kourai H., *Biol. Pharm. Bull.*, **21**, 1057—1061 (1998).
12) Maeda T., Manabe Y., Yamamoto M., Yoshida M., Okazaki K., Nagamune H., Kourai H., *Chem. Pharm. Bull.*, **47**, 1020—1023 (1999).
13) Yoshida M., Maeda T., Okazaki K., Nagamune H., Kunikata K., Tsuchiya H., Namba T., Kourai H., *Biocontrol Sci.*, **5**, 65—71 (2000).
14) Shirai A., Maeda T., Hara I., Yoshinari A., Nagamune H., Kourai H., *Biocontrol Sci.*, **8**, 151—157 (2003).
15) Shirai A., Maeda T., Nagamune H., Matsuki H., Kaneshina S., Kourai H., *Eur. J. Med. Chem.*, **40**, 113—123 (2005).
16) Sumitomo T., Maeda T., Nagamune H., Kourai H., *Biocontrol Sci.*, **9**, 1—9 (2004).
17) Ohkura K., Sukeno A., Yamamoto K., Nagamune H., Maeda T., Kourai H., *Bioorg. Med. Chem.*, **11**, 5035—5043 (2003).
18) Maeda T., Yoshida M., Manabe Y., Okazaki K., Nagamune H., Kourai H., *Biocontrol Sci.*, **4**, 75—81 (1999).
19) Ohkura K., Sukeno A., Nagamune H., Kourai H., *Bioorg. Med. Chem.*, **13**, 2579—2587 (2005).
20) Maeda T., Goto S., Manabe Y., Okazaki K., Nagamune H., Kourai H., *Biocontrol Sci.*, **1**, 41—49 (1996).
21) Arami E., Beinert G., Marie P., Zana R., *Langmuir*, **9**, 1465—1467 (1993).