Aminimides: III Antimicrobial Effect of Various Hexadecyl and Quaternary Derivatives

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ABSTRACT AND SUMMARY

Aminimides, a new class of surfactants, have been screened for in vitro antimicrobial activity. Greatest activity against gram (+) and yeast organisms is achieved when a chain length of C_{16} is reached. This generalization is true whether or not the chain length is joined to the imide or amine group. The type of functional group associated with the surfactant is less important than chain length. Gram (-) organisms do not fit this generalization, since shorter ($<C_{16}$) rather than longer chain derivatives are more active. The importance of surfactant chain length to biodegradability is discussed.

INTRODUCTION

Soaps, whose antibacterial action was appreciated for many years, belong to a category known as surfactants—surface active agents. Because of their limited spectrum of antimicrobial activity, thousands of synthetic compounds with more desirable properties have been prepared. In recent years, this has been the most prolific field in the development of new antiseptic agents, apart from antibiotics. Surfactants are commonly classified into three main groups: anionic, cationic, and nonionic. The history of surface active agents as bacteriocides and detailed aspects of their chemistry, germicidal properties, and application were reviewed by Glassman (1) and Lawrence (2).

Previous reports from our laboratory have focused attention on fatty acids as examples of anionic surfactants (3,4). Data on structure-function relationships were also presented on cationic amines, (5) and nonionic compounds, fatty acid esters (6). A unique and new group of compounds, aminimides, which were prepared as industrial surfactants, were also reported (7,8). Aminimides are neutral surfactants that contain a dipolar moiety as their principal functional group. These dipolar compounds have remarkable antifungal and antibacterial activity. In a continuation of these studies on aminimides a number of derivatives were screened in order to further our knowledge on the relationship between structure and function for these kinds of chemical agents. Many of the compounds to be presented are quaternary compounds.

MATERIAL AND METHODS

The aminimides screened in our laboratory were prepared by the organic group of Ashland Chemical Company (Dublin, OH).

Compounds were tested for in vitro antimicrobial activity by the broth dilution technique (3,5). Test results of these compounds obtained from disc-agar or agar-dilution techniques were not always comparable (unpublished data). In the latter two methods, the physical and chemical interaction of the aminimides derivatives and the agar gave in certain instances divergent results.

Using the broth-dilution method, 200 mg of compound was dissolved in 0.5-1.0 ml of methanol. Two hundred milliliters of Trypticase soy broth (TSB, Baltimore Biological Laboratories, Baltimore, MD) was added aseptically.

If the resulting suspension was granular or turbid, the suspension was carefully heated (ca. 70 C) to increase drug solubilization. Standard solutions (or suspensions) containing 1000 μg/ml were serially diluted with additional broth to achieve desired concentrations. The serial dilutions were then dispensed into screw cap tubes (16 x 125 mm).

The organisms used in this survey were either American Type Culture Collection (ATCC) or clinical isolates maintained in our laboratory. The organisms had been stored in skim milk broth at -80 C (1).

A test inoculum consisted of 0.05 ml of an 18- to 24-hr TSB culture (ca. 10^8-10^9 organism/ml). The inoculum was aseptically delivered into all dilutions of the compound, mixed well, and incubated at 36 C in a 5% CO_2-95% air atmosphere. A tube of inoculated broth without drugs served as the positive control; also, the uninoculated set of drug dilutions was incubated. After 18-hr incubation, the minimal inhibitory concentration (MIC) of each compound against each organism was determined. In this study, the MIC is defined as the lowest concentration of compound at which no evidence of growth was observed when turbidity of the inoculated broth dilutions was compared to the control tubes.

In cases in which the test compound caused turbidity and the MIC could not accurately be determined, a sample (0.1 ml) of the well agitated broth in question was inoculated onto a Trypticase soy agar plate containing 5% defibrinated sheep blood, incubated at 35 C, and examined after 18 hr for bacteriostatic and bactericidal end points. Usually, there was only one tube difference between the bactericidal and bacteriostatic concentrations.

The pH of the broth was monitored throughout the study by an Accutint set (Anachemia, Montreal, Quebec, Canada) and was found to be within the range of 7.3 ± 0.2. Also, at the concentration used, methanol was found to be noninhibitory.

Hexachlorophene was always used as a chemical control in our screening experiments. This procedure served as a statistical control for the procedure. Repetitive MIC values obtained from tenfold dilution rarely deviated from single observations (8).

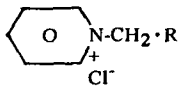
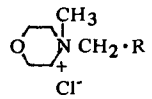
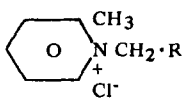
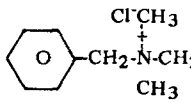
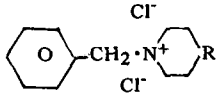
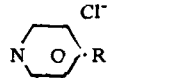
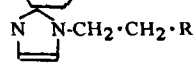
Acute toxicity experiments were carried out on select aminimide derivatives. The compounds were suspended in 0.25% methylcellulose or saline and injected intraperitoneally into 20-22 g male mice (CF-1). Animals in groups of five or ten were observed for 72 hr after drug injection. The mode of dying and the number of survivors were recorded. The LD_{50} was calculated according to the method of Litchfield and Wilcoxon (9).

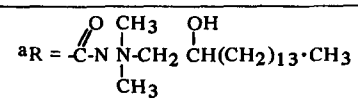
RESULTS

Aminimides representing a diverse collection of compound structures were screened. To date, over 150 different derivatives have been screened. Only the more active derivatives, most with an amine chain length of C_{16}, are presented. In Table I, a number of aminimides were screened which have a hexadecylamine group in common. The acyl portion of the molecule varies greatly and in some cases contains a quaternary nitrogen group. Although not always predictable, quaternary aminimides are active, albeit slightly, against *Escherichia coli*. This is in contrast to most aminimides which have little or no activity against gram (-) organisms. Although no clear advantage can be gleaned from structure/function comparisons, the overriding

TABLE I

Structure-Function Activity of Hexadecylamine Imides

No.	Compound[a]	MIC (μg/ml) Ec	Pa	Sf	Sp	Sa	Csp	Na	Ca	Sc	LD_{50}
M-125		100	NI	10	1	10	1	10	10	10	
M-66		100	NI	10	1	10	10	10	100	10	85[b]
M-62		1000	NI	10	1	10	1	10	10	10	35
M-31		100	NI	10	1	1	1	10	100	10	50
M-32	$(CH_3 \cdot CH_2)_3N^+ \cdot CH_2R$ Cl^-	1000	>1000	10	10	10	10	10	10	10	
M-65		NI	NI	100	1	1000	10	100	10	100	
M-54	$HO \cdot CH_2 \cdot CH_2 \cdot N^+(CH_3)_2 \cdot CH_2 \cdot R$ Cl^-	NI	NI	10	1	10	10	10	100	10	160
M-126	$HO \cdot (CH_2)_5 \cdot R$	NI	NI	10	10	10	10	10	100	10	
M-101	$CH_3\text{-}O\text{-}CH_2 \cdot R$	NI	NI	10	10	1	10	10	100	10	
M-102	$(CH_3)_2N^+(CH_2)_2 \cdot R$ Cl^-	NI	NI	10	10	1	10	10	100	10	
M-63		NI	NI	1000	1	100	100	10	1000	1000	
M-61		NI	NI	10	10	10	10	10	10	10	125

[a] R = 

[b] mg/kg.

common denominator seems to be the hexadecylamine group. The data in Table I indicate several important principles relating structure to compound activity. First, all compounds having an alkyl group of C_{16} chain length are active against gram (+) and yeast organisms regardless of the diversity in structure of the acyl derivative. Also, where the acyl portion contains or is a quaternary nitrogen, the derivative is active against gram (-) as well as gram (+) and yeast organisms. Subtle changes in structure as found in comparing compounds M-62, 66, and 125 produced no noticeable differences in aminimide antimicrobial activity. More diverse changes in acyl moiety as, for example, substitution of hydrocarbons for aromatic groups (M-32, 54, and 102) gave derivatives which had no activity against gram (-) organisms but still indicated high activity against gram (+) and yeast organisms.

In Table II, several striking examples can be seen which substantiate the importance of a C_{16} chain length. In the first instance, M-56, which contains a C_{12} chain length, can be compared to its analog containing C_{16} (M-31) from Table I. The hexadecylamine derivative is more active.

Of particular interest was the comparison of two aminimide imidazole derivatives. The hexadecyl amine derivative was very active against gram (+) and yeast while the shorter 1,1-dimethyl,-2-hydroxypropylamine derivative was totally inactive even at concentrations of 1000 μg/ml.

Again, if one compares compound M-27 (C_{14}) with its C_{16} counterpart, (M-28), higher activity is recorded for the C_{16} analog. A point to be made is that compound activity, when tested against gram (-) organisms, favors shorter rather than longer chain lengths, i.e. M-27 activity against *E. coli*.

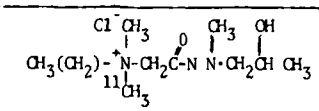
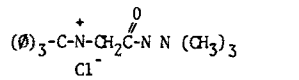
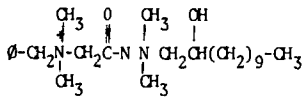
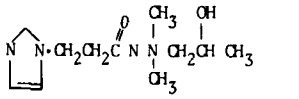
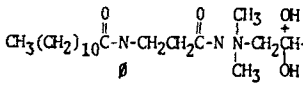
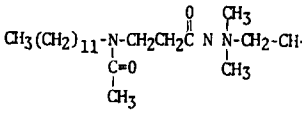
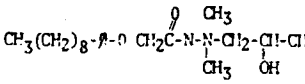
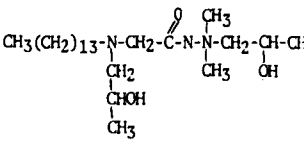
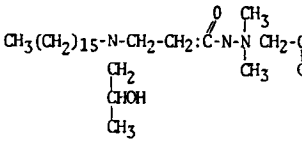
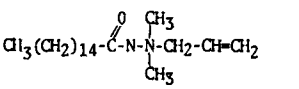
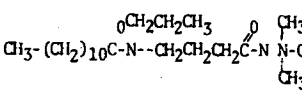
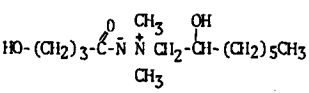
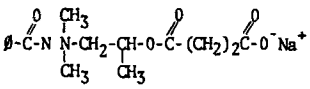
Compounds such as M-7 or M-38 contain short chains both at the acyl and acyl position and are less active. Neither aromatic structures or short aliphatic structures impart any special activity to these aminimides. In Table II, some quaternary aminimides showed activity against either Ec or Pa but not both.

The universal consideration for compound activity seems to be the length (C_{16}) of the chain on either the imide or amine portion of the aminimide.

Most of the aminimides tested are solid derivatives. In an attempt to discover the effect of changes of physical property by ethoxylation (EO) or propoxylation (PO) on aminimide antimicrobial activity, select aminimides representing three acyl chain lengths were screened (Table III). These compounds were reacted with 5-10 moles of either EO or PO. Ethylene oxide will react with any compound

TABLE II

Minimal Inhibitory Concentration of Acyl Aminimides

No.	Compound [a]	MIC (μg/ml)									
		Ec	Pa	Sf	Sp	Sa	Csp	Na	Ca	Sc	LD_{50}
M-49		1000	NI	NI	10	100	10	100	1000	100	
M-33		NI	NI	1000	1000	1000	100	1000	1000	1000	
M-56		NI	1000	100	10	100	10	100	100	100	
M-46		NI	NI	NI	NI	NI	NI	NI	NI	NI	
M-52		NI	NI	10	1	10	10	10	10	10	85
M-64		NI	NI	100	10	100	100	100	100	100	
M-55		NI	NI	1000	100	1000	10	10	NI	100	
M-27		1000	NI	100	10	100	100	100	100	100	42
M-28		NI	NI	10	1	10	10	100	100	100	
M-12		NI	NI	10	1	10	10	10	100	10	140
53		NI	1000	10	100	100	100	1000	100		
M-7		NI	NI	NI	NI	NI	1000	1000	1000	NI	
M-38		NI	NI	NI	1000	NI	1000	NI	NI	1000	

possessing reactive hydrogen atoms, i.e. acid, alcohol, amines, and amides. The fatty long chain forms the lipophilic part of the molecule. Higher alkylene oxides such as propylene or butylene oxide can be reacted similarly. The polypropoxy or polybutoxy groups, however, tend to be hydrophobic rather than hydrophilic.

Significantly, the alkoxylated compounds lost little antibacterial activity, but in contrast lost much or all of their yeast activity. The latter effect was dependent on the molar concentration of EO or PO used, but independent of the chain length of the alkoxylation reactant.

On a molar basis, the alkoxylated derivatives were more active against gram (+) bacteria than were the nonalkoxylated aminimides.

DISCUSSION

Our laboratory has reported on the structure-function

TABLE III

Oxylation of Acyl Aminimides

No.	Compound	MIC (μg/ml) Ec	Pa	Sf	Sp	Sa	Csp	Na	Ca	Sc	LD_{50}
M-1	$CH_3(CH_2)_{10}C(=O)N(CH_3)CH_2CH(CH_3)OH$	NI	NI	100	10	100	10	100	100	100	178[a]
M-108	+ 5 EO	NI	NI	1000	100	100	100	100	1000	1000	
M-109	+10 EO	NI	NI	1000	100	100	100	100	NI	1000	
M-114	+ 5 PO	NI	NI	1000	100	100	100	100	NI	1000	
M-115	+10 PO	NI	NI	NI	100	1000	100	100	NI	NI	
M-2	$CH_3(CH_2)_{12}C(=O)N(CH_3)N(CH_3)CH_2CH(CH_3)OH$	NI	NI	100	10	10	10	10	10	10	
	+ 5 EO	NI	NI	100	100	100	100	100	NI	100	
	+10 EO	NI	NI	1000	100	100	100	100	NI	NI	
M-3	$CH_3(CH_2)_{14}C(=O)N(CH_3)N(CH_3)CH_2CH(CH_3)OH$	NI	NI	100	10	10	10	10	10	10	
	+ 5 EO	NI	NI	10	10	100	10	10	NI	10	
	+10 EO	NI	NI	100	10	100	10	10	NI	NI	
	+10 PO	NI	NI	NI	10	NI	10	10	NI	NI	

[a]mg/kg

activity of such surface active agents as soaps (3,4), nonionic derivatives (6), and simple aliphatic amides and amines (5). Recently, a new industrial family of surfactants, aminimides, was made available for study. The chemistry and potential uses of these chemicals have been reviewed (10) and their antimicrobial properties were recently reported (7,8). In these two reports the importance of chain length of either the acyl or amine portion of the aminimides was emphasized. Our conclusions on the importance of chain length to antimicrobial activity with these compounds substantiates the general nature of such structure-function relationships as previously noted for cationic agents (11,12).

Our past and present results continue to support Ferguson's Rule (13): where chain length is important to compound activity, the most active derivatives contain chain lengths of C_{12}-C_{16}. In the case of cationic surfactants or the dipolar aminimides, optimal biological activity is reached at C_{16}. This generalization was reinforced by our current screening efforts presented in Table I. In the table where a number of hexadecylamines aminimides with unrelated acyl structures are compared, compound activity depended more on the chain length of the group on the amine than on the functionality of the acyl group. Data support the fact that where the size of the chain is satisfied, i.e. C_{16} in length, it makes very little difference whether the chain is attached to the imide or the amine group.

The relationship between chain length and surface activity of compounds has been previously reviewed (14). The importance of chain length in determining the surface activity of soaps (15), alcohols (16-19), and aliphatic acids (20) has been demonstrated. While all surface active agents that are efficient bacteriocides have been found to reduce surface tension, nonionic compounds that have surface activity have little or no effect on microorganisms. It should be pointed out that the extent of biodegradation for surfactants, like aminimides, is a function of chain length. It goes without saying that the more biologically active derivatives (C_{12}-C_{16}) will inactivate those microorganisms that are responsible for compound degradation. Therefore, in looking for biodegradability in surfactant compounds, a balance between surface activity and antimicrobial activity needs to be reached. These properties, in turn, are more a function of chain length than of the functional group of a surfactant.

While aminimides have good antimicrobial activity against gram (+) and yeast organisms, their activity against gram (-) organisms is low. In regard to gram (-) activity, two structural properties seem to be important: first, shorter ($<C_{16}$) chain lengths seem to be more effective than longer chain lengths ($>C_{16}$); second, quaternary nitrogen imparts some activity to the compound against gram (-) strains. In any case, one should consider the activity of aminimides against gram (-) strains only of very minor importance.

The majority of compounds in this series, except for three, can all be considered relatively toxic ($LD_{50}<100$ mg/kg). The three compounds–M-12, M-54, and M-61–have LD_{50} values between 125 and 160 mg/kg. These are not the least toxic members of the aminimides family, since some of the methacrylimide and acetimide derivatives have LD_{50}'s greater than 300 mg/kg (8). From the large series of aminimides studied to date, there is no relationship between toxicity and antimicrobial activity. In fact, the most active antimicrobial derivative is also the least toxic (8). All of our studies to date indicate that aminimides are unusual chemical structures which have wide spectrum biocidal activity. Their most promising property, antifungal activity, is currently being explored.

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REFERENCES

1. Glassman, H.N., Bact. Rev. 12:105 (1948).

2. Lawrence, C.A., in "Disinfection, Sterilization and Preservation," Edited by C.A. Lawrence, and S.S. Block, Lea and Febiger, Philadelphia, PA, 1968, pp. 430.
3. Kabara, J.J., D.M. Swieczkowski, A.J. Conley, and J.P. Truant, Antimicrob. Ag. Chemother. 2:23 (1972).
4. Kabara, J.J., A.J. Conley, D.M. Swieczkowski, I.A. Ismail, M. Lie Ken Jie, and F.D. Gunstone, J. Med. Chem. 16:1060 (1973).
5. Kabara, J.J., A.J. Conley, and J.P. Truant, Antimicrob. Ag. Chemother. 2:492 (1972).
6. Conley, A.J., and J.J. Kabara, Ibid. 4:501 (1973).
7. Kabara, J.J., W.J. McKillip, and E.A. Sedor, JAOCS 52:316 (1975).
8. Kabara, J.J., and G.V. Haitsma, Ibid. 52:444 (1975).
9. Litchfield, J.T., and F. Wilcoxon, J. Pharmacol. Exp. Therap. 96:99 (1949).
10. McKillip, W.J., E.A. Sedor, B.M. Bulbertson, and S. Wawzonek, Chem. Rev. 73:255 (1973).
11. Jacobs, W.A., J. Exp. Med. 23:563 (1916).
12. Domagk, G., Drut. Med. Wochenschr. 61:829 (1935).
13. Ferguson, P., Proc. Roy. Soc. London B127:387 (1939).
14. Ralston, A.W., and C.W. Hoerr, J. Am. Chem. Soc. 64:772 (1942).
15. Shirolkar, G.V., and K. Venkataraman, J. Soc. Dyers Colour. 55:503 (1940).
16. Cowles, P.B., Yale J. Biol. Med. 11:127 (1938).
17. Dreger, E.E., G.I. Keim, G.D. Miles, L Shedlovsky, and J. Ross, Ind. Eng. Chem. 36:610 (1944).
18. Powney, J., and C.C. Addison, Trans. Faraday Soc. 33:1243 (1937).
19. Shedlovsky, L., Ann. NY Acad. Sci. 46:427 (1946).
20. Stanley, W.M., and R. Adams, J. Am. Chem. Soc. 54:1548 (1932).

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