Aminimides: I. Antimicrobial Effect of Some Long Chain Fatty Acid Derivatives

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ABSTRACT

Aminimides are a new class of surface active agents which have antimicrobial activity. These bipolar compounds have activity against both gran-positive and yeast organisms. Their activity against gram-negative organisms is low or absent. The acyl derivatives tested showed maximum activity at chain lengths of C_{14} and C_{16}. Fatty acid derivatives, shorter ($<C_{14}$) or longer ($>C_{16}$), were less active. Unsaturation was an important factor contributing to aminimide activity. Their low toxicity and wide spectrum of antimicrobial activity make aminimides ideal candidates for further investigation.

INTRODUCTION

Aminimides or ammonio-amidates are compounds representing a relatively new functional group [R $\overset{O}{\overset{\|}{C}}$-N-N-]. The functionality consists of a quaternary nitrogen attached to an imide nitrogen. The compounds exist as bipolar ions. The first aminimides were prepared in 1930 by Curtiss (1), but systematic investigation remained inactive until 1954. The chemistry of aminimides has recently been surveyed by Timpe (2) and McKillip, et al., (3).

Acyl aminimides possess interesting surface tension or wetting properties and emulsifying characteristics. They behave like nonionic surfactants with respect to cloud point (4,5) and exhibit a Kraft Point phenomena similar to that associated with ionic surfactants (6).

Because of their surfactant functions (7), these structures are generally good candidates as antimicrobial agents (8,9). Our own interest in the effect of amines and amides on bacteria and fungi (10) added further stimulus to the screening of aminimides as potential antimicrobial agents.

MATERIALS AND METHODS

The acyl aminimide derivatives were prepared by the organic group of Ashland Chemical Company (Dublin, Ohio) using methods previously reported (11,12). Most of the samples were recrystallized five or six times and were chromatographically pure.

A 0.2 g amount of compound was dissolved in 0.5 ml absolute methanol and quantitatively transferred to 200 ml Trypticase soy broth (TBS) (BBL Div. Becton, Dickinson & Co., Cockeyville, MD.). 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 clinical isolates (Providence Hospital, Southfield, Michigan, 1970-1972); maintained in our laboratory. The organisms had been stored in skim milk broth at -80 C.

A test inoculum consisted of 0.05 ml of an 18- to 24-hr TSB culture (ca. 10^9 organism/ml). The inoculum was aseptically delivered into all dilutions of the compound, well mixed and incubated at 36 C in a 5% CO_2: 95% O_2 atmosphere. A tube of inoculated broth without drugs served as the positive control; also, an uninoculated set of 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.

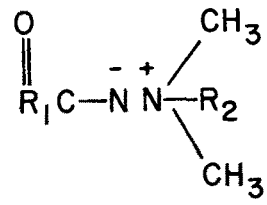
In cases in which the test compound caused turbidity and the MIC could not accurately be determined, a sample (0.015 ml) of the well agitated broth in question was inoculated onto a Trypticase soy agar plate containing 5 percent defibrinated sheep blood, incubated at 35 C and examined after 18 hr for bacteriostatic and bactericidal end points. Usually there was only one tube difference between the bactericidal and bacteriostatic concentrations. It was found that turbidity from the compounds did not confuse the readings. Most of the compounds were inhibitory at low concentrations where solubility was almost complete.

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, in controlled test experiments.

RESULTS AND DISCUSSION

In our initial studies (10,13), 8 gram-negative and 12 gran-positive organisms were used. To achieve greater efficiency, a less ambitious screening program was developed which included two gram-negative, five gram-positive, and two representative yeast cultures. The present results are on this miniscreen. Because of the preliminary nature of this report, ten-fold rather than two-fold dilutions were used to determine MIC values.

The acyl aminimides (R_1) reported here are derived from C_{12} through $C_{18:1}$ fatty acids and have the general structure shown below. Series I is represented when R_2 is methyl, Series II when R_2 is 2-hydroxyethyl and Series III when R_2 is 2-hydroxypropyl in the general formula:



Where R_1+C_{11} to C_{17} and $C_{17:1}$, then R_2=CH_3 (Series I), or R_2 = CH_2CH_2OH (Series II), or R_2 = $CH_2CHOHCH_3$ (Series III).

The results from tests on these three series are given in Table I. Included in the table are data on the effects of hexachlorophene, which was used as a chemical control against these same organisms.

Acyl aminimides, for the most part, exhibit activity chiefly against gran-positive and yeast organisms. In our study, levels of 1000 μg/ml showed little or no inhibition

TABLE I

The Minimal Inhibitory Concentration (μg/ml) For Long Chain Acyl Aminimides

Aminimide[a]	Organism[b]								
	Gram (−)		Gram (+)					Yeast	
Acyl derivatives	1	2	3	4	5	6	7	8	9
1,1,1 Trimethyl									
C_{12}	NI[c]	NI	1000	1000	1000	1000	1000	1000	1000
C_{14}	NI	NI	100	10	10	10	10	100	10
C_{16}	NI	NI	10	10	10	10	10	10	10
C_{18}	NI	NI	100	10	100	10	100	NI	100
1,1-Dimethyl-1-(2-hydroxyethyl)-									
C_{12}	NI	NI	100	100	100	100	100	100	100
C_{14}	NI	NI	10	10	10	10	10	10	10
C_{16}	NI	NI	100	10	10	10	10	10	10
C_{18}	NI	NI	1000	10	100	100	10	NI	1000
1,1-Dimethyl-1-(2-hydroxypropyl)-									
C_{12}	NI	NI	100	10	100	10	100	100	100
C_{14}	NI	NI	100	10	10	10	10	10	10
C_{16}	NI	NI	100	10	10	10	10	10	10
C_{18}	NI	NI	100	10	100	10	100	1000	100
$C_{18:1}$	NI	1000	1000	1	10	10	100	1000	10
Hexachlorophene	NI	1000	10	1	1	1	10	10	100

[a] C_n = Carbon number of acyl chain.

[b] 1) *Escherichia coli*, 2) *Pseudomonas aeruginosa*, 3) *Streptococcus faecalis* (Group D), 4) *Streptococcus pyrogenes*, 5) *Staphylococcus aureus* 6) *Corynebacterium* sp., 7) *Nocardia asteroides*, 8) *Candida albicans*, and 9) *Saccharomyces cerevisiae* (ref. 8).

[c] NI = Non-inhibitory.

against gram-negative organisms. In this respect, these compounds are similar to hexachlorophene, which also has low activity against most gram-negative organisms (14).

Maximum activity of acyl derivatives was achieved with chain length corresponding to myristic (C_{14}) or palmitic (C_{16}) acid; fatty acids shorter ($<C_{14}$) or longer ($>C_{16}$) were less active. This generalization held true for both gram-positive and yeast microorganisms, and supports previous findings for long chain acid amides (10).

The influence of unsaturation is an aminimide derivative was examined because it was previously (15) confirmed that unsaturation was an important factor in contributing to the antimicrobial property of long chain fatty acids. The preparation of 1,1-dimethyl-1-(2-hydroxypropyl)-amine stearimide and oleimide allowed the comparison of a saturated and a Δ^9 unsaturated aminimide. Accumulated MIC's for the five gram-positive organisms indicated only a slight difference between saturated and the Δ^9 unsaturated derivatives. The unsaturated derivative was more active than the saturated aminimide, except for an increased MIC against *Streptococcus faecalis*. This structure-function activity follows the generalization made for fatty acids themselves (10).

These initial investigations with aminimides indicate that this group of compounds possess antimicrobial spectra and activity similar to hexachlorophene. These derivatives may be less toxic than hexachlorophene. Acute toxicity experiments with mice indicated LD_{50} values (180-400 mg/kg) after intraperitoneal injection. Because only a few classes of the myriad of possible derivatives have been screened, an unlimited number of variations are available for further screening. The wide spectrum activity for these kinds of drugs is evident because other derivatives currently being screened suggest that gram-negative organisms may also be affected. The low toxicity and wide spectrum antimicrobial activity of aminimide, especially against bacteria and yeast, make them ideal for further investigation.

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