Aminimides IV: Antimicrobial Activity of 1,1,1-Tris(2-hydroxyethyl)amine-2-acylimides

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ABSTRACT AND SUMMARY

The newest member of the aminimide family of surfactants was screened for antimicrobial activity. The present report supports past conclusions that chain lengths of C_{14} or C_{16} gave maximum activity to the aminimide derivative. When a number of hexadecane compounds with different polar groups were compared, the aminimide exhibited wide spectrum antimicrobial activity. Although the functional group was important, the length and character of the acyl chain also help to determine antimicrobial activity. A new amide antibiotic, Cerulenin, with a 4-keto-2-enyl chain, was more active than the unsaturated isomer. Thus, both the functional group as well as the chain length contribute to biocidal activity.

INTRODUCTION

Previous reports from our laboratory have focused on the effect of short and long chain acyl animides on bacteria, fungi, and yeast type organisms (1-3). These industrial surfactants have remarkable wide-spectrum antimicrobial activity, especially antifungal activity. In almost every case, the compounds screened were derivatives from 1,1-dimethyl hydrazine which were produced by rather complicated processes (4). Recently, one of the authors of this paper (5) has been successful in preparing aminimides using the more accessible and inexpensive hydrazine hydrate instead of the unsymmetrically substituted hydrazine (5). Because of past activity indicated for aminimides, it was of interest to screen 1,1,1-Tris(2-hydroxyethyl)-amine-2-acylimides of varying chain length against microorganisms. Also, functional group composition of various acyl derivatives was compared to antimicrobial activity.

MATERIAL AND METHODS

The 1,1,1-Tris(2-hydroxyethyl)amine-2-acylimides screened in our laboratory were prepared by methods previously reported (5). Crude products were recrystallized from hexane or benzene. Other compounds used were from previous reports (1,2,4) or from various commercial sources. S. Omura (Japan) supplied 4-oxo-2-ene-dodecanoyl dimethylamide for comparison purposes (6).

Compounds were tested for in vitro antimicrobial activity by the broth dilution technique (7,8). Test results of these lipophilic compounds obtained from disc-agar or agar dilution techniques are not always comparable and should not be used.

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 (7).

A test inoculum consisted of 0.05 ml of an 18- to 24-hr Trypticase Soy Broth (TSB) culture (ca. 10^8-10^9 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% air atmosphere. A tube of inoculated borth without drugs served as the positive control: also, an 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 be accurately determined, a sample (011 ml) of the well agitated broth in question was inoculated onto a Trypticase soy agar plate containing 5% deribrinated 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

TABLE I

MIC[a] Values (μg/ml) for 1,1,1-Tris(2-hydroxyethyl)amine-2-acylimide

Organism	Chain Length			
	C_{12}	C_{14}	C_{16}	C_{18}
Escherichia coli - clinical isolate	125	NI	NI	NI
Pseudomonas aeruginosa -ATCC 10145	NI	NI	NI	NI
Corynebacterium pseudodiptheriae - ATCC 10700	15.6	<3.9	<3.90	15.6
Norcardia asteroides - ATCC 3308	62.5	7.81	15.6	NI
Staphylococcus aureus - ATCC 27543	31.2	7.81	7.81	NI
Streptococcus agalactiae - NCDO 1520	31.2	7.81	3.90	NI
Streptococcus mutans - Great Lakes 655	31.2	7.81	3.90	62.5
Streptococcus mutans - ATCC 6715	15.6	7.81	3.90	62.5
Streptococcus mutans - ATCC 10441	31.2	7.81	3.90	31.2
Candida albicans (Sero Type A) - CI	62.5	15.6	125	NI
Saccharomyces cerevisiae	125	31.2	125	NI

[a]MIC = minimal inhibitory concentration.

obtained from such experiments rarely deviated from single observations (2).

RESULTS

Because of previous experience, it was necessary to screen only four acyl derivatives in order to determine the chain length necessary for optimum activity (Table I). In general and for most organisms, chain lengths of C_{14} to C_{16} were most effective. Some notable exceptions were obvious when specific organisms are considered. For instance, *Escherchia coli* (gram negative) is affected by shorter (C_{12}) rather than longer chain lengths. In the case of yeast organisms, both *Candida albicans* and *Saccharomyces cerevisiae* were more affected by C_{14} chain length than by C_{16}. Inactivation by a C_{14} derivative was most specific for these two classes of organisms.

Nine hexadecane derivatives representing different functional groups were also tested and compared (Table II). The simplest derivative, palmitic acid, was weakly active; the hydroxamic derivative of the acid was completely inactive; the N,N-dimethylamide had reasonable activity (MIC between 12.5 and 50.0 μg/ml); the N,N-dimethylhydrazide was inactive (MIC >1000 μg/ml). In considering the various aminimides, there was no significant difference between the various hexadecane derivatives with different polar functional groups.

In Table III, the activity of a new antibiotic cerelenin (4-oxo-2-ene-dodecandimetylamide) was compared to the N,N-dimethyl dodecanamide and the trimethylaminimide dodecane compound. When these saturated dodecane derivatives are compared with the antibiotic, it can be seen that chain structure of the antibiotic imparted additional activity to the amide compound as compared to the saturated chain.

DISCUSSION

A new type of aminimide [1,1,1-Tris(2-hydroxyethyl)-amine-2-acylimide] was found to be as active as previously screened compounds in this family (1-3). As in previous cases, the C_{14} to C_{16} derivatives were most active. The data also confirms our other study indicating activity of short chain derivatives against gram negative strains (2). Gram positive and yeast type organisms, on the other hand, are more affected by longer ($>C_{12}$) rather than short ($<C_{12}$) chain aminimides (1,2). These conclusions continue to be supported by our present experiments.

In addition to chain length, the present data indicates that the character of the functional group is also important. The conversion of an amide of imide to a carboxyl, hydroxamic acid, amide, or hydrazine group decreases biocidal activity of the hexadecane compound. On the other hand, the activity of the amide group can be enhanced by the presence of a -4-oxo-2-ene group instead of a straight chain.

In comparing the various hexadecanoyl aminimides there seems to be little advantage gained in changing the structure of the amine group. The simplest compound, the trimethyl derivative, is as active as some of the more complex derivatives. What needs to be considered further in terms of the most useful form of aminimides is the toxicity between the various compounds, the in vivo half-life of the compound, and the economics of the chemical processes fo making the drug. Additional studies of this nature need to be carried out before this unique set of compounds, the aminimides, finds its way into the pharmacological armamentarium. To date, they represent one of the most promising antifungal agents known because of their high in vitro activity and low mammalian toxicity. Additional data is being accumulated in order to substantiate their early promise.

TABLE II

MIC[a] Values (μg/ml) of Various Hexadecane Derivatives

Organism	Hexadecane derivative: RCOOH[b]	$R-C(=O)-NHOH$	$R-C(=O)-NH_2$	$R-C(=O)-NH-N(CH_3)_2$	$R-C(=O)-N^{+}(CH_3)_2-N^{-}-CH_3$	$R-C(=O)-N^{+}(CH_3)_2-N^{-}-CH_2-CH_2OH$	$R-C(=O)-N^{+}(CH_3)_2-N^{-}-CH_2-CH(OH)-CH_3$	$R-C(=O)-N^{+}(CH_3)_2-N^{-}-CH_2-CH=CH_2$
Escherichia coli	NI[c]	NI	NI	NI	NI	NI	NI	NI
Pseudomonas aeruginosa	NI	NI	NI	NI	NI	NI	NI	NI
Streptococcus faecalis (Grp. D)	>100	NI	NI	NI	6.25	6.25	12.5	12.5
Streptococcus pyogenes	>100	NI	NI	NI	3.12	3.12	3.12	3.12
Staphylococcus aureus	NI	NI	NI	NI	3.12	3.12	3.12	3.12
Corynebacterium sp.	>100	NI	NI	NI	3.12	6.25	3.12	6.25
Norcardia asteroides	NI	NI	NI	NI	3.12	6.25	6.25	6.25
Candida albicans	NI	NI	NI	NI	12.5	12.5	25.0	50.0
Saccharomyces cerevisiae	NI	NI	NI	NI	6.25	3.12	6.25	6.25

[a]MIC = minimal inhibitory concentration.
[b]R = C_{15}
[c]NI = >1000 μg/ml

TABLE III

MIC[a] Values (μg/ml) for Dodecyl Derivatives

Organism	dodecyl-4-oxo 2-ene- dodecane-dimethylamide	N,N dimethyl- dodecanamide	1,1,1-Trimethylamine- 2-dodecanimide	Hexachlorophene
Escerichia coli	NI[b]	NI[c]	NI	100
Pseudomonas aeruginosa	NI	NI	NI	100
Streptococcus faecalis (Grp. D)	25.0	50.0	1000	(1)
Streptococcus pyrogenes	6.25	12.5	1000	(1)
Staphylococcus aureus	12.5	50.0	1000	(1)
Corynebacterium sp.	1.56	12.5	1000	(1)
Nocardia asteroides	12.5	25.0	1000	(1)
Candida albicans	12.5	50.0	NI	100
Saccharomyces cerevisiae	6.25	25.0	1000	10

[a]MIC = minimal inhibitory concentration.

[b]100 μg/ml was the highest conc. tested – -4-oxo-2-ene-dodecane-dimethylamide.

[c]200 μg/ml was the highest conc. tested.

REFERENCES

1. Kabara, J.J., W.J. McKillip, and E.A. Sedor, JAOCS 52:316 (1975).
2. Kabara, J.J., and G.V. Haitsma. Ibid. 52:444 (1975).
3. Kabara, J.J., Ibid. 54:202 (1977).
4. McKillip, W.J., E.A. Sedor, B.M. Bulbertson, and S. Wawzonek, Chem. Rev. 73:255 (1973).
5. Ikeda, I., T.F. Katsura, and M. Okahara, JAOCS 53:537 (1976).
6. Omura, S., M. Katagiri, J. Awaya, T. Furuka, I. Umezawa, N. Oi, M. Mizoguchi, B. Aoki, and M. Shindo, Antimicrob. Agents Chemother. 6:207 (1974).
7. Kabara, J.J., D.M. Swieczkowski, A.J. Conley, and J.P. Truant, Ibid. 2:23 (1972).
8. Kabara, J.J., A.J. Conley, and J.P. Truant. Ibid. 2:492 (1972).

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