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✗Fatty Acid Amides and Anilides, Syntheses and Antimicrobial Properties[1]

R.G. BISTLINE, JR., E.W. MAURER, F.D. SMITH, and W.M. LINFIELD, Eastern Regional Research Center[2], Philadelphia, PA 19118

ABSTRACT

A series of fatty acid amides and anilides was prepared and a number of compounds in the series were found to be highly active against gram positive bacteria but ineffective against gram negative organisms. The N,N-dimethyl- and N,N-diethylamides of C_{12}-C_{14} fatty acids had minimal inhibitory concentration (MIC) values of 100 ppm or less. Substituted anilides of C_6-C_{11} fatty acids were active when the following groups were attached to the aromatic ring: 3,4-dichloro, 3-nitro, 4-nitro, 5-chloro-2-hydroxy, 4-chloro-3-nitro, and 2-hydroxy-5-nitro. Some of these compounds had a MIC value of 0.1 ppm. Significantly, the presence of soap did not reduce the activity of these bacteriostats, whereas polysorbate 80 at high concentrations deactivated the compounds.

INTRODUCTION

In spite of substantial research activity in the sanitizing field, the number of suitable antimicrobial agents used remains relatively small. Quaternary ammonium compounds, for example, are adversely affected by proteinaceous materials and hard water ions and are incompatible with anionic surfactants such as soap. Halogens, particularly chlorine, sodium hypochlorite, iodine and iodine complexes, are effective germicidal agents, but they lose their effectiveness because of volatility and instability due to their nonselective oxidation of organic matter. Halogens also attack textiles, some plastics and many metals.

Halogenated aromatic compounds, such as hexachlorophene, 3,4,4'-trichlorocarbanilide, 3,4,5-tribromosalicylanilide and 2,4,4'-trichloro-2-hydroxydiphenyl ether are fairly compatible with anionic surfactants and thus have been widely used in santizing cleaners, surgical scrub soaps, and deodorant toilet soaps. However, these antibacterial agents have drawbacks, and some have been banned because of toxicity, photosensitization and/or chemical instability. The fatty acid amides (1,2,3), on the other hand, are neutral derivatives of fatty acids and are compatible with

soaps and anionic surfactants. Mitchell and Reid (4) made a series of fatty acid amides by passing ammonia gas through heated fatty acids, the method of commercial amide production. Similarly, D'Alelio and Reid (5) synthesized the series of N-methylamides. Earlier Robertson (6) characterized the N-phenylamides or anilides. Novak and coworkers (7,8) found that some N,N disubstituted amides, such as N,N-bis-(2-hydroxyethyl) lauramide, possessed antimicrobial activity. Kabara and coworkers (9) reported that N,N-dimethyllauramide is highly effective against *Staphylococcus aureus.* However, information in the literature concerning the antimicrobial activity of the fatty acid anilides is sparse (10), although mention is made of their effectiveness as herbicides (11) and as inhibitors of photosynthesis (12,13).

Beaver et al. (14) made a comprehensive study of the bacteriostatic properties of substituted ureas. These were remarkably specific in that activity was greatly enhanced or completely lost with slight changes in chemical structure. A similar systematic study of the fatty acid amides and anilides has not been reported in the literature.

The objective of our investigation was to develop chemical agents which ideally would be effective against both gram positive and gram negative bacteria but would not be rendered inactive by soaps and detergents. Fatty amides appeared to be promising agents, and their structures can be readily modified by: (a) varying the size of the fatty alkyl group; (b) placing one or two substituents on the amido nitrogen atom, and (c) changing the structure of the substituents on the nitrogen atom. The large number of substituted anilines commercially available thus made it feasible to synthesize a variety of fatty acid anilides.

A slight modification of the bacteriological screening technique of Beaver et al. (14) was used to determine antibacterial properties against five different microorganisms. Linfield et al. (15) reported that certain nonionic surfactants act as potentiating agents for antibacterial compounds. Accordingly, we also examined those fatty amides and anilides which displayed high antimicrobial activity for this potentiating effect.

EXPERIMENTAL

Materials

All chemicals were used as received from chemical supply houses without further purification. Aldrich Chemical Co., Milwaukee, WI, supplied fatty acid chlorides — butyryl through palmitoyl chloride, octyl through dodecyl fatty amines, and all substituted anilines. Eastman Kodak Co., Rochester, NY, provided acetyl, myristoyl, stearoyl and oleoyl chlorides and hexylamine. Fisher Scientific Co., Pittsburgh, PA, provided aniline. ICI United States Inc., Wilmington, DE, furnished polysorbate 80. Original Bradford Soap Works, West Warwick, RI, supplied tallow soap. Difco, Detroit, MI, supplied nutrient broth and agar. Givaudan Corp., Clifton, NY, furnished hexachlorophene (G-11).

Methods of Synthesis

The fatty acid amides and anilides were prepared via the Schotten-Baumann Reaction.

Amide synthesis. For ammonia and water soluble amines. The following is illustrative of the procedure: myristoyl chloride, 20 g (0.08 mole), was added to a 60% aqueous solution of dimethylamine, 30 ml (0.4 mole). The product, dissolved in hexane, was recovered in a separatory funnel, washed until neutral, filtered and crystallized. The product was recrystallized from absolute ethanol. Yield of N,N-dimethylmyristamide = 17.7 g, 86.8% (M.P. 28.5 C).

Amide and anilide synthesis. For water insoluble amines and anilines. Decanoyl chloride, 17.9 g (0.09 mole), was added to octaylamine, 12.2 g (0.09 mole), dissolved in dichloroethane (The National Cancer Institute has determined that dichloroethane [ethylene dichloride] is carcinogenic to rats and mice. [CEN, Sept. 25, 1978, p. 6]. Extreme care should be used in handling this solvent.), 200 ml, containing pyridine, 25 ml. Excess pyridine was removed with 4N hydrochloric acid, and the product was washed until neutral. The organic phase was passed through a Florisil (Florisil = adsorbent magnesium silicate, Floridin Co., Berkley Springs, WV), column to remove impurities. The product was crystallized from dichloroethane, then recrystallized from absolute ethanol. N-Octyldecanamide, 21.4 g (M.P. 63.0 C), was obtained in 84% yield. Except for the nitro derivatives, which are yellow, all solid compounds are white needle-like crystals.

Purity of amides and anilides was determined by elemental analyses which in all cases agreed to within 0.5% of theory. Melting points, obtained on the Fisher-Johns melting point apparatus, and infrared spectra were also used as criteria for purity. Refractive indices (n_D^{20}) of the liquid amides and anilides were measured on the Abbe-3L Bausch and Lomb refractometer.

Test Organisms

The following microorganisms were used: *Staphylococcus aureus* ATCC No. 6538, *Escherichia coli* ATCC No. 11229, *Pseudomonas aeruginosa* ATCC No. 8709, *Salmonella typhimurium* (U.S. HEW, CDC), and *S. enteritidis* (U.S. HEW, CDC). Stock cultures were maintained on nutrient agar slants. Hexachlorophene (G-11) was used as the control germicidal standard.

Screening Procedures

In the screening tests for bacteriostatic activity, 1% stock solutions were prepared by dissolving 100 mg of test compound in either 10 ml of 95% ethanol or water. The stock solutions were serially diluted by successively pipetting 2 ml of solution in 18 ml of sterile nutrient agar at 47

C to obtain 1,000, 100, 10, 1, and 0.1 ppm concentrations of compound. The agar was poured into sterile plastic petri dishes (100 x 15 mm), allowed to harden, dried at 37 C for ½ hr with covers off, then inoculated with one drop of a 24 hr culture of test microorganism in nutrient broth. The inoculated dishes were then incubated for 48 hr and examined for presence or absence of growth.

Those compounds found active against *S. aureus* in the range of 1-10 ppm were tested for their activity in the presence of 1000 ppm soap. In this test the concentration range was extended to 0.1 ppm. Two concentrations of soap solutions (1% and 0.9% in 50% EtOH) were dispensed in the following manner into 16 ml of sterile agar: 2 ml of 1% solution were added to the first of five test tubes, and 2 ml of the 0.9% solution were added to each of the four remaining tubes in the series. Then 2 ml of test compound was serially diluted as previously described. To test the possibility that nonionic surfactants at low concentrations might act as potentiating agents for these antibacterial agents (15), polysorbate 80 was tested in combination with compounds effective against *S. aureus* in the 1-10 ppm range. The procedure was similar to that used for soap combinations.

RESULTS AND DISCUSSION

The fatty acid amides and anilides are conveniently prepared compounds which lend themselves readily to a study of correlation between chemical structure and antibacterial properties. Antibacterial activity is greatly affected by slight changes in molecular structure and by position of functional group attachment. The saturated amides, acetamide through stearamide, as well as the unsaturated 10-undecenamide and oleamide, did not exhibit bacteriostatic properties. Regardless of chain length and unsaturation, the primary amides had a MIC = >1000 ppm against the 5 microorganisms tested. The N-hexyl- to N-dodecylpelargonamides and the N-octylamides of butanoic through 10-undecenoic acids (not shown in Table I) had MIC = >1000 ppm, indicating no antimicrobial activity of N-substituted amides whose N-alkyl substituent has a higher molecular weight. Examination of amides with a short N-alkyl substituent proved to be more fruitful (Table I). Although the monosubstituted amides, N-methyl (No. 1), N-ethyl (No. 10), and N-isopropyl (No. 15), were not bacteriostats, the N,N-dimethylamides of C_9-C_{16} fatty acids (Nos. 2-6) displayed activity against *S. aureus*. Novak (7) and Kabara (9) reported that N,N-dimethyllauramide had a MIC value of 25 ppm. In this study the lowest MIC (10 ppm) was observed for N,N-dimethylmyristamide (No. 6). Insertion of an hydroxy group into the aliphatic chain, as in N,N-dimethyl-2-hydroxylauramide (No. 9), did not alter bacteriostatic activity. The N,N-diethylamides (Nos. 11-14) also exhibited antimicrobial activity, with an optimum acyl chain length at C_{11}, the N,N-diethyllauramide (No. 12). The introduction of an additional methylene group into the alkyl chain, as for example in the N,N-diisopropyllauramide (No. 16), resulted in loss of antimicrobial activity. The fatty acid ethanolamides are reported to have antimicrobial properties (7,9). However, amides, such as N-(2-hydroxyethyl)lauramide (No. 17), had essentially no antimicrobial activity. Among the diethanolamides, only the N,N-bis-(2-hydroxyethyl)lauramide (No. 18) showed antimicrobial activity. These results indicate that only some dialkyl amides are moderately active antimicrobial agents.

In contrast to the amides, many of the fatty anilides tested in the present study were highly effective against *S. aureus* at very low concentrations. Correlations could be made between chemical structure and bacteriostatic properties, as shown in Tables II and III. The unsubstituted

anilides, such as pelargonanilide and lauranilide (No. 20), were inactive, as was diphenyllauramide (No. 24). N-Methylpelargonanilide (No. 21) and N-methyllauranilide (No. 22), similar in structure to the N,N-dimethylamides (Nos. 2-6), displayed some activity, as did N,N-diphenyl-pelargonamide (No. 23).

The effect of substituents on the aromatic ring was next evaluated. No antimicrobial activity was observed for

anilides with the following ring substituents: monomethyl in ortho, meta or para positions (No. 25); dimethyl in the 2,6- or 3,4-positions (No. 26); monohydroxy (No. 27); cyclopropyl (No. 28); trifluoromethyl (No. 29); and monochloro (No. 30). Among the N-(dichlorophenyl)-pelargonamides with chlorine atoms in the 2,3 (No. 31), 2,4 (no. 32), 2,5 (No. 33), 2,6 (No. 34), and 3,4 (No. 38) positions, only the N-(3,4-dichlorophenyl)pelargonamide

TABLE I

Antimicrobial Activity, Fatty Acid Amides

Compd. No.	Amide	Melting point C	n_D^{20}	S. aureus MIC, ppm
1	$C_{11}H_{23}CONHCH_3$	76.0-77.0		>1,000
2	$C_8H_{17}CON(CH_3)_2$	liq	1.4524	1,000
3	$C_9H_{19}CON(CH_3)_2$	liq	1.4532	100
4	Δ^{10}-$C_{10}H_{19}CON(CH_3)_2$	liq	1.4658	1,000
5	$C_{11}H_{23}CON(CH_3)_2$	liq	1.4566	100
6	$C_{13}H_{27}CON(CH_3)_2$	28.0-28.5		10
7	$C_{15}H_{31}CON(CH_3)_2$	37.5-38.5		1,000
8	Δ^9-$C_{17}H_{33}CON(CH_3)_2$	liq	1.4690	>1,000
9	$C_{10}H_{21}CHOHCON(CH_3)_2$	42.5-43.0		100
10	$C_{11}H_{23}CONHC_2H_5$	54.0-54.5		>1,000
11	$C_8H_{17}CON(C_2H_5)_2$	liq	1.4502	1,000
12	$C_{11}H_{23}CON(C_2H_5)_2$	liq	1.4552	100
13	$C_{13}H_{27}CON(C_2H_5)_2$	liq	1.4574	1,000
14	Δ^9-$C_{17}H_{33}CON(C_2H_5)_2$	liq	1.4651	1,000
15	$C_{11}H_{23}CONHCH(CH_3)_2$	61.0-61.5		>1,000
16	$C_{11}H_{23}CON[CH(CH_3)_2]_2$	liq	1.4542	>1,000
17	$C_{11}H_{23}CONHC_2H_4OH$	85.5-86.0		>1,000
18	$C_{11}H_{23}CON(C_2H_4OH)_2$	43.5-44.0		100
19	$C_{13}H_{27}CON(C_2H_4OH)_2$	53.5-54.0		1,000

TABLE II

Antimicrobial Activity, Fatty Anilides RCONR′ (ring with X and Y substituents)

Compd. No.	R	R′	X	Y	Melting point C	n_D^{20}	S. aureus MIC, ppm
20	$C_{11}H_{23}$	H	H	H	75.5-76.0		>1,000
21	C_8H_{17}	CH_3	H	H	liq	1.5020	100
22	$C_{11}H_{23}$	CH_3	H	H	liq	1.4930	100
23	C_8H_{17}	C_6H_5	H	H	50.5-51.0		100
24	$C_{11}H_{23}$	C_6H_5	H	H	54.5-55.0		>1,000
25	C_8H_{17}	H	4-CH_3	H	81.0-81.5		>1,000
26	C_8H_{17}	H	3-CH_3	4-CH_3	88.0-88.5		>1,000
27	C_8H_{17}	H	4-OH	H	121.5-122.0		>1,000
28	C_8H_{17}	H	4-CH⟨CH_2/CH_2 (cyclopropyl)	H	100.5-101.0		>1,000
29	C_8H_{17}	H	4-CF_3	H	100.5-101.0		>1,000
30	C_9H_{19}	H	4-Cl	H	93.0-93.5		>1,000
31	C_8H_{17}	H	2-Cl	3-Cl	68.5-69.0		>1,000
32	C_8H_{17}	H	2-Cl	4-Cl	90.0-90.5		>1,000
33	C_8H_{17}	H	2-Cl	5-Cl	82.0-82.5		>1,000
34	C_8H_{17}	H	2-Cl	6-Cl	100.0-100.5		>1,000
35	C_3H_7	H	3-Cl	4-Cl	76.5-77.0		100
36	C_5H_{11}	H	3-Cl	4-Cl	74.0-74.5		1
37	C_7H_{15}	H	3-Cl	4-Cl	39.0-39.5		0.1
38	C_8H_{17}	H	3-Cl	4-Cl	68.5-69.0		0.1
39	Δ^{10}-($C_{10}H_{19}$)		3-Cl	4-Cl	58.0-58.5		0.1
40	$C_{11}H_{23}$	H	3-Cl	4-Cl	78.0-78.5		1,000
41	$C_{15}H_{31}$	H	3-Cl	4-Cl	79.5-80.0		>1,000

(SD&C 24)

TABLE III

Antimicrobial Activity, Fatty Anilides RCONH

Compd. No.	R	X	Y	Melting point C	S. aureus, MIC, ppm
42	C_8H_{17}	2-NO$_2$	H	37.5-38.0	>1,000
43	C_5H_{11}	3-NO$_2$	H	62.5-63.0	100
44	C_7H_{15}	3-No$_2$	H	65.0-65.5	1
45	C_8H_{17}	3-NO$_2$	H	63.0-63.5	0.1
46	C_9H_{19}	3-NO$_2$	H	72.0-72.5	1
47	$C_{11}H_{23}$	3-NO$_2$	H	80.0-80.5	>1,000
48	C_5H_{11}	4-NO$_2$	H	66.5-67.0	100
49	C_7H_{15}	4-NO$_2$	H	77.0-77.5	0.1
50	C_8H_{17}	4-NO$_2$	H	76.0-76.5	0.1
51	C_9H_{19}	4-NO$_2$	H	73.5-74.0	0.1
52	$C_{11}H_{23}$	4-NO$_2$	H	78.5-79.0	>1,000
53	C_8H_{17}	2-NO$_2$	4-NO$_2$	179.5-180.0	1,000
54	C_8H_{17}	2-NO$_2$	6-NO$_2$	138.5-139.0	100
55	C_8H_{17}	3-NO$_2$	5-NO$_2$	72.5-73.0	0.1
56	C_5H_{11}	2-OH	5-NO$_2$	136.0-137.0	10
57	C_7H_{15}	2-OH	5-NO$_2$	127.0-128.0	0.1
58	C_8H_{17}	2-OH	5-NO$_2$	140.0-141.0	0.1
59	C_9H_{19}	2-OH	5-NO$_2$	126.5-127.0	1
60	$C_{11}H_{23}$	2-OH	5-NO$_2$	110.0-111.0	1,000
61	C_8H_{17}	3-NO$_2$	4-OH	99.5-100.5	1,000
62	C_8H_{17}	2-Cl	4-NO$_2$	60.0-60.5	>1,000
63	C_8H_{17}	2-Cl	5-NO$_2$	100.0-101.0	>1,000
64	C_8H_{17}	2-NO$_2$	4-Cl	69.5-70.0	>1,000
65	C_5H_{11}	3-NO$_2$	4-Cl	43.5-44.0	0.1
66	C_7H_{15}	3-NO$_2$	4-Cl	51.0-51.5	0.1
67	C_8H_{17}	3-NO$_2$	4-Cl	44.5-45.0	0.1
68	C_9H_{19}	3-NO$_2$	4-Cl	58.0-58.5	0.1
69	$C_{11}H_{23}$	3-NO$_2$	4-Cl	66.5-67.0	>1,000
70	C_5H_{11}	2-OH	5-Cl	97.5-98.0	0.1
71	C_7H_{15}	2-OH	5-Cl	94.0-94.5	1
72	C_8H_{17}	2-OH	5-Cl	93.5-94.0	0.1
73	C_9H_{19}	2-OH	5-Cl	90.0-90.5	0.1
74	$C_{11}H_{23}$	2-OH	5-Cl	73.5-74.0	1

was highly bacteriostatic. The highest degree of activity was observed for compounds with chlorine in the 3- and 4-positions on the aromatic ring and with acyl groups in the 6-11 carbon atom range (Nos. 35-39). The presence of a double bond in the fatty acid chain increased effectiveness. Beyond a C_{11} acyl group, the MIC increased from 0.1 ppm (No. 39) to 1,000 ppm for N-(3,4-dichlorphenyl)lauramide (No. 40) and to >1,000 ppm for N-(3,4-dichlorophenyl)-palmitamide (No. 41).

These findings parallel those of Beaver et al. (14) for the chlorinated carbanilides. To achieve maximum activity, both benzene rings were chlorinated, and effectiveness was at a maximum when chlorine was present in the 3- and 4-positions on one ring and 3- or 4-position on the second ring. The effectiveness of the N-(3,4-dichlorophenyl) amides was affected by the presence of soap and polysorbate 80, as discussed below.

The properties of the nitro substituted anilides (Table III) were next studied. When the nitro group was in a position ortho to the amido nitrogen atom (No. 42), inactivation resulted, probably because of hydrogen bonding between the adjacent amido and nitro functions. Several of the 3- and 4-substituted nitroanilides were highly active. The N-(3-nitrophenyl) amides (Nos. 43-46) and N-(4-nitrophenyl) amides (Nos. 48-51) of C_6-C_{10} fatty acids, in which the amido group is meta or para to the nitro group, were effective bacteriostats. Activity decreased sharply as the

acyl group chain length was further increased (Nos. 47 and 52).

Among the dinitroanilides, those containing nitro groups ortho to the amido function (Nos. 53 and 54) did not exhibit high activity. This was analogous to observations made with the mononitroanilides (Table III). Thus, the presence of the nitro group in the ortho position deactivated the para-nitro group so that the 2,4 dinitro compound was much less active than the analogous 4-nitroanilide. The 3,5-dinitro derivative (No. 55) was highly active but no more so than the 3-nitroanilide. The activity of the N-dinitrophenylpelargonamides was no greater than that of the N-nitrophenyl derivatives and did not extend into the area of gram negative bacteria. For these reasons no further N-dinitroanilides were investigated.

Some unlike pairs of functional groups on the aromatic ring, such as nitro and hydroxy, nitro and halogen, and halogen and hydroxy, were also examined (Table III). The N-(2-hydroxy-5-nitrophenyl) amides of C_6-C_{10} fatty acids (Nos. 56-59) had high antimicrobial activity which, however, dropped off with increased chain length of the acyl group (No. 60), as previously observed for other systems. Proximity of the amido function to an adjacent nitro or hydroxy group did not result in deactivation. However, when a hydroxy group was located ortho to a nitro group, as in No. 61, substantial deactivation occurred, probably because of hydrogen bonding between the adjacent groups.

The nitrochloroanilides behaved differently. Whenever the 2-position on the aromatic ring was occupied by a chloro or nitro group (Nos. 62-64), inactivity resulted. However, when the chloro or nitro groups were removed from a position ortho to the amido group, as for example the N-(3-nitro-4-chlorophenyl) amides of C_6-C_{10} fatty acids (Nos. 65-68), high antibacterial activity occurred. When the acyl chain length was extended beyond C_{10}, as in No. 69, activity was lost. Baker (10) observed similar results for the N-(3-chloro-4-nitrophenyl) amides. Monochlorophenyl- and monohydroxyphenylamides did not display antimicrobial activity, and only when both chlorine and hydroxy groups

were present in specific positions on the aromatic ring were the resulting compounds active. The N-(5-chloro-2-hydroxyphenyl) amides of C_6-C_{12} fatty acids (Nos. 70-74) possessed high bacteriostatic activity.

Table IV illustrates the importance of position attachment to the aromatic amido group. The study of the 3,4-dichloroanilides was extended to determine whether insertion of a methylene group between the amido nitrogen and the aromatic ring would alter antimicrobial activity. The resulting N,(3,4-dichlorobenzyl)pelargonamide (No. 75) was inactive. Introduction of an α-sulfo group into the fatty acid moiety, as in sodium N-(3,4-dichlorophenyl)α-

TABLE IV

Effect of Structure Modifications on Antimicrobial Activity

Compd. No.	Compound	Melting point C	S. aureus MIC, ppm
38	$C_8H_{17}CONH$⟨⟩Cl (Cl)	68.5-69.0	0.1
75	$C_8H_{17}CONHCH_2$⟨⟩Cl (Cl)	39.5-40.0	>1,000
73	$C_7H_{15}CH(SO_3Na)CONH$⟨⟩Cl (Cl)	—	1,000
77	$C_8H_{17}NHCO$⟨⟩Cl (Cl)	65.5-66.0	>1,000
78	$C_8H_{17}NHCO$⟨⟩Cl (Cl)	52.5-53.0	>1,000

TABLE V

Effect of Soap and Polysorbate 80 on Antimicrobial Properties of RCONH⟨⟩X Y

Compd. No.	R	X	Y	S. aureus, MIC, ppm			
				Alone	With 1000 ppm soap	With 1:1 Polysorbate 80	With 1000 ppm Polysorbate 80
36	C_5H_{11}	3-Cl	4-Cl	1	1	1	10
37	C_7H_{15}	3-Cl	4-Cl	0.1	1	0.1	10
38	C_8H_{17}	3-Cl	4-Cl	0.1	1	0.1	10
39	Δ^{10}-$(C_{10}H_{19})$	3-Cl	4-Cl	0.1	1	0.1	10
44	C_7H_{15}	3-NO$_2$	H	1	1	0.1	100
45	C_8H_{17}	3-NO$_2$	H	0.1	10	0.1	100
46	C_9H_{19}	3-NO$_2$	H	1	10	0.1	1,000
49	C_7H_{15}	4-NO$_2$	H	0.1	0.1	0.1	100
50	C_8H_{17}	4-NO$_2$	H	0.1	0.1	0.1	>1,000
51	C_9H_{19}	4-NO$_2$	H	0.1	0.1	0.1	100
55	C_8H_{17}	3-NO$_2$	5-NO$_2$	0.1	0.1	0.1	100
57	C_7H_{15}	2-OH	5-NO$_2$	0.1	0.1	0.1	10
58	C_8H_{17}	2-OH	5-NO$_2$	0.1	0.1	0.1	10
59	C_9H_{19}	2-OH	5-NO$_2$	0.1	0.1	0.1	10
65	C_5H_{11}	3-NO$_2$	4-Cl	0.1	1	0.1	100
66	C_7H_{15}	3-NO$_2$	4-Cl	0.1	0.1	0.1	100
67	C_8H_{17}	3-NO$_2$	4-Cl	0.1	0.1	0.1	100
68	C_9H_{19}	3-NO$_2$	4-Cl	0.1	1	0.1	100
70	C_5H_{11}	2-OH	5-Cl	0.1	1	10	100
71	C_7H_{15}	2-OH	5-Cl	10	1	1	100
72	C_8H_{17}	2-OH	5-Cl	0.1	1	0.1	100
73	C_9H_{19}	2-OH	5-Cl	0.1	1	1	100
74	$C_{11}H_{23}$	2-OH	5-CL	1	1	0.1	100
	Hexachlorophene			0.1	0.1	0.1	10
	Soap			>1,000			
	Polysorbate 80			>1,000			

sulfopelargonamide (No. 76), also resulted in loss of bacteriostatic activity. When the -CONH- group was reversed, as in the dichloro-N-alkyl-benzamides (Nos. 77-78) prepared from C_8-C_{12} fatty amines, activity was likewise lost. These results illustrate close correlation between antimicrobial activity and chemical structure.

Some nonionic surfactants, such as polysorbate 80, can have a potentiating effect on certain germicidal agents (15). The effectiveness of those compounds which displayed high antimicrobial activity was therefore determined in the presence of polysorbate 80, as well as soap, as shown in Table V. Compound numbers in this table refer to positions in Tables I-III. The incorporation of soap led to a more uniform dispersion of water insoluble compounds and had little or no adverse effect on antimicrobial activity. Because MIC values for 1:1 mixtures with soap were the same as those for the compound itself, they are not reported separately. Only a slight decrease in antimicrobial activity was found in the presence of 1000 ppm soap for some of the compounds examined. Polysorbate 80 did not act as a potentiating agent at low levels, but instead acted as a neutralizing agent at high concentrations (15). Thus, the MIC values for 1:1 polysorbate 80 mixtures in Table V were similar to those found for the compounds alone, and values increased significantly when the concentration of polysorbate 80 was increased to 1000 ppm.

The mechanisms by which these antibacterial compounds act on microorganisms have not been fully established. It appears that the enzyme systems within the cells are inactivated. Highest activity was observed for amides and anilides prepared from the medium chain length (C_6-C_{14}) fatty acids. Activity decreased markedly with derivatives of lower or higher molecular weight fatty acids, probably due to solubility effects. The substituted anilides were more effective bacteriostats than the substituted amides. In the anilides the high degree of specificity with respect to position of substituents on the armoatic ring suggests that the compounds interfere with or possibly denature one or several of the enzymes produced by the microorganism. The high antimicrobial activity of the number of substituted anilides and their compatibility with soap suggest the possibility of incorporation of these compounds into sanitizing formulations. However, the toxicity of these compounds in unknown and related biological properties have not been evaluated.

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