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BACTERIOSTATIC, FUNGISTATIC, AND ALGISTATIC ACTIVITY OF FATTY NITROGEN COMPOUNDS

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Bacteriostatic, Fungistatic, and Algistatic Activity of Fatty Nitrogen Compounds

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ABSTRACT

HUECK, HENDRIK J. (Central Laboratory TNO, Delft, The Netherlands), DOROTHEA M. M. ADEMA, AND JOHN R. WIEGMANN. Bacteriostatic, fungistatic, and algistatic activity of fatty nitrogen compounds. Appl. Microbiol. **14**:308–319. 1966.—A total of 164 fatty nitrogen compounds, consisting of quaternary ammonium compounds, alkylamines, *N*-alkyl-1,3-propylene diamines, substituted amino hydroxystearonitriles, substituted amino hydroxystearyl amines, and nitrogen-containing surfactants, were screened for bacteriostatic, fungistatic, and algistatic activity. The most active compounds were dodecylamine and dodecylamine acetate. A number of compounds were very active against gram-negative bacteria. Most of the surfactants were virtually nontoxic to all of the test organisms.

It is well known that a number of nitrogen-containing compounds, like quaternary ammonium compounds, possess excellent bactericidal activity. We screened 164 fatty nitrogen compounds provided by the Central Research and Chemical Divisions of General Mills, Inc., for their biostatic (a general term indicating the static condition against bacteria, fungi, and algae) activity, and compared it with that of benzalkonium chloride and other well-known disinfectants. Most of the compounds belonged to the following classes of fatty nitrogens: quaternary ammonium compounds, alkylamines, alkylamine acetates, substituted amino hydroxystearonitriles, substituted amino hydroxystearyl amines, and nitrogen-containing surfactants. Inhibiting concentrations for these compounds are reported.

MATERIALS AND METHODS

The fatty nitrogen compounds were not chemically pure. The percentage of active ingredient was given for each compound so that actual concentrations could be calculated. All the quaternaries were supplied as solutions of either isopropanol, aqueous isopropanol, or water.

The test organisms were *Escherichia coli* ATCC 11229, *Pseudomonas fluorescens* ATCC 9721, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Aspergillus niger* CMI 17454, *Chaetomium globosum* ATCC 6205, *Myrothecium verrucaria* ATCC 9095, *Trichoderma viride* ATCC 8678, *Chlorella vulgaris* strain A (Agricultural University, Wageningen, The Netherlands), *Stigeoclonium* sp. strain 448 (Indiana University, Bloomington), *Anabaena cylindrica* strain 1403/2 (Culture Collection of Algae and Protozoa, Cambridge, England), and *Oscillatoria tenuis* strain 1459/4 (Culture Collection of Algae and Protozoa, Cambridge, England).

Bacteriostatic activity was determined by a microtechnique roll-culture method (2). Sterilized roller-flasks were filled with 1.5 ml of nutrient agar (Difco), *p*H 6.8, plugged with cotton, sterilized, and kept in a water bath (45 C) so that the medium would remain liquid. A 0.1-ml amount of a stock solution of the test substance was introduced into the roller-flask with a calibrated screw-controlled pipette. Duplicate roller-flasks were inoculated with one drop (0.05 ml) of a suspension of each organism used. Immediately after inoculation, the roller-flasks were inserted in a horizontal position into the roller-flask apparatus (Fig. 1) where they were simultaneously cooled and rotated (approximately 1,000 rev/min). Consequently, the medium solidified in an even layer on the wall of the roller-flask, and at the same time the inoculum and the test substance were mixed with the medium.

The inoculated roller-flasks were incubated for 24 hr at 37 C (*P. fluorescens* at 24 C). The lowest concentration (parts per million) of the test compound that inhibited growth in both roller-flasks was reported as the inhibiting concentration in Table 2.

To prepare the inoculum, the bacteria were cultured in nutrient broth (Difco) at 30 C on a shaker apparatus. Vigorous growth was obtained by subculturing twice at intervals of 24 hr before the test. The culture was then centrifuged. Next, the packed cells were suspended in sterile water to give a density of 10^9 viable cells per milliliter for the gram-negative bacteria and *S. aureus*, and 10^8 viable cells per milliliter for *B. subtilis* as revealed by plate-counts.

Fungistatic activity was determined by a modified

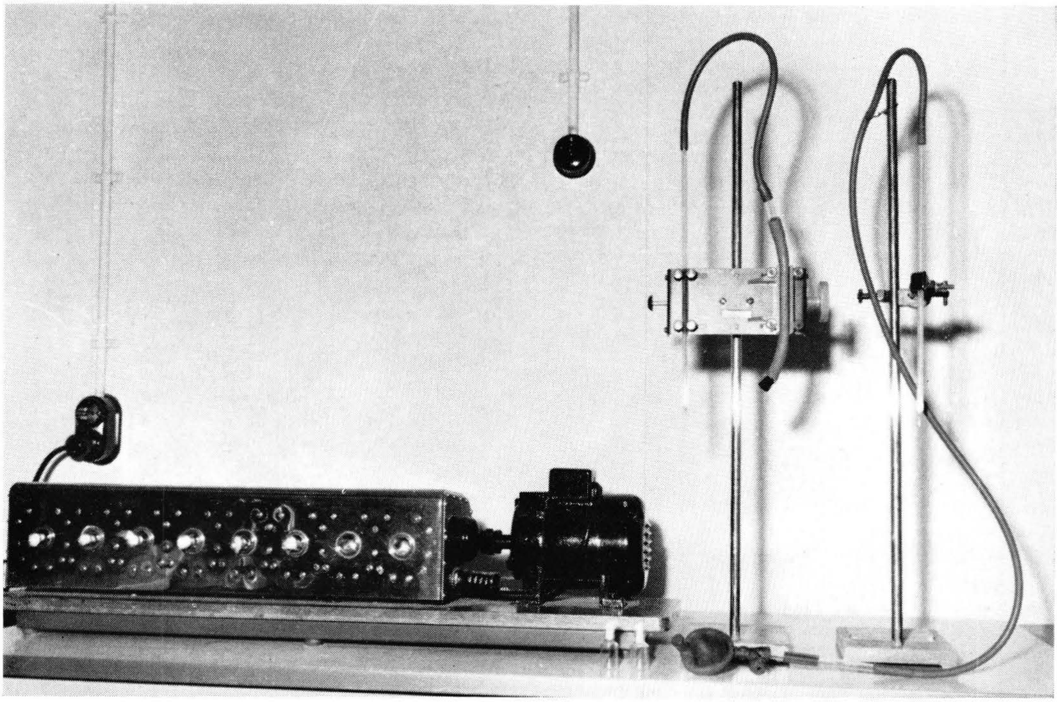


FIG. 1. *Roller-flask apparatus and screw-controlled pipettes for delivering the test solution and the test inoculum.*

roll-culture method (4). The roller-flasks contained a glucose agar with the following formulation: distilled water, 1,000 ml; NH_4NO_3, 3 g; KH_2PO_4, 2.5 g; K_2HPO, 2 g; $MgSO_4 \cdot 7H_2O$, 2 g; glucose, 10 g; and Noble agar (Difco), 20 g; at *p*H 6.4. After incubation at 24 C for 3 days, the presence or absence of growth was recorded.

To obtain spores for testing, the fungi were cultured alternately for 14 days on oatmeal agar and on Malt Agar (Difco). The spores were collected in distilled water and filtered through sterile cotton to remove mycelial threads. The filtered spore suspension was diluted so that 1 ml contained approximately 125,000 spores. One drop (0.05 ml) of the suspension was used as the inoculum.

For determination of algistatic activity, the culture tubes were filled with 4.15 ml of a nutrient solution devised by Chu (1), plugged with cotton, and sterilized. A 0.3-ml amount of the stock test solution was added to the medium, and the tubes were inoculated with 0.5 ml of an algal suspension, incubated at 24 C, and illuminated with fluorescent tubes that provided 600 ft-c for the green algae and 200 ft-c for the blue-green algae. The results were recorded after 1 week. The lowest concentration of the test substance that completely inhibited algal growth in duplicate tests was recorded.

Algae used as inoculum were obtained from stock cultures maintained on soil extract-agar and proteose agar (6).

Preparation and addition of test compounds. Whenever possible, the test compounds were dissolved in water. Stock solutions were prepared so that after addition to the nutrient medium in the roller-flasks, final concentrations of 1, 0.316, 0.1, 0.316, 0.01, 0.00316, 0.001, 0.000316, 0.0001, 0.0000316, and 0.00001% for the fungistats and bacteriostats were obtained. Because of the greater susceptibility of the algae, concentrations of 0.01, 0.00316%, etc., to 0.000001% were used.

Compounds not water-soluble were dissolved in one of the following: acetone, acetone-water, isopropanol-water, or acetic acid-water; or they were emulsified with organic solvents containing carboxymethyl cellulose and Tween 80 (Atlas Powder Co., Wilmington, Del.). The vehicle for each compound is shown in Table 2. When a compound dissolved in acetone was used, only 0.03 ml of the stock solution was added to the medium, because higher concentrations of acetone were toxic to the organisms.

With some compounds, water was used as the solvent in the algistatic tests and organic solvents were used in the bacteriostatic and fungistatic tests, because the former required a lower concentration of the test compound. Acetic acid was never used in the algal media because of the organism's sensitivity to it. In general, the solvents in Table 2 refer only to those used in the bacteriostatic and fungistatic tests.

Standard test compounds. The standard reference compound used in each biostatic test was benzalkonium chloride (Zephirol, Farbenfabriken Bayer A.G., Leverkusen-Bayerwerk, Germany; Zephiran, Winthrop Laboratories, New York, N.Y.), which was selected because of its stability (3), good biostatic activity, and widely accepted usage. Throughout the investigation, the biostatic activity of benzalkonium chloride was found to be rather constant. No effort was made to adjust the data presented to compensate for slight changes in sensitivity of the organisms to benzalkonium chloride which occurred during the 2-year test period. In addition to the standard included in each test, other chemicals (Table 1) having biocidal properties were included once to facilitate a comparison of our results with those of other investigators.

Results and Discussion

The bacteriostatic, fungistatic, and algistatic activity for the fatty nitrogen compounds is recorded in Table 2. The inhibiting concentration is the lowest concentration which completely inhibits the growth in both cultures in a duplicate test. If no inhibition was found at the highest concentration tested, the inhibiting concentration was reported as being greater than the highest concentration tested.

From the results in Table 2, the following conclusions can be drawn.

Monoquaternaries. In the homologous series of monoquaternaries $RMe_3N^+Cl^-$, the highest biostatic activity is found when R contains 14 carbon atoms. This agrees with a previous report (5) that the antiseptic activity in this homologous series reaches its peak when R contains between 12 and 16 carbon atoms. In the homologous series $R_2Me_2N^+Cl^-$, the highest biostatic activity occurs when R contains 8 carbon atoms. In the homologous series $R_3MeN^+Cl^-$, the compounds where R contains 8 carbon atoms are the most active. In comparing monoquaternaries having one, two, or three long alkyl chains, the compounds with more than one alkyl chain have the lowest biological activity except where chain length is eight or fewer.

Diquaternaries. In the homologous series of diquaternaries $[RN^+(CH_3)_2N(CH_3)_3]2Cl^-$, there is no clear optimal level of activity. In general, the biostatic activity of this group appears to be similar to that of the monoquaternaries except for the few rather active members of the group.

Amines. The primary amines with alkyl groups containing 12 to 16 carbon atoms had higher algistatic activity but lower bacteriostatic activity against the gram-positive bacteria than the monoquaternaries having the same alkyl groups. Dodecylamine had the highest biostatic activity because it was quite active against all the organisms tested. For the secondary amines with two long alkyl chains, only those with the lower carbon chain lengths were soluble in the solvents used. Dioctylamine was the most active compound. The *N*-substituted propylene diamines had approximately the same activity as the corresponding primary amines, with the dodecyl-substituted compound possessing the highest activity. In compounds containing *N*-methyl groups, these groups had very little influence on the bacteriostatic and fungistatic activity; however, the *N*,*N*-methyl compounds had better algistatic activity than the *N*-methyl compounds. The acetic acid salts of amines of the monoacetates had approximately the same activity as the free amines or diamines, and the diacetates were generally less active than the monoacetates. Some of the substituted amino hydroxystearonitriles and amino hydroxystearyl amines, e.g., propylamino-10 (9)-hydroxystearyl amine, exhibited a good activity against gram-negative bacteria. None of the surfactants screened was a good general biostat. All were inactive against the gram-negative bacteria, whereas only a few exhibited some activity toward gram-positive bacteria, fungi, or algae, but never against all organisms simultaneously. A large number of these surfactants exhibited rather low activity towards all the organisms.

Table 2 indicates that the biostatic activity of the standard compound, benzalkonium chloride, was not easily surpassed. However, dodecylbenzyldimethyl ammonium chloride and cocobenzyldimethyl ammonium chloride had better activity against gram-positive bacteria. Other compounds with reasonably good activity for gram-positive bacteria were the mono- and diquaternaries with one long alkyl chain, and the $R_2Me_2N^+Cl^-$ and $R_2MeN^+Cl^-$ where R contains 8 or 10 carbon atoms. Benzalkonium chloride is less effective against gram-negative than gram-positive bacteria. Compounds superior to the standard in this respect were: dodecyl amine, three *N*-alkyl propylene diamines, 9 (10)-*m*-amino methyl benzylamino-10 (9)-hydroxystearonitrile, six substituted amino hydroxystearyl amines; and dioctyldimethyl ammonium chloride. The fungistatic activity of the standard was surpassed by a number of monoquaternaries (see Table 3).

All compounds with good bacteriostatic or fungistatic activity, or both, also had good algistatic activity. Compounds having high algistatic activity but low bacteriostatic or fungistatic activity, or both, were tetradecylamine, 9 (10)-dimethyl-amino-10 (9)-hydroxystearyl amine, and a 50:50 mixture of cotton trimethyl ammonium chloride and dicocodimethyl ammonium chloride.

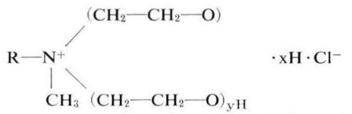
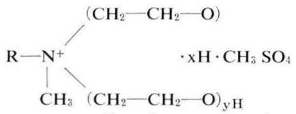
The most biologically active compounds are indicated in Table 3. The dodecyl and the mixed carbon chain lengths of coco were the predom-

TABLE 1. *Inhibiting concentrations (in ppm) of some biostatical chemicals*

Compound*	Solvent system stock solution	Bacteria				Fungi				Algae			
		Gram-negative		Gram-positive						Green		Blue-green	
		Escherichia coli	*Pseudomonas fluorescens*	*Bacillus subtilis*	*Staphylococcus aureus*	*Aspergillus niger*	*Chaetomium globosum*	*Myrothecium verrucaria*	*Trichoderma viride*	*Chlorella vulgaris*	*Stigeoclonium* sp.	*Anabaena cylindrica*	*Oscillatoria tenuis*
Phenol	Water	>1,000	1,000	1,000	>1,000	1,000	1,000	300	1,000	>100	100	>100	60
Na-pentachlorophenol	Water	300	300	3	3	30	3	10	100	10	3	30	—
Dichlorophene (D.D.M.)	Acetone	100	300	3	10	100	6	10	60	6	0.3	—	1
Hexachlorophene (G 11)	Acetone	30	10	0.3	0.6	1	0.3	0.6	1	3	0.2	1	0.6
Na-*p*-toluenesulfonchloramide (Chloramine T)	Water	300	300	2,000	100	100	300	20	100	—	—	—	—
Chlorhexidine dihydro chloride (Hibitane)	Ethyl alcohol-water	10	10	3	3	100	30	100	30	1	0.3	0.3	0.6
$HgCl_2$	Water	3	10	2	3	3	10	0.6	3	10	0.3	0.3	—
Phenylmercuric acetate	Acetone	10	30	0.3	1	0.1	0.1	0.1	0.1	0.3	—	0.03	0.03
$CuSO_4$	Water	1,000	1,000	300	1,000	300	100	100	300	3	1	3	—
Cu-8-oxyquinolinolate	Suspension in water	>100	>100	1	10	0.3	0.3	0.3	0.2	0.3	—	0.3	—
Tributyl tin oxide	Emulsion in water	>3,000	>1,000	3	30	10	10	3	20	0.3	0.03	0.3	0.1
Salicyl anilide (Shirlan NA)	Suspension in water	300	300	100	100	200	30	30	100	30	30	100	100
Benzethonium chloride (Hyamine 1622)	Water	1,000	300	3	3	300	30	300	200	3	1	1	1
Formaldehyde	Water	30	30	30	30	100	30	100	1,000	30	30	30	—

* Hibitane—Imperial Chemical Ltd., Imperial Chemical House, Millbank, London, England; Shirlan NA—Imperial Chemical Ltd.; Hyamine—Rohm & Haas Co., Philadelphia, Pa.

Table 2. *Inhibiting concentrations (in ppm) of fatty nitrogen compounds for some bacteria, fungi, and algae*

RC no.	Compound*	Solvent used in stock solution†	Bacteria: Gram-negative: *Escherichia coli*	Bacteria: Gram-negative: *Pseudomonas fluorescens*	Bacteria: Gram-positive: *Bacillus subtilis*	Bacteria: Gram-positive: *Staphylococcus aureus*	Fungi: *Aspergillus niger*	Fungi: *Chaetomium globosum*	Fungi: *Myrothecium verrucaria*	Fungi: *Trichoderma viride*	Algae: Green: *Chlorella vulgaris*	Algae: Green: *Stigeoclonium* sp.	Algae: Blue-green: *Anabaena cylindrica*	Algae: Blue-green: *Oscillatoria tenuis*
Standard	Benzalkonium chloride	1	200	300	3	4	60	10	40	80	1	0.7	1	.06
	Monoquaternaries $R_4N^+X^-$ *(one R group is fatty)*													
4529	(Octyl-decyl) trimethyl ammonium chloride	1	500	500	50	50	5,000	500	5,000	5,000	50	50	50	50
4528	Dodecyl trimethyl ammonium chloride, Aliquat 4	1	500	500	5	5	500	50	500	500	50	5	5	0.5
4527	(Dodecyl-tetradecyl) trimethyl ammonium chloride	1	150	500	5	5	50	50	50	500	5	1.5	1.5	1.5
5132	Tetradecyl trimethyl ammonium chloride	1	150	100	1.5	5	50	30	50	150	5	3	5	0.5
4526	Hexadecyl trimethyl ammonium chloride	1	5,000	5,000	5	5	500	50	50	150	5	5	5	15
4525	9-Octadecenyl trimethyl ammonium chloride	1	5,000	5,000	5	5	500	50	50	500	5	5	5	5
4524	Octadecyl trimethyl ammonium chloride	1	5,000	5,000	5	15	500	50	500	500	5	5	5	5
4530	Tallow trimethyl ammonium chloride, Aliquat 26	1	5,000	5,000	5	15	500	50	50	150	1.5	1.5	1.5	1
4531	Soya trimethyl ammonium chloride	1	5,000	5,000	5	15	500	50	150	150	5	5	5	0.5
4532	Cotton trimethyl ammonium chloride	1	5,000	5,000	5	50	150	50	50	50	1.5	0.5	5	0.5
4533	Tall oil trimethyl ammonium chloride	1	500	5,000	5	5	500	50	50	500	5	5	5	0.5
4534	Coco trimethyl ammonium chloride, Aliquat 21	1	500	500	5	5	500	—	50	500	5	5	5	5
5625	Dodecyl benzyl dimethyl ammonium chloride	1	750	750	2	2	75	7	150	75	0.2	0.2	0.2	0.2
5624	Coco benzyl dimethyl ammonium chloride	1	225	225	2	2	20	7	20	20	2	0.5	2	0.7
5138	Hydrogenated-tallow benzyl dimethyl ammonium chloride	1	2,250	2,250	7	20	225	75	150	225	2	2	0.7	1.5
5136	Dodecyl-benzyl hydrogenated-tallow dimethyl ammonium chloride	2	>750	>750	>2,250	4,500	>7,500	7,500	>7,500	>7,500	20	45	2.5	7
5137	Dodecyl-benzyl tri(octyl-decyl) ammonium chloride	2	>2,250	7,500	2,250	2,250	>7,500	7,500	>7,500	>7,500	7	4.5	15	75
5134	 R = from coco; x + y = 15	1	3,000	3,000	30	30	>10,000	100	1,000	>10,000	10	3	>100	30
5135	 R = from coco; x + y = 15	1	>10,000	6,000	30	100	>10,000	30	100	>10,000	10	10	100	30

	Monoquaternaries $R_4N^+X^-$ (two R groups are fatty)													
5626	Di-octyl dimethyl ammonium chloride	1	40	75	20	20	225	75	75	75	7	2	0.7	2
5627	Di-decyl dimethyl ammonium chloride	1	225	750	<0.7	7	75	7	20	20	2	0.7	0.2	0.7
4538	Di(octyl-decyl) dimethyl ammonium chloride	1	50	500	5	5	50	50	50	50	0.5	0.5	0.5	0.5
4537	Di-dodecyl dimethyl ammonium chloride, Aliquat 204	3	2,250	2,250	20	225	225	150	225	225	5	5	2	7
5144	Di(dodecyl-tetradecyl) dimethyl ammonium chloride	2	2,250	2,250	20	225	225	225	750	225	7	7	4.5	7
5146	Di-tetradecyl dimethyl ammonium chloride	2	2,250	>2,250	225	750	2,250	750	2,250	2,250	7	7	2	7
5147	Di-hexadecyl dimethyl ammonium chloride	2	>2,500	>800	>800	1,500	8,000	2,400	8,000	8,000	8	2	2	8
4541	Di-octadecyl dimethyl ammonium chloride	2	>750	>750	>750	>750	>750	>750	>750	>750	20	75	15	20
5142	Di-oleyl dimethyl ammonium chloride	2	7,500	>750	75	750	7,500	750	7,500	2,250	7	7	2	7
4535	Di(hydrogenated-tallow) dimethyl ammonium chloride	3	>2,250	>2,250	225	750	>7,500	2,250	2,250	750	20	20	7	7
5145	Di(tall oil) dimethyl ammonium chloride	2	2,250	>750	750	2,250	7,500	750	7,500	4,500	7	7	2	7
4536	Di-coco dimethyl ammonium chloride, Aliquat 221	3	2,250	2,250	15	225	225	225	225	225	2	7	2	7
4539	50:50 4530:4536	3	500	1,500	15	50	150	150	50	150	1.5	1.5	1.5	1.5
4540	50:50 4532:4536	1	5,000	5,000	5	50	500	50	50	50	0.05	0.5	0.05	0.05
5139	Benzyl didococyl methyl ammonium chloride	2	2,250	2,250	75	225	225	225	750	225	7	4.5	1.5	7
5140	Benzyl dicoco methyl ammonium chloride	2	2,250	2,250	22	225	750	225	750	225	2	4.5	2	7
5141	Benzyl di(hydrogenated-tallow) methyl ammonium chloride	2	>750	>750	>750	>750	>750	>750	>750	>750	7	7	7	20
	Monoquaternaries $R_4N^+X^-$ (three R-groups are fatty)													
4542	Triostyl methyl ammonium chloride	2	500	150	3	3	150	50	50	50	0.5	1	0.3	1.5
5148	Tri isooctyl methyl ammonium chloride	2	500	50	1.5	5	50	50	30	50	5	1.5	0.5	1.5
4543	Tri(octyl-decyl) methyl ammonium chloride	2	500	500	3	5	150	100	50	100	1.5	1.5	1.5	1
4545	Tridodecyl methyl ammonium chloride	2	>5,000	>5,000	500	1,500	1,500	300	—	1,500	50	15	30	15
4546	Tricoco methyl ammonium chloride	2	>5,000	>5,000	1,500	1,500	>5,000	—	>5,000	3,000	>50	>50	50	>50
4547	Tri(hydrogenated-tallow) methyl ammonium chloride	2	>5,000	>5,000	500	1,500	5,000	5,000	>5,000	5,000	30	>50	15	50
	Diquaternaries $RN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3 2Cl^-$													
4552	1-(Octyl-decyl) dimethyl, 3-trimethyl, propane diammonium chloride	1	1,500	5,000	150	150	1,500	500	1,500	1,500	50	50	50	5
4551	1-(Dodecyl) dimethyl, 3-trimethyl, propane diammonium chloride	1	500	500	5	5	500	150	500	500	5	5	5	5
4550	1-(Dodecyl-tetradecyl) dimethyl, 3-trimethyl, propane diammonium chloride	1	1,500	500	5	15	500	50	500	500	5	0.5	5	0.5
5149	1-(Hexadecyl)dimethyl, 3-trimethyl, propane diammonium chloride	1	500	500	5	15	150	150	150	150	1.5	1.5	5	3
5150	1-(Octadecyl) dimethyl, 3-trimethyl, propane diammonium chloride	1	1,500	5,000	15	50	150	150	150	150	1.5	1.5	1.5	1.5
4549	1-(9-Octadecenyl) dimethyl, 3-trimethyl, propane diammonium chloride	1	500	5,000	5	50	500	50	500	500	5	5	0.5	0.5

* Aliquat, Versamid, Alamine, Dram, Alamac, and Deriphat are products of General Mills, Inc. Primene is a product of Rohm & Haas Co.

† Solvents used in preparing stock solutions: (1) water; (2) acetone; (3) isopropanol-water 1:3; (4) acetic acid-water (In the highest concentration, 10,000 ppm, the test substance was dissolved in glacial acetic acid. Further dilutions were made with distilled water only.); (5) emulsion with acetone, carboxymethyl cellulose, and Tween 80 (1 ml of acetone + 9 ml of water with 0.1% carboxymethyl cellulose, and 0.1% Tween 80); (6) emulsion with isopropanol, carboxymethyl cellulose, and Tween 80 (1 ml of isopropanol + 9 ml of water with 0.1% carboxymethyl cellulose and 0.1% Tween 80).

TABLE 2—*Continued*

RC no.	Compound*	Solvent used in stock solution†	Bacteria				Fungi				Algae			
			Gram-negative		Gram-positive						Green		Blue-green	
			Escherichia coli	*Pseudomonas fluorescens*	*Bacillus subtilis*	*Staphylococcus aureus*	*Aspergillus niger*	*Chaetomium globosum*	*Myrothecium verrucaria*	*Trichoderma viride*	*Chlorella vulgaris*	*Stigeoclonium* sp.	*Anabaena cylindrica*	*Oscillatoria tenuis*
4553	1-(Tallow) dimethyl, 3-trimethyl, propane diammonium chloride	1	1,500	1,500	5	15	150	150	150	150	0.5	1.5	0.5	1.5
4554	1-(Soya) dimethyl, 3-trimethyl, propane diammonium chloride	1	500	1,500	5	15	150	150	150	150	5	5	5	5
4555	1-(Cotton) dimethyl, 3-trimethyl, propane diammonium chloride	1	500	1,500	5	50	150	150	150	150	0.5	0.5	1.5	0.5
4556	1-(Tall oil) dimethyl, 3-trimethyl, propane diammonium chloride	1	500	1,500	5	50	500	500	500	500	0.5	1.5	0.5	0.15
4557	1-(Coco) dimethyl, 3-trimethyl, propane diammonium chloride	1	500	500	1.5	5	150	50	150	150	0.5	0.5	1	0.5
	Diquaternaries (other)													
4560	1-Tallow, dimethyl, 3-methyl morpholino propane diammonium chloride	1	500	1,500	5	15	150	150	150	150	0.5	1.5	0.5	1.5
4559	Bis-(methyl) morpholino dilinoleyl ammonium chloride	1	1,500	5,000	100	300	1,500	150	1,500	500	1.5	1.5	1.5	5
4561	1-Di(hydrogenated-tallow) methyl trimethyl propane diammonium chloride	3	5,000	5,000	1,500	1,500	>5,000	1,500	5,000	5,000	10	10	5	5
4562	1-Dicoco, methyl trimethyl propane diammonium chloride	1	500	1,500	50	150	500	50	500	150	3	1.5	3	1.5
	Triquaternaries $RN^+(CH_3)[(CH_2)_3N^+(CH_3)_3]_2 3Cl^-$													
5151	R = tallow	1	450	450	15	140	450	140	140	450	5	3	1.5	1.5
4566	R = hydrogenated-tallow	1	500	500	15	100	500	150	150	300	5	3	5	5
4567	R = coco	1	500	500	15	50	500	150	500	500	1.5	5	1.5	1.5
	Polyquaternaries (Versamid polyamide resins quaternized by methyl-chloride)													
4563	Quaternized Versamid 100	3	5,000	>5,000	5,000	5,000	>5,000	1,500	>5,000	>5,000	15	15	15	50
4564	Quaternized Versamid 115	1	5,000	5,000	500	1,500	5,000	500	1,500	1,500	15	15	50	15
4565	Quarternized Versamid 125	1	5,000	5,000	1,000	1,500	>5,000	500	5,000	1,500	15	15	30	15
	Primary amines													
5153	Octyl amine	2	300	300	300	300	300	300	300	300	0.6	1	1	1
4603	(Octyl-decyl) amine	4	300	100	200	1,000	300	300	300	300	3	0.6	0.6	1
5154	Tetradecyl amine	2	2,000	3,000	300	1,000	300	300	200	300	0.6	0.3	0.1	0.1
4605	Dodecyl amine, Alamine 4	4	30	30	20	30	60	30	30	30	0.3	0.3	0.3	0.3
4606	Tall oil fatty amine	4	300	300	300	300	300	200	300	300	10	3	6	3
5158	Tall oil amine (mixture of fatty and rosin amines)	2	300	1,000	30	30	100	60	100	60	0.6	1	1	0.3

4607	Phenyl stearyl amine	4	300	300	300	1,000	1,000	300	300	600	100	100	—	10
	Secondary amines													
5632	Dioctyl amine	2	100	100	10	10	30	10	30	30	1	0.3	0.3	0.3
4608	Di(octyl-decyl) amine	2	1,000	>1,000	30	30	30	10	100	100	3	1	0.3	0.3
	Diamines													
5633	*N*-octyl propylene diamine	2	100	100	100	300	300	100	300	100	6	3	0.3	0.3
4616	*N*-(octyl-decyl) propylene diamine	4	100	100	30	300	300	300	1,000	300	3	1	0.3	0.3
5634	*N*-decyl propylene diamine	2	30	30	10	100	300	100	300	100	3	0.2	0.3	0.3
5635	*N*-dodecyl propylene diamine, Diam 4	2	30	30	10	100	60	60	100	100	3	0.3	0.2	0.06
5172	*N*-(dodecyl-tetradecyl) propylene diamine	2	100	30	30	100	100	100	100	100	0.3	0.1	1	0.3
5636	*N*-tetradecyl propylene diamine	2	100	100	30	100	100	60	100	100	3	1	0.3	0.3
5173	*N*-hexadecyl propylene diamine	2	1,000	300	100	300	1,000	600	1,000	1,000	6	10	0.3	0.3
5174	*N*-octadecyl propylene diamine	2	1,000	>1,000	600	1,000	>1,000	1,000	>1,000	>1,000	6	10	1	6
5175	*N*-oleyl propylene diamine	2	300	300	300	300	300	300	1,000	300	3	3	0.2	0.3
4618	*N*-coco propylene diamine	4	30	30	30	100	100	30	100	100	3	1	0.3	0.3
4619	*N*-tallow propylene diamine	4	100	60	100	300	300	100	100	100	10	30	0.3	0.6
4620	*N*-tall oil propylene diamine	4	100	100	100	300	300	100	100	200	10	10	3	2
5178	*N*-cotton propylene diamine	2	300	300	300	300	300	300	600	300	1	1	0.3	0.3
4621	*N*-phenylstearyl propylene diamine	4	100	100	100	300	300	100	300	200	30	30	10	10
5179	$RNHCH_2CH(CH_3)CH_2NH_2$ when R = dodecyl	2	100	100	30	100	100	60	100	100	1	0.3	0.3	0.1
5180	$RNHCH_2CH(CH_3)CH_2NH_2$ when R = tall oil derived	2	300	600	300	300	600	300	300	200	3	3	3	3
	Triamines													
5637	$RN(CH_2CH_2CH_2NH_2)_2R$ = dodecyl	2	30	100	10	100	100	100	100	300	3	1	0.3	0.3
5638	$RN(CH_2CH_2CH_2NH_2)_2R$ = tallow	2	1,000	100	30	200	300	100	300	100	10	3	0.6	1
5639	$RN(CH_2CH_2CH_2NH_2)_2R$ = oleyl	2	600	300	100	300	100	100	100	100	20	6	0.6	1
	Miscellaneous polyamines													
4622	$R(NH_2)_2$ R = dimerized oleyl-linoleyl	4	1,000	300	1,000	1,000	2,000	1,000	1,000	1,000	10	30	2	10
4623	$R(NC_3H_8O)_2$ R = dimerized linoleyl	4	100	100	100	300	1,000	300	300	300	>100	>100	>30	>100
4624	$R_2NCH_2CH_2CH_2NH_2$ R = coco	4	100	100	300	300	600	300	300	300	10	10	3	3
4625	$RN(CH_2CH_2CH_2NH_2)_2$ R = coco	4	100	100	20	300	300	100	300	100	6	3	0.6	0.3
	Methylated amines													
5170	Mono methyl didodecyl amine	6	>1,000	>1,000	1,000	>1,000	>1,000	>1,000	>1,000	>1,000	30	10	>3	10
5171	Mono methyl dioleyl amine	6	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	>100	>100	10	30
4614	Mono methyl di(hydrogenated tallow) amine	4	100	100	300	300	300	300	300	300	100	100	>10	>30
4615	Mono methyl di coco amine	4	300	300	300	1,000	1,000	200	300	300	10	3	10	3
5629	*N,N*-dimethyl dodecyl amine	2	100	100	30	100	30	30	30	30	1	0.3	1	0.6
5630	*N,N*-dimethyl hexadecyl amine	2	>1,000	>1,000	60	60	300	100	200	300	3	3	0.3	1
4613	*N,N*-dimethyl coco amine	4	100	60	30	300	100	30	30	100	1	0.3	1	0.3
4612	*N,N*-dimethyl hydrogenated-tallow amine	4	300	600	100	300	600	100	300	300	3	3	0.6	1
	Primary amine acetates													
5643	Distilled decyl amine acetate	1	30	100	60	300	100	100	100	60	10	1	1	3
5644	Distilled dodecyl, amine acetate, Almac 4	1	30	100	10	30	20	10	60	30	0.6	0.6	0.3	0.3
5645	Distilled hexadecyl amine acetate	1	>1,000	>1,000	300	1,000	1,000	100	300	>1,000	6	3	0.3	1

TABLE 2—*Continued*

RC no.	Compound*	Solvent used in stock solution†	Bacteria				Fungi				Algae			
			Gram-negative		Gram-positive						Green		Blue-green	
			Escherichia coli	*Pseudomonas fluorescens*	*Bacillus subtilis*	*Staphylococcus aureus*	*Aspergillus niger*	*Chaetomium globosum*	*Myrothecium verrucaria*	*Trichoderma viride*	*Chlorella vulgaris*	*Stigeoclonium* sp.	*Anabaena cylindrica*	*Oscillatoria tenuis*
4568	Distilled coco amine acetate	1	100	100	30	100	100	60	30	100	1	0.3	0.3	1
4569	Distilled tallow amine acetate	1	3,000	1,000	200	1,000	300	100	300	300	10	10	1	2
4570	Distilled cotton amine acetate	2	1,000	1,000	200	1,000	300	30	300	300	3	3	1	3
5642	Tall oil amine acetate	1	1,000	>1,000	10	100	300	30	300	300	3	10	6	3
	Diamine acetates													
4571	Distilled N-coco propylene diamine mono acetate	2	100	30	30	100	100	100	100	100	3	1	3	0.3
4572	Distilled N-coco propylene diamine di acetate	3	100	100	30	100	100	100	100	300	3	1	3	0.6
4573	*N*-tallow propylene diamine mono acetate	2	1,000	300	100	300	300	200	300	300	3	6	3	3
4574	*N*-tallow propylene diamine di acetate	3	300	300	100	1,000	3,000	2,000	3,000	3,000	10	10	6	10
4575	*N*-oleyl propylene diamine mono acetate	2	1,000	300	30	300	300	100	300	300	10	10	3	2
4576	*N*-oleyl propylene diamine di acetate	3	300	300	60	600	3,000	2,000	3,000	1,000	10	10	3	1
4577	*N*-tall oil propylene diamine mono acetate	2	1,000	300	30	1,000	300	30	300	300	10	3	1	2
4578	*N*-tall oil propylene diamine di acetate	3	300	300	100	300	3,000	1,000	3,000	1,000	10	6	1	1
	Miscellaneous acetates													
4579	Coco-morpholine acetate	2	1,000	1,000	300	300	100	6	60	30	3	3	3	3
4580	1-Tallow 3-morpholino-propylene diamine di acetate	3	1,000	1,000	200	600	3,000	1,000	3,000	3,000	10	6	10	3
	Substituted amino hydroxy stearonitriles													
5102	9(10)-Amino-10(9)-hydroxystearonitrile	2	100	100	30	100	100	100	300	100	1	1	3	1
5103	9(10)-β Aminoethylamino-10(9)-hydroxystearonitrile	2	10	30	3	100	300	100	300	100	3	3	1	1
5104	9(10)-*m*-Aminomethyl benzylamino-10(9)-hydroxystearonitrile	2	30	100	10	100	300	100	300	100	1	3	0.3	0.3
5105	9(10)-Anilino-10(9)-hydroxystearonitrile	2	>300	>300	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000	>100	30	>100	>100
5106	9(10)-Diethanolamino-10(9)-hydroxystearonitrile	2	1,000	300	30	100	1,000	100	1,000	300	10	10	10	20
5107	9(10)-Dimethylamino-10(9)-hydroxystearonitrile	2	1,000	300	30	100	100	100	100	100	1	1	0.3	3
5108	9(10)-Dodecylamino-10(9)-hydroxystearonitrile	5	>100	>100	>100	>100	>100	>100	>100	>100	20	3	10	30
5109	9(10)-Methylamino-10(9)-hydroxystearonitrile	2	300	100	30	100	100	100	100	60	1	1	3	3
5110	9(10)-Morpholino-10(9)-hydroxystearonitrile	5	>100	>100	>100	>100	>100	>100	>100	>100	100	10	3	60
5111	9(10)-*N* cyanoethylamino-10(9)-hydroxystearonitrile	5	>100	>100	100	100	>100	100	>100	>100	3	3	10	10

	Substituted aminohydroxystearyl amines													
5112	9(10)-Amino-10(9)-hydroxystearyl amine	5	30	100	30	100	>100	100	>100	>100	3	1	1	1
5113	9(10)-β Amino ethylamino-10(9)-hydroxystearyl amine	5	300	30	30	30	300	100	300	100	3	3	1	3
5114	9(10)-*m*-Aminomethylbenzylamino-10(9)-hydroxystearyl amine	2	60	100	30	100	3,000	200	2,000	300	3	3	0.3	1
5115	9(10)-Anilino-10(9)-hydroxystearyl amine	2	2,000	3,000	30	300	300	200	300	300	0.6	3	0.3	3
5116	9(10)-Diethanolamino-10(9)-hydroxystearyl amine	2	30	100	100	200	1,000	300	3,000	1,000	10	3	0.3	1
5117	9(10)-Dimethylamino-10(9)-hydroxystearyl amine	2	100	100	30	100	1,000	300	1,000	1,000	0.3	0.3	0.1	0.3
5118	9(10)-Dodecylamino-10(9)-hydroxystearyl amine	2	3,000	3,000	100	200	300	200	300	300	10	3	1	3
5652	9(10)-Methylamino-10(9)-hydroxystearyl amine	2	30	100	30	100	300	300	300	300	6	3	0.3	1
5119	9(10)-Morpholino-10(9)-hydroxystearyl amine	2	30	100	30	300	300	300	300	300	1	0.3	0.1	1
5120	9(10)-γ-Amino propylamino-10(9)-hydroxystearyl amine	5	30	30	10	10	300	100	600	100	3	3	0.3	1
5121	9(10)-*N*,*N*-dicyanoethylamino-10(9)-hydroxy-*N*,*N*-dicyanoethylstearyl amine	5	>1,000	>1,000	>1,000	>1,000	>1,000	1,000	>1,000	>1,000	100	30	10	60
	Surfactants													
4581	Deriphat 170 B‡	2	>5,000	1,500	50	150	150	150	150	150	5	5	50	50
4582	Deriphat 170 C	1	1,500	500	50	150	150	50	100	150	1.5	1	1.5	3
4583	Deriphat 160 C	1	>3,000	900	300	900	300	90	300	300	>30	10	>30	>30
4584	Deriphat 151 C	1	>4,500	>4,500	45	100	140	15	140	140	30	15	15	15
4585	Deriphat 151	1	10,000	3,000	30	100	100	60	100	100	10	6	10	10
4586	Deriphat 160	1	10,000	3,000	300	10,000	300	100	300	300	100	30	30	>100
4587	Deriphat 170	1	10,000	300	30	100	100	30	100	100	10	3	10	30
4588	Deriphat 154	1	>10,000	10,000	100	>10,000	100	30	100	100	10	30	20	>100
4589	Deriphat 157	3	>10,000	10,000	100	100	300	30	300	300	1	10	10	>100
4590	Deriphat 150	1	3,000	300	100	300	100	30	100	30	10	3	30	30
4591	Sodium *N*-rosin β-amino propionate	1	>10,000	>10,000	30	100	100	30	100	100	30	10	30	100
4592	Sodium *N*-Primene JM-T β-amino propionate	3	9,000	>2,500	9	25	25	25	90	260	9	9	15	9
4593	Sodium *N*-Sunaptyl B- β-amino propionate	3	>3,000	>3,000	30	100	100	100	3,000	10,000	30	30	20	100
4594	Sodium *N*-alkane aspartate	1	10,000	3,000	3,000	600	10,000	3,000	10,000	6,000	>100	>100	>100	>100
4595	Disodium *N*-Sunaptyl B-β-imino	1	>10,000	>10,000	100	>10,000	>10,000	100	1,000	10,000	>100	60	100	>100
4596	Sodium *N*-dodecyl β-amino butyrate	1	3,000	300	300	300	300	30	300	300	30	3	10	30
4597	Sodium *N*-tetradecyl aspartate	1	10,000	10,000	1,000	>10,000	10,000	100	1,000	3,000	100	6	30	30
4598	Sodium *N*-dodecyl aspartate	1	10,000	3,000	300	>10,000	1,000	100	300	300	30	10	10	30
4599	Sodium *N*-hexadecyl aspartate	1	10,000	10,000	3,000	10,000	10,000	300	10,000	10,000	>100	>100	>100	>100
4600	Sodium α-sulfo stearate	3	>3,000	>3,000	200	2,000	3,000	60	300	3,000	>100	>100	>100	>100
Standard	Benzalkonium chloride	1	200	300	3	4	60	10	40	80	1	0.7	1	0.6

‡ Deriphat amphoteric surfactants include sodium salts and free acids of both *N*-fatty aminopropionates and *N*-fatty imiopropionates.

TABLE 3. *Compounds with good activity towards all or some of the test groups of organisms*

RC no.	Compound	Bacteria				Fungi				Algae			
		Gram-negative		Gram-positive						Green		Blue-green	
		Escherichia coli	*Pseudomonas fluorescens*	*Bacillus subtilis*	*Staphylococcus aureus*	*Aspergillus niger*	*Chaetomium globosum*	*Myrothecium verrucaria*	*Trichoderma viride*	*Chlorella vulgaris*	*Stigeoclonium* sp.	*Anabaena cylindrica*	*Oscillatoria tenuis*
	Compounds outstanding in activity towards all organisms tested												
4605	Dodecyl amine, Alamine 4	30	30	20	30	60	30	30	30	0.3	0.3	0.3	0.3
5644	Distilled dodecyl amine acetate, Alamac 4	30	100	10	30	20	10	60	30	0.6	0.6	0.3	0.3
	Compounds reasonably good in activity towards all organisms tested												
5626	Dioctyl dimethyl ammonium chloride	40	75	20	20	225	75	75	75	7	2	0.7	2
5632	Dioctyl amine	100	100	10	10	30	10	30	30	1	0.3	0.3	0.3
5635	*N*-dodecyl propylene diamine	30	30	10	100	60	60	100	100	3	0.3	0.2	0.06
5172	*N*-(dodecyl-tetradecyl) propylene diamine	100	30	30	100	100	100	100	100	0.3	0.1	1	0.3
5636	*N*-tetradecyl-propylene diamine	100	100	30	100	100	60	100	100	3	1	0.3	0.3
4618	*N*-coco-propylene diamine, Diam. 21	30	30	30	100	100	30	100	100	3	1	0.3	0.3
5179	$RNHCH_2CH(CH_3)CH_2NH_2$ R = dodecyl	100	100	30	100	100	60	100	100	1	0.3	0.3	0.1
5637	$RN(CH_2CH_2CH_2NH_2)_2$ R = dodecyl	30	100	10	100	100	100	100	300	3	1	0.3	0.3
5629	*N,N*-dimethyl dodecylamine	100	100	30	100	30	30	30	30	1	0.3	1	0.2
4613	*N,N*-dimethyl coco amine	100	60	30	300	100	30	30	100	1	0.3	1	0.3
4568	Distilled coco amine acetate, Alamac 21	100	100	30	100	100	60	30	100	1	0.3	0.3	1
4571	Distilled *N*-coco propylene diamine monoacetate	100	30	30	100	100	100	100	100	3	1	3	0.3
4572	Distilled *N*-coco propylene diamine diacetate	100	100	30	100	100	100	100	300	3	1	3	0.6
	Fungistatic compounds not active towards gram-negative bacteria												
5624	Coco benzyl dimethyl ammonium chloride	225	225	2	2	20	7	20	20	3	0.3	3	1
5627	Dodecyl dimethyl ammonium chloride	225	750	0.7	7	75	7	20	20	2	0.7	0.2	0.7
4538	Di(octyl-decyl) dimethyl ammonium chloride	50	500	5	5	50	50	50	50	0.5	0.5	0.5	0.5
5148	Tri isooctyl methyl ammonium chloride	500	150	3	3	150	50	50	50	0.5	1.5	0.3	1.5
4606	Di(octyl-decyl) amine	1,000	>1,000	30	30	30	10	100	100	3	1	0.3	0.3
4579	Coco morpholine acetate	1,000	1,000	300	300	100	6	60	30	3	3	3	3
	Compounds with good activity towards gram-negative bacteria, not falling in the groups mentioned before												
5634	*N*-decyl propylene diamine	30	30	10	100	300	100	300	100	3	0.3	0.3	0.3
5103	9(10)-β Aminoethylamino-10(9)-hydroxy stearonitrile	10	30	3	100	300	100	300	100	3	3	1	1
5120	9(10)-γ Aminopropylamino-10(9)-hydroxy stearyl amine	30	30	10	10	300	100	600	100	3	3	0.3	1

inant chain lengths in the compounds having the best biostatic activity. Except for the coco derivatives there was no substantial difference in the biostatic activity among the other fatty nitrogen compounds containing mixed carbon chain lengths derived from cotton, tallow, soya, and tall oil. The designations of coco, cotton, tallow, soya, and tall oil refer to the mixture of carbon atom chain lengths in the respective natural products—coconut oil, cottonseed oil, soybean oil, and tall oil.

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