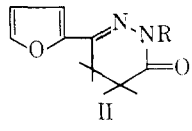
TABLE I



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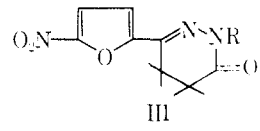

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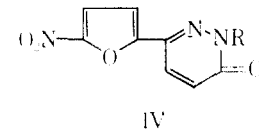

IV

No.	R	Mp, °C	Yield, %	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found
IIa	H	144–145[a]	78	$C_8H_8N_2O_2$	58.53	58.62	4.91	4.89	...	...
b	CH_3	90.5–91.5	79	$C_9H_{10}N_2O_2$	60.66	60.71	5.66	5.68	15.72	15.75
c	C_2H_5	65–66	97	$C_{10}H_{12}N_2O_2$	62.48	62.51	6.29	6.37	14.58	14.65
d	$(CH_2)_2CH_3$	73–74	72	$C_{11}H_{14}N_2O_2$	64.06	63.99	6.84	6.78	13.58	13.54
IIIa	H	249.5–250	42	$C_8H_7N_3O_4$	45.94	45.81	3.37	3.26	20.09	20.04
b	CH_3	198–199	42	$C_9H_9N_3O_4$	48.43	48.43	4.06	4.03	18.83	18.80
c	C_2H_5	134.5–135.5	20	$C_{10}H_{11}N_3O_4$	50.63	50.75	4.67	4.74	17.72	17.71
d	$(CH_2)_2CH_3$	101–102	31.5	$C_{11}H_{13}N_3O_4$	52.58	52.64	5.22	5.22	16.73	16.62
IVa	H	303–304	94	$C_8H_5N_3O_4$	46.38	46.43	2.43	2.65	20.29	20.16
b	CH_3	232	89[b]	$C_9H_7N_3O_4$	48.87	49.05	3.19	3.21	19.00	18.88
c	C_2H_5	149–149.5	39.5[c]	$C_{10}H_9N_3O_4$	51.06	51.06	3.86	3.86	17.87	17.88
d	$(CH_2)_2CH_3$	106.5–107.5	32.5[c]	$C_{11}H_{11}N_3O_4$	53.01	53.10	4.45	4.57	16.86	16.77

[a] Lit.[2] mp 145°. [b] By oxidation. [c] By alkylation.

TABLE II

BIOLOGICAL ACTIVITY OF 2-ALKYL-6-(5-NITRO-2-FURYL)-3(2H)-PYRIDAZINONE AND 4,5-DIHYDRO DERIVATIVES

No.	ED_{50} Orice, mg/kg/MIC (μg/ml) *Staphylococcus aureus*	*Salmonella typhosa*	Effective dose, % in feed *Eimeria tenella*
IIIa	50/6	100/3	0.0055
b	>200/25	>200/6	0.0055
c	100/12.5	>100/6.2	0.0055
d	>200/12.5	−/50	0.011
IVa	146/3	112/1.5	0.0055
b	59/25	87/1.5	0.0055
c	57/12	66/3	0.011
d	70/12	63/6	0.011
Nitrofurazone[a]	50/12.5	100/3	0.0055

[a] See ref 11.

orous stirring into 3 l. of cracked ice and water. The crude product was collected by filtration and washed thoroughly with water. Recrystallization from glacial acetic acid (charcoal) gave the product as short yellow needles. Other derivatives of III were prepared similarly.

6-(5-Nitro-2-furyl)-3(2H)-pyridazinone (IVa).—A mixture of 41.8 g (0.2 mole) of IIIa in 200 ml of glacial acetic acid was heated to 90°. Bromine (2 ml) was added with stirring. When HBr evolution began, the remaining bromine (total of 32 g, 0.2 mole) was added at such a rate as to maintain a temperature of 90–95°. When the addition was completed, the mixture was heated at 100° for 30 min. The mixture was cooled, diluted with water, and filtered, and the residue was washed thoroughly with water. The yield of crude product melting at 293–295° was 39.0 g (94%). Four recrystallizations from dimethylformamide (charcoal) gave pale yellow crystals. IVb was prepared similarly.

2-Ethyl-6-(5-nitro-2-furyl)-3(2H)-pyridazinone (IVc).—A mixture of 50.0 g (0.24 mole) of IVa and 13.0 g (0.24 mole) of sodium methoxide in 1000 ml of methanol was refluxed with stirring for 3 hr. Ethyl iodide (50 ml) was added and refluxing was continued overnight. The solvents were evaporated under diminished pressure on a steam bath and the residue was shaken with 500 ml of cold 5% NaOH solution. The crude product was filtered, washed thoroughly with water, and recrystallized from aqueous ethanol (charcoal). The product separated as pale yellow needles. IVb and IVd were prepared from the appropriate alkyl iodide.

Acknowledgments.—We wish to express our gratitude to W. O. Smith and B. F. Stevenson and group for technical assistance during the preparation of these compounds, to G. Gustin and M. Tefft for the elemental analyses, and to R. A. Dobson and A. Engle for the screening results.

The Bacteriostatic Effectiveness of 1-Alkyl-3-(3,4-dichlorophenyl)ureas

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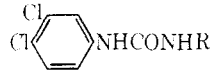
Because of their effectiveness against staphylococci and other gram-positive bacteria, substituted ureas have been extensively studied for their bacteriostatic activity. In a comprehensive investigation of such compounds, Beaver, *et al.*,[1] screened dozens of phenylureas, thioureas, and anilides against *Staphyloccus aureus* strain FDA No. 209. Two 1-alkyl-3-(3,4-dichlorophenyl)ureas were included, the 1-ethyl and the 1-*t*-octyl derivatives. The minimum concentra-



R = C_2H_5 or t-C_8H_{17}

tions of these compounds able to inhibit the test organism were found to be 100 μg/ml. On the other hand, two halogenated carbanilides, 3,3′,4-trichlorocarbanilide (I) and 3,4,4′-trichlorocarbanilide (II) were found to give complete inhibition at 0.033 μg/ml.

I, X = 3-Cl
II, X = 4-Cl

(1) D. J. Beaver, D. P. Roman, and P. J. Stoffel, *J. Am. Chem. Soc.*, **79**, 1236 (1957).

Recently, we had occasion to prepare **1**-(3,4-dichlorophenyl)-3-*n*-nonylurea. Although there was no reason to expect unusual bacteriostatic activity, this compound was routinely tested against *S. aureus* 209. Since it was found to be nearly as effective as I and II, a series of **1**-alkyl-3-(3,4-dichlorophenyl)ureas was prepared and the activity was determined against *S. aureus* 209.

Experimental Section

The 1-alkyl-3-(3,4-dichlorophenyl)ureas were obtained by the reaction of 3,4-dichlorophenyl isocyanate with the proper alkylamine by the method of Beaver, *et al.*[1] These compounds were white crystalline solids, soluble in alcohol, benzene, and dimethylformamide; slightly soluble in ether; and insoluble in water. Several alkylene-α,ω-bis(3,4-dichlorophenylureas) were prepared by the reaction of 2 equiv of 3,4-dichlorophenyl isocyanate with the appropriate diamine in dimethylformamide. The following preparation is representative.

1,5-Bis(3,4-dichlorophenylureido)pentane.—To a stirred solution of 10.2 g (0.1 mole) of cadaverine in 150 ml of dimethylformamide was carefully added a solution of 40.4 g (0.22 mole) of 3,4-dichlorophenyl isocyanate. A white precipitate began to to form, whereupon the reaction mixture was heated until a clear solution again resulted. After 15 min, water was added to precipitate the crude product. This material was filtered and washed with water; yield 38 g (74%). Recrystallization from dimethylformamide–water gave white crystals, mp 208–210°.

The compounds were tested by the agar streak dilution technique, in the presence of soap.[2] A 24-hr broth culture of *S. aureus* 209 was used as the test organism. The concentrations of soap used (100 times the concentration of the test compound) would not inhibit this organism under our test conditions.

Results and Discussion

The minimum concentrations (MIC) of these compounds able to inhibit the growth of *S. aureus* 209 are listed in Tables I and II. These results confirm the observations of Beaver, *et al.*,[1] that 1-(3,4-dichlorophenyl)-3-ethylurea (**1**) is relatively ineffective against this organism when compared with I or II. As the length of the alkyl chain is extended from ethyl to *n*-octyl, the bacteriostatic activity increases regularly. **1**-(3,4-Dichlorophenyl)-3-*n*-octylurea (**10**) is as effective as I or II. With further increase in chain length, however, the activity decreases. The *n*-undecyl derivative (**15**) is no more effective than the *n*-propyl compound (**2**). The tridecyl (**17**), tetradecyl (**18**), and octadecyl (**19**) derivatives showed no inhibition at the highest concentrations tested.

Such a relationship of bacteriostatic activity to the size of an alkyl substituent is not an isolated phenomenon. A similar effect has been observed for the activity of *n*-alkylbenzoylacrylic acids against staphylococci,[3a] where the most effective compound is the nonyl, and for *n*-alkylphenols against *Salmonella typhosa*,[3b] where the optimum chain length is amyl.

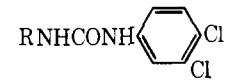
However, there is no certain explanation why the *n*-octyl compound should be more effective against the test organism than compounds with longer or smaller alkyl chains. A second finding of interest is the fact that the minimum inhibitory concentrations of **3**, **4**, **5**,

(2) D. R. Noel, R. E. Casely, W. M. Linfield, and L. A. Harriman, *Appl. Microbiol.*, **8**, 1 (1960). A commercial toilet soap consisting of the sodium salts of mixed tallow and coconut oil fatty acids (80% tallow) was used (Ivory®).

(3) (a) F. Kirchner, J. Bailey, and C. Cavilito, *J. Am. Chem. Soc.*, **71**, 1210 (1949); (b) E. G. Klarman and E. S. Wright in "Antiseptics, Disinfectants, Fungicides, and Chemical and Physical Sterilization," G. F. Reddish Ed., Lea and Febiger, Philadelphia, Pa., 1954, p 429.

TABLE I

1-ALKYL-3-(3,4-DICHLOROPHENYL)UREAS

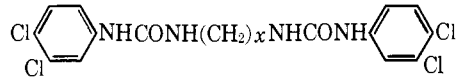


No.	R	Mp, °C[a]	Chlorine, % Calcd	Chlorine, % Found[b]	MIC, μg/ml[c]
1	Ethyl	179–180[d]	30.4	30.6	20
2	*n*-Propyl	129–131[e]	28.7	28.5	10
3	*n*-Butyl	121–123[e]	27.2	27.3	5
4	*sec*-Butyl	132–134	27.2	27.4	5
5	Isobutyl	138–139[f]	27.2	27.0	5
6	*t*-Butyl	194–195	27.2	27.5	5
7	*n*-Pentyl	117–118	25.9	26.0	1.0
8	*n*-Hexyl	104–106[e]	24.6	24.4	0.5
9	*n*-Heptyl	97–98	23.4	23.4	0.3
10	*n*-Octyl	80–82[g]	22.4	22.5	0.2[h]
11	1,1,3,3-Tetramethylbutyl	145–147	22.4	22.4	0.4[h]
12	*n*-Nonyl	87–88	21.5	21.7	0.4
13	*n*-Decyl	89–90	20.6	20.8	1.0
14	9-*n*-Decenyl	82–83	20.6	21.0	1.0
15	*n*-Undecyl	94–96	19.8	19.5	10.0
16	*n*-Dodecyl	94–95	19.0	18.8	15
17	*n*-Tridecyl	87–88	18.3	18.5	>20
18	*n*-Tetradecyl	96–97	17.7	18.0	>20
19	*n*-Octadecyl	77–79	16.0	16.3	>20

[a] All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. [b] Chlorine analyses were by the Schoniger combustion technique. [c] Minimum inhibitory concentration against *Staphylococcus aureus* 209. [d] Lit.[1] mp 180°. [e] N. E. Good [*Plant Physiol.*, **36**, 788 (1961)] reports mp 128–129°, 121–122°, and 104–105° for **2**, **3**, and **8**, respectively. [f] A second crystalline modification, melting 122–124°, was observed. On melting and refreezing, it reverted to the 138–139° form. [g] A second crystalline modification, melting 100–101°, was observed. Its analyses, infrared spectrum, and MIC against *S. aureus* 209 were identical with those of the 80–82° form. [h] Compounds **10** and **11** were also tested in the absence of soap. The MIC under these conditions were 0.3 and 0.4 μg/ml, respectively.

TABLE II

ALKYLENE-α,ω-BIS(3,4-DICHLOROPHENYLUREAS)



No.	*x*	Mp, °C[a]	Chlorine, % Calcd	Chlorine, % Found[b]	MIC,[c,d] μg/ml
20	0	227–228	34.8	34.6	>5
21	2	255–256	32.5	32.8	>5
22	3	227–228	31.5	31.8	>5
23	4	259–260	30.6	30.3	>5
24	5	208–210	29.7	29.8	>5
25	6	228–229	28.8	28.4	>5
26	8	213–216	27.3	27.0	>5

No.	Compd	MIC,[c] μg/ml
27	1-(4-Chlorophenyl)-3-*n*-octylurea[e]	>20
I	3,3′,4-Trichlorocarbanilide	0.2[f]
II	3,4,4′-Trichlorocarbanilide	0.2[f]
	3,4-Dichloroaniline	>5[d]

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] See footnote *c*, Table I. [d] Highest concentration tested. [e] Mp 131–132°. *Anal.* Calcd for $C_{15}H_{22}Cl_2N_2O$: Cl, 12.5. Found: Cl, 12.3. [f] These compounds were obtained from the Monsanto Chemical Co. These values for the minimum inhibitory concentrations are higher than those reported by Beaver, *et al.*,[1] for the same compounds (0.033 μg/ml). Slight variations in test method or conditions may account for the discrepancy.

and **6** are the same(5 μg/ml), although the chemical and physical properties of the *n*-butyl, isobutyl, *sec*-butyl, and *t*-butyl compounds vary significantly. Likewise, unsaturation in the alkyl chain does not seem to influence the bacteriostatic activity. The MIC of the 9-*n*-decenyl compound (**14**) is the same as that of the *n*-decyl derivative (**13**). However, slight changes in the anilide portion of the molecule cause considerable variation in activity. For example, 1-(3,4-dichlorophenyl)-3-*n*-octylurea (**10**) inhibited the test organism at 0.2 μg/ml, whereas 1-(4-chlorophenyl)-3-*n*-octylurea (**27**) did not inhibit the organism at 20 μg/ml.

Since the bisureas (**20–26**) were not effective at the highest concentration tested (5 μg/ml), no relationship of chain length to activity can be postulated. It is interesting to note that 3,4-dichloroaniline, an expected hydrolysis product from all these compounds, is not as active under our test conditions as most of the ureas.

These compounds were also tested against *Escherichia coli* ATCC 11229. They were found to be inactive at 50 μg/ml.

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Halonitroanilides and Their Bacteriostatic Activity

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The antibacterial activity of a variety of halo- and nitrosalicylanilides is well documented.[1] Many of these compounds possess good bacteriostatic activity and also are substantive to skin and cloth. We wish to report the preparation and bacteriostatic activity of additional anilides in which the *o*-hydroxyphenyl moiety has been changed to alkyl, haloalkyl, alkenyl, cycloalkyl, benzyl phenethyl, phenoxymethyl, and phenyl groups. The N-aryl portions of the compounds possess both nitro and halo substituents.

The methods of preparation depended upon the anilide or starting materials and were generally modifications of known procedures. For the acid anhydride reactions a trace of sulfuric acid was added to catalyze the condensations with the aniline. When an acid chloride was employed, it was allowed to react in a solvent with the aniline either with or without triethylamine as the hydrogen chloride acceptor. It was necessary to reflux the reaction mixture several hours to remove the HCl when no acceptor was used. The N-methylanilide (**15**) was prepared by the action of dimethyl sulfate on the sodium salt of **14**.

(1) H. Lamaire, C. H. Schramm, and A. Cohn, *J. Pharm. Sci.*, **50**, 831 (1961), and references cited therein.

The infrared spectra of the anilides were examined and characteristic absorptions[2] were observed with the NH stretching band occurring at 3210–3310 cm^{-1} and the amide I and II bands at 1670–1690 and at 1570–1600 cm^{-1}, respectively. The amide II band was obscured somewhat by other absorptions. Amide bands for those derivatives (*e.g.*, **45** and **52**) with *ortho* substituents were shifted to the lower limits of the ranges. The characteristic NH stretching band was absent for **15**, the N-methylanilide.

Bacteriostatic activity of the halonitroanilides against *Staphylococcus aureus* was determined *in vitro*. The scope of activity is limited to those compounds which are substituted in the 3, 4, and 5 positions of the N-phenyl ring with a nitro and one or two halogen groups and in which the acid-derived moiety incorporates alkyl, haloalkyl, alkenyl, or cycloalkyl groups and contains from 5 to 13 carbon atoms. The phenyl, benzyl, phenethyl, and phenoxymethyl derivatives were inactive. Substitution of an alkyl group on the nitrogen forming the N-methylanilide (**15**) destroys the bacteriostatic activity. Those anilides containing a tertiary α-carbon exhibited a lower order of activity.

The requirements for optimum activity attributed to ring substitution parallels, in general, the findings of Beaver, *et al.*,[3] for series of substituted carbanilates and carbanilides in which substitution in the 3 and 4 positions gave maximum activity. Substituents in the *ortho* position reduced drastically or completely suppressed activity. The correlation of activity with the salicylanilides[1,4] is not readily apparent. In the latter case, an *ortho* substituent is allowable for 2′,4′-substituted derivatives but not for those containing groups in the 2′,5′ positions.

Experimental Section

Chemical Procedures.—Most of the acid anhydrides, acid chlorides, and substituted anilines were obtained commercially. 3-Chloro-4-nitroaniline,[5] 3-bromo-4-nitroaniline,[6] 3-chloro-5-nitroaniline,[7] 3,5-dichloro-4-nitroaniline,[8] α-bromononanoyl chloride,[9] 2,4-dichlorophenoxyacetyl chloride,[10] and 2,4,5-trichlorophenoxyacetyl chloride[10] were prepared in a manner similar to those reported in the literature.

α-Chlorononanoyl chloride was prepared from the corresponding acid[11] and $SOCl_2$ by a procedure employed for the preparation of similar acid chlorides;[12] bp 91–95° (2 mm), yield 64%. This intermediate was characterized by conversion to **23**.

Anilides. Method 1.—A mixture of 0.10 mole of required aniline, 15–18 ml of the acid anhydride, and a drop of concentrated H_2SO_4 was heated at reflux for 1–2 hr and poured into cold water. The crude product was collected, washed with water, dried, and recrystallized.

Method 2.—The acid chloride (0.055 mole) was added dropwise to a stirred and refluxed solution of the aniline (0.050 mole) in 150–200 ml of toluene or methylcyclohexane or a mixture of these solvents. The mixture was refluxed until HCl evolution ceased.

(2) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, pp 203–223.

(3) D. J. Beaver, D. P. Roman, and P. J. Steffel, *J. Med. Chem.*, **6**, 501 (1963); *J. Org. Chem.*, **24**, 1676 (1959); *J. Am. Chem. Soc.*, **79**, 1236 (1957).

(4) R. E. Stenseth, J. W. Baker, and D. P. Roman, *J. Med. Chem.*, **6**, 212 (1963).

(5) H. H. Hodgson and A. Kersbaw, *J. Chem. Soc.*, 2917 (1929).

(6) A. Claus and W. Scheulen, *J. Prakt. Chem.*, **43**, 200 (1891).

(7) J. B. Cohen and D. McCandlish, *J. Chem. Soc.*, 1257 (1905).

(8) F. Beilstein and A. Kurbatow, *Ann.*, **196**, 214 (1879).

(9) A. Hopwood and C. Werzmann, *J. Chem. Soc.*, 1577 (1911).

(10) J. W. Wood and T. D. Fontaine, *J. Org. Chem.*, **17**, 891 (1952).

(11) H. H. Guest, *J. Am. Chem. Soc.*, **69**, 300 (1947).

(12) A. W. Ralston, E. W. Segerbrecht, and S. T. Bauer, *J. Org. Chem.*, **4**, 502 (1939).