Antibacterially Active Substituted Anilides of Carboxylic and Sulfonic Acids[1]

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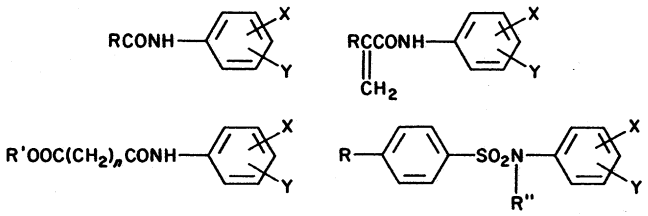
Anilides of carboxylic and sulfonic acids were prepared and tested for antimicrobial activity. While these anilides were ineffective against Gram-negative organisms, there was a good correlation between chemical structure and biological activity against Gram-positive species. Both the nature and position of the benzene ring substituents and the length of the carbon side chain affected the activity and specificity of the compounds. The highest activity was observed when the acyl or sulfuryl moiety had a C_7–C_9 side chain attached. The CONH and SO_2NH bridging groups were equally effective. The attachment of COOH or $COOCH_3$ groups in the ω-position did not effect activity, but the substitution of the acidic proton of the sulfonamide group by an alkyl group rendered the compound inactive. Six compounds, which were substituted anilides of sulfonic acids, fatty acids, or the analagous α-methylene-substituted acids, were bacteriostatic at 10 ppm against *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Lactobacillus plantarum*. One of these compounds, 2-hydroxy-5-nitroanilide of α-methylenedecanoic acid, was bactericidal at 1 ppm.

The need for effective antibacterial agents, nontoxic to mammals and useful in the disinfection of skin and hard surfaces, has posed a continuing research problem, particularly in view of the banning of hexachlorophene and tribromosalicylanilide. This study was carried out to gain greater insight into the chemical structure–biological activity relationship of substituted aniline derivatives. Comprehensive studies by Beaver et al.[3,4] on diarylureas pinpointed the high antibacterial properties of 3,4,4′- and 3,3′,4-trichlorocarbanilides, while Hoffman et al.[5] investigated *N*-hydroxycarbanilides. Substituted alkylureas also possess bacteriostatic properties,[6] as do some substituted acylureas.[7] The antibacterial activity of anilides of carboxylic and sulfonic acids have received little attention. Baker et al.[8] found some nitrohaloanilides to be bacteriostatic, and Chase and Weller[9] described several active carbanilides and sulfonanilides not covered by previous investigators. A recent study from our laboratory reported the correlation between chemical structure and bacteriostatic properties of substituted anilides of fatty acids. In spite of some inconsistencies, all of the above-mentioned studies reveal parallels in the structure–activity correlations of these compounds. This study extends the work of Bistline et al.[10] in an effort to broaden our knowledge of biologically active anilides. The following structural types were examined:



X and Y are substituents such as Cl, NO_2, or OH; R is an alkyl, alkylaryl, or arylalkyl group; R′ is H or CH_3; R″ is H, CH_3, or p-$ClC_6H_4CH_2$; and n is an integer from 2 to 8.

While the synthesis of the above compounds is not new, their bacteriostatic properties have not been reported. This study focuses on the effect of side-chain length and structure, unsaturation of the side chain, introduction of a carboxyl or carboxymethyl group into the side chain, the nature of the bridging group CONH vs. SO_2NH, and the nature and position of substituents on the aromatic ring upon antibacterial activity.

Results and Discussion

The initial screening of the compounds showed that while some were active against *S. aureus*, all were ineffective against Gram-negative organisms. Considering the highly lipophilic nature of the compounds, the lack of activity against Gram-negative bacteria is not surprising. The structure of the Gram-negative cell wall is complex, as well as higher in lipids, than the simpler Gram-positive cell wall. Compounds that are most effective against Gram-negative bacteria are considerably less lipophilic

(1) Presented in part at the 182nd National Meeting of the American Chemical Society, Aug. 1981, New York. See "Abstracts of Papers"; American Chemical Society: Washington, DC, 1981.
(2) Agricultural Research Service, U.S. Department of Agriculture.
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(5) Hoffman, P. P.; Madison, R. K.; Hardy, W. B. *J. Med. Chem.* **1964**, *7*, 665.
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(9) Chase, B. H.; Weller, W. T. *J. Pharm. Pharmacol.* **1964**, *16*, 163.
(10) Bistline, R. G., Jr.; Maurer, E. M.; Smith, F. D.; Linfield, W. M. *J. Am. Oil Chem. Soc.* **1980**, *57*, 98.

Table I. Substituted Anilides of Fatty Acids and Analogous α-Alkylacrylic Acids

	$RCH_2CONHAr$					$RC(=CH_2)CONHAr$			
no.	R	Ar	mp, °C	*S. aureus* MIC, ppm	anal.	no.	mp, °C	*S. aureus* MIC, ppm	anal.
1	C_4H_9	$3,4\text{-}Cl_2C_6H_3$	74–75[a]	10	Cl	31	50–51[c]	10	Cl
2	C_4H_9	$3,5\text{-}Cl_2C_6H_3$	66–67[b]	10	Cl	32	oil[h]	10	Cl
3	C_4H_9	$2\text{-OH-5-}ClC_6H_3$	97–98[c]	10	Cl	33	114–115[b]	100	Cl
4	C_4H_9	$2\text{-OH-5-}NO_2C_6H_3$	136–137[c]	10	N				
5	C_4H_9	$3\text{-}NO_2\text{-4-}ClC_6H_3$	51–52[d]	10	Cl				
6	C_5H_{11}	$3,4\text{-}Cl_2C_6H_3$	58–59[d]	10	Cl	34	42–43[i]	10	Cl
7	C_5H_{11}	$3,5\text{-}Cl_2C_6H_3$	72–73[e]	1	Cl	35	oil[h]	1	Cl
8	C_5H_{11}	$2\text{-OH-5-}ClC_6H_3$	95–96[f]	10	Cl	36	108–109[b]	100	Cl
9	C_5H_{11}	$2\text{-OH-5-}NO_2C_6H_3$	171–172[b]	10	N	37	169–171[b]	10	N
10	C_5H_{11}	$3\text{-}NO_2\text{4-}ClC_6H_3$	38–39[d]	10	Cl	38	43–44[i]	10	Cl
11	C_6H_{13}	$3,4\text{-}Cl_2C_6H_3$	39–40[f]	1	Cl	39	53–54[i]	1	Cl
12	C_6H_{13}	$3,5\text{-}Cl_2C_6H_3$	55–56[f]	1	Cl	40	37–39[i]	1	Cl
13	C_6H_{13}	$2\text{-OH-5-}ClC_6H_3$	94–95[f]	10	Cl	41	92–93[f]	1	Cl
14	C_6H_{13}	$2\text{-OH-5-}NO_2C_6H_3$	127–128[f]	1	N	42	173–174[b]	1	N
15	C_6H_{13}	$3\text{-}NO_2\text{-4-}ClC_6H_3$	51–52[d]	1	Cl	43	42–43[f]	1	Cl
16	C_7H_{15}	$3,4\text{-}Cl_2C_6H_3$	69–70[e]	1	Cl	44	43–45[f]	1	Cl
17	C_7H_{15}	$3,5\text{-}Cl_2C_6H_3$	69–70[e]	1	Cl	45	52–54[j]	10	Cl
18	C_7H_{15}	$2\text{-OH-5-}ClC_6H_3$	93–94[f]	1	Cl	46	101–102[b]	1	Cl
19	C_7H_{15}	$2\text{-OH-5-}NO_2C_6H_3$	140–141[f]	10	N	47	160–161[f]	1	N
20	C_7H_{15}	$3\text{-}NO_2\text{-4-}ClC_6H_3$	44–45[d]	1	Cl	48	49–50[f]	1	N
21	C_8H_{17}	$3,4\text{-}Cl_2C_6H_3$	64–65[g]	10	Cl	49	46–47[f]	1	N
22	C_8H_{17}	$3,5\text{-}Cl_2C_6H_3$	65–66[g]	10	Cl	50	oil[h]	10	N
23	C_8H_{17}	$2\text{-OH-5-}ClC_6H_3$	90–91[f]	1	Cl	51	84–85[f]	1	Cl
24	C_8H_{17}	$2\text{-OH-5-}NO_2C_6H_3$	126–127[j]	1	N	52	163–164[f]	1	N
25	C_8H_{17}	$3\text{-}NO_2\text{-4-}ClC_6H_3$	57–58[b]	10	Cl	53	37–38[f]	1	Cl
26	$C_{10}H_{21}$	$3,4\text{-}Cl_2C_6H_3$	78–79[k]	100	Cl	54	44–45[f]	1000	Cl
27	$C_{10}H_{21}$	$3,5\text{-}Cl_2C_6H_3$	58–59[b]	10	Cl	55	44–45[f]	>1000	Cl
28	$C_{10}H_{21}$	$2\text{-OH-5-}ClC_6H_3$	73–74[f]	1	Cl	56	79–80[f]	1	Cl
29	$C_{10}H_{21}$	$2\text{-OH-5-}NO_2C_6H_3$	110–111[e]	1000	N				
30	$C_{10}H_{21}$	$3\text{-}NO_2\text{-4-}ClC_6H_3$	66–67[f]	>1000	N				

Recrystallization solvents were: [a] Hexane. [b] Absolute EtOH. [c] $Cl_2CH_2CH_2Cl$. [d] EtOH + H_2O. [e] 95% EtOH. [f] ClC_2H_4Cl. [g] Pyridine + EtOH. [h] Not applicable. [i] C_6H_{14} + EtOH. [j] ClC_2H_4Cl + C_6H_{14}. [k] C_6H_{14}. [l] ClC_2H_4Cl + EtOH.

Table II. Substituted Anilides of Aromatic Carboxylic Acids

	RCONHAr				
no.	R	Ar	mp, °C	*S. aureus* MIC, ppm	anal.
57	$C_6H_5CH_2$	$3,4\text{-}Cl_2C_6H_3$	129–130[f]	>1000	Cl
58	$C_6H_5CH_2$	$2\text{-OH-5-}ClC_6H_3$	164–166[b]	100	Cl
59	$C_6H_5CH_2CH_2$	$3,4\text{-}Cl_2C_6H_3$	120–121[f]	1	Cl
60	$C_6H_5CH_2CH_2$	$2\text{-OH-5-}ClC_6H_3$	156–158[f]	100	Cl
61	$CH_3CH(C_6H_5)CH_2$	$3,4\text{-}Cl_2C_6H_3$	107–108[e]	10	Cl
62	$C_2H_5CH(C_6H_5)$	$3,4\text{-}Cl_2C_6H_3$	81–83[d]	10	Cl
63	$C_2H_5CH(C_6H_5)$	$2\text{-OH-5-}ClC_6H_3$	142–143[f]	10	Cl
64	$3,4\text{-}Cl_2C_6H_3$	$3,4\text{-}Cl_2C_6H_3$	225–227[g]	>1000	Cl
65	$3,4\text{-}Cl_2C_6H_3$	$2\text{-OH-5-}ClC_6H_3$	232–233[b]	>1000	Cl
66	$3,4\text{-}Cl_2C_6H_3$	$2\text{-OH-5-}NO_2C_6H_3$	>250[b]	10	Cl
67	$4\text{-}CH_3C_6H_4$	$3,4\text{-}Cl_2C_6H_3$	168–169[b]	1000	Cl
68	$4\text{-}C_2H_5C_6H_4$	$3,4\text{-}Cl_2C_6H_3$	125–126[b]	>1000	Cl
69	$4\text{-}(n\text{-}C_3H_7)C_6H_4$	$3,4\text{-}Cl_2C_6H_3$	131–132[b]	>1000	Cl
70	$4\text{-}(n\text{-}C_3H_7)C_6H_4$	$2\text{-OH-5-}ClC_6H_3$	171–173[b]	>1000	Cl
71	$4\text{-}(n\text{-}C_3H_7)C_6H_4$	$2\text{-OH-5-}NO_2C_6H_3$	214–216[b]	10	N
72	$4\text{-}(n\text{-}C_4H_9)C_6H_4$	$3,4\text{-}Cl_2C_6H_3$	127–128[b]	1000	Cl
73	$4\text{-}(n\text{-}C_4H_9)C_6H_4$	$2\text{-OH-5-}ClC_6H_3$	177–178[b]	1000	Cl
74	$4\text{-}(n\text{-}C_4H_9)C_6H_4$	$2\text{-OH-5-}NO_2C_6H_3$	208–210[b]	1	N
75	$4\text{-}(n\text{-}C_6H_{13})C_6H_4$	$3,4\text{-}Cl_2C_6H_3$	111–112[f]	>1000	Cl

Recrystallization solvents were: [a] Hexane. [b] Absolute EtOH. [c] $Cl_2CH_2CH_2Cl$. [d] EtOH + H_2O. [e] 95% EtOH. [f] ClC_2H_4Cl. [g] Pyridine + EtOH.

than compounds effective against the Gram-positive bacteria.[11]

Because the compounds were ineffective against Gram-negative bacteria, only data for *S. aureus* are presented and discussed in Tables I–IV. The minimum inhibitory concentration (MIC) data represent a range that establishes activity to an order or magnitude. We took MIC values of 1 and 10 to indicate high activity, and in our correlations did not make a great distinction between these values.

Some data for fatty acid anilides from our previous study[10] are repeated here for comparison with the corresponding α-alkylacrylanilides (Table I). Table II gives data for substituted anilides of arylalkanoic and *p*-alkylbenzoic acids. Data for anilides of dicarboxylic acids are shown in Table III and those for sulfonanilides in Table IV.

(11) Franklin, T. J.; Snow, G. A. "Biochemistry of Antimicrobial Action"; Chapman and Hall: New York, 1981; pp 141, 160, and 165.

Table III. Substituted Anilides of Dicarboxylic Acids

			$ROOC(CH_2)_nCONHAr$			
no.	R	n	Ar	mp, °C	*S. aureus* MIC, ppm	anal.
76	H	2	$3,4\text{-}Cl_2C_6H_3$	158–159[d]	1000	Cl
77	CH_3		$3,4\text{-}Cl_2C_6H_3$	119–120[d]	1000	Cl
78	H		$2,6\text{-}Cl_2C_6H_3$	211–212[d]	>1000	Cl
79	CH_3		$2,6\text{-}Cl_2C_6H_3$	158–159[d]	1000	Cl
80	H	4	$3,4\text{-}Cl_2C_6H_3$	127–129[l]	1000	Cl
81	CH_3		$3,4\text{-}Cl_2C_6H_3$	125–127[l]	1000	Cl
82	H		$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	143–145[f]	100	Cl
83	CH_3		$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	112–113[f]	100	Cl
84	H	6	$3,4\text{-}Cl_2C_6H_3$	131–132[f]	100	Cl
85	CH_3		$3,4\text{-}Cl_2C_6H_3$	113–114[f]	1000	Cl
86	H		$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	109–110[f]	100	Cl
87	CH_3		$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	98–99[f]	1000	Cl
88	H	7	$3,4\text{-}Cl_2C_6H_3$	103–104[f]	1	Cl
89	CH_3		$3,4\text{-}Cl_2C_6H_3$	63–64[i]	1	Cl
90	H		$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	114–115[f]	100	Cl
91	CH_3		$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	66–67[i]	1000	Cl
92	H	8	$3,4\text{-}Cl_2C_6H_3$	147–149[f]	>1000	Cl
93	CH_8		$3,4\text{-}Cl_2C_6H_3$	101–103[f]	>1000	Cl
94	H		$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	111–112[f]	>1000	Cl
95	CH_3		$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	100–102[f]	>1000	Cl

Recrystallization solvents were: [a] Hexane. [b] Absolute EtOH. [c] $Cl_2CH_2CH_2Cl$. [d] EtOH + H_2O. [e] 95% EtOH. [f] ClC_2H_4Cl. [g] Pyridine + EtOH. [h] Not applicable. [i] C_6H_{14} + EtOH. [j] ClC_2H_4Cl + C_6H_{14}. [k] C_6H_{14}. [l] ClC_2H_4Cl + EtOH.

Table IV. Substituted Sulfonanilides

			$Ar^1SO_2NRAr^2$			
no.	Ar^1	R	Ar^2	mp, °C	*S. aureus* MIC, ppm	anal.
96	$3,4\text{-}Cl_2C_6H_3$	H	$3,4\text{-}Cl_2C_6H_3$	140–142[d]	1	Cl
97	$3,4\text{-}Cl_2C_6H_3$	H	$2\text{-}OH\text{-}5\text{-}ClC_6H_3$	170–171[d]	10	Cl
98	$3,4\text{-}Cl_2C_6H_3$	H	$2\text{-}OH\text{-}5\text{-}NO_2C_6H_3$	197–198[f]	10	Cl
99	$3,4\text{-}Cl_2C_6H_3$	H	$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	137–138[e]	1	Cl
100	$2,5\text{-}Cl_2C_6H_3$	H	$3,4\text{-}Cl_2C_6H_3$	132–133[k]	1000	Cl
101	$4\text{-}CH_3C_6H_4$	H	$3,4\text{-}Cl_2C_6H_3$	139–141[f]	10	Cl
102	$4\text{-}C_2H_5C_6H_4$	H	$3,4\text{-}Cl_2C_6H_3$	125–127[d]	10	Cl
103	$4\text{-}(n\text{-}C_3H_7)C_6H_4$	H	$3,4\text{-}Cl_2C_6H_3$	96–98[d]	1	Cl
104	$4\text{-}(n\text{-}C_3H_7)C_6H_4$	CH_3	$3,4\text{-}Cl_2C_6H_3$	112–113[b]	>1000	Cl
105	$4\text{-}(n\text{-}C_3H_7)C_6H_4$	$4\text{-}ClC_6H_4CH_2$	$3,4\text{-}Cl_2C_6H_3$	139–141[b]	>1000	Cl
106	$4\text{-}(n\text{-}C_3H_7)C_6H_4$	H	$2\text{-}OH\text{-}5\text{-}ClC_6H_3$	103–105[f]	1	Cl
107	$4\text{-}(n\text{-}C_3H_7)C_6H_4$	H	$2\text{-}OH\text{-}5\text{-}NO_2C_6H_3$	117–118[f]	1	Cl
108	$4\text{-}(n\text{-}C_3H_7)C_6H_4$	H	$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	93–95[d]	10	Cl
109	$4\text{-}(CH_3)_2CHC_6H_4$	H	$3,4\text{-}Cl_2C_6H_3$	118–120[d]	10	Cl
110	$4\text{-}(CH_3)_2CHC_6H_4$	H	$2\text{-}OH\text{-}5\text{-}ClC_6H_3$	136–138[f]	10	Cl
111	$4\text{-}(n\text{-}C_4H_9)C_6H_4$	H	$3,4\text{-}Cl_2C_6H_3$	105–106[d]	10	Cl
112	$4\text{-}(n\text{-}C_4H_9)C_6H_4$	H	$2\text{-}OH\text{-}5\text{-}ClC_6H_3$	88–89[k]	10	Cl
113	$4\text{-}(n\text{-}C_4H_9)C_6H_4$	H	$2\text{-}OH\text{-}5\text{-}NO_2C_6H_3$	118–119[j]	10	N
114	$4\text{-}(n\text{-}C_4H_9)C_6H_4$	H	$3\text{-}NO_2\text{-}4\text{-}ClC_6H_3$	99–101[d]	1	Cl
115	$4\text{-}(s\text{-}C_4H_9)C_6H_4$	H	$3,4\text{-}Cl_2C_6H_3$	91–92[d]	10	N
116	$4\text{-}(t\text{-}C_4H_9)C_6H_4$	H	$3,4\text{-}Cl_2C_6H_3$	61–62[k]	>1000	Cl

Recrystallization solvents were: [a] Hexane. [b] Absolute EtOH. [c] $Cl_2CH_2CH_2Cl$. [d] EtOH + H_2O. [e] 95% EtOH. [f] ClC_2H_4Cl. [g] Pyridine + EtOH. [h] Not applicable. [i] C_6H_{14} + EtOH. [j] ClC_2H_4Cl + C_6H_{14}. [k] C_6H_{14}.

The nature and position of substituents on the aromatic ring were key elements in the structure–activity relationship. Since this aspect of correlation was thoroughly covered in our previous study,[10] it suffices to state here that substitution on the aniline ring by OH, Cl, or NO_2 in the appropriate positions enhanced activity. Florestano and Bahler[12] have demonstrated the importance of the nature and position of ring substituents in diphenylmethanes, suggesting that the occurrence of this structure–activity relationship may be widespread among general classes of compounds. The amides of 3,4-dichloroaniline, 2-hydroxy-5-chloroaniline, 2-hydroxy-5-nitroaniline, and 3-nitro-4-chloroaniline were highest in activity. The activity of the amides of a given fatty acid was usually similar for these four aniline derivatives. Amides of fatty and α-alkylacrylic acids of recently commercially available 3,5-dichloroaniline were synthesized and evaluated as shown in Table I. The 3,5-dichloroanilides showed a slight edge over the 3,4-dichloroanilides (**6** vs. **7**, and **26** vs. **27**).

Jerchel[13] noted that unsaturation of the acyl moiety enhanced antimicrobial activity. We, however, determined that the substituted anilides of α-alkylacrylic acids[14] had activity similar to the corresponding saturated fatty acids (Table I).

The pronounced effect of side-chain length on activity is evident in all four tables. Thus, the anilides of RCH_2COOH and $RCH(=CH_2)COOH$ (Table I) had the highest activity when R was six or seven carbon atoms long.

(12) Florestano, H. J.; Bahler, M. E. *J. Am. Pharm. Assoc.* **1953**, *42*, 576.

(13) Jerchel, D. (to C. H. Boehringer Sohn), U.S. Patent 2978465, 1961.

(14) Serota, S.; Simon, J. R.; Murray, E. B.; Linfield, W. M. *J. Org. Chem.* **1981**, *46*, 4147.

A chain length longer or shorter resulted in reduced activity. Replacement of part of the alkyl chain by a phenyl group (**57–63**), which is equivalent to about four carbon atoms, resulted in highly active compounds (Table II) when a total equivalent side-chain length of six or seven carbon atoms was reached. Replacement of a fatty acyl group by an alkylbenzoyl group generally resulted in low activity. Only the 2-hydroxy-5-nitroanilides of *n*-propyl- and *n*-butylbenzoic acids (**71** and **74**) had high activity. If an equivalent side-chain length of four carbons is also assumed for the benzene ring of the alkylbenzoic acids, the side chain optimum for activity would be seven and eight carbon atoms, which is in good agreement with our findings for fatty acid and acrylic acid anilides. Similar chain-length effects exist for esters of gallic[15] and hydroxybenzoic[16] acids, N-substituted aminodiamides,[17] and aliphatic straight-chain alcohols.[18]

The compounds in Table II also demonstrate the importance of the nature and position of ring substituents. The 3,4-dichloroanilides did not have the same activity as the corresponding 2-hydroxy-5-chloroanilides or the 2-hydroxy-5-nitroanilides. The 2-hydroxy-5-nitroanilides of the alkylbenzoic acids showed substantially higher activity. The 2-hydroxy-5-nitroanilide of 3,4-dichlorobenzoic acid showed an MIC of 10, whereas the other two anilides of this acid were inactive. While there is no ready explanation for this phenomenon, it may be related to the ability of the compounds to enter the cell or their ability to react at the (unknown) target site(s).

The results from anilides of dicarboxylic acids and their methyl esters are shown in Table III. The 3,4-dichloroanilide of azelaic acid (**88**) and its methyl ester (**89**) were the only highly active compounds of this series. The chain length (including the COOH group) of eight carbon atoms is in agreement with the previously discussed correlation between chain length and biological activity. As the chain length was increased or decreased by one carbon atom, activity dropped rapidly. There was a substituent effect in this series too, insofar as 3,4-dichloroanilide was far more active than 3-nitro-4-chloroanilide. Esterification with methanol often decreased the activity of the anilides. The presence of an acidic proton apparently enhanced activity somewhat. This was also observed for some of the Gram-negative organisms and was noticeable for anilides of maleic acid. These data are not reported, since the maleamic acid derivatives were no more active than the succinamic derivatives.

The structure–activity relationships of sulfonanilides (Table IV) paralleled that of fatty acid anilides, indicating that the SO_2NH bridging group and the CONH bridge had equal biological activity. As with the fatty acid anilides, an MIC minimum was observed at a critical side-chain length. Thus, the highest activity among the 3,4-dichlorosulfonanilides was reached when the benzenesulfonyl moiety had an *n*-propyl side chain. Assuming an equivalent of four carbon atoms for the benzene ring, this would correspond to a total of seven carbons for the side chain attached to the sulfonyl group. Replacement of the alkyl side chain on the benzenesulfonyl group by two halogen atoms gave rise to high activity, provided the halogens were in the proper position on the ring. Gump[19] has shown that halogenated bis(phenols) are potent uncouplers of oxidative phosphorylation. The anilides of 3,4-dichlorobenzenesulfonic acid (**96–99**) showed high activity, whereas the derivative of 2,5-dichlorobenzenesulfonic acid (**100**) was low in activity. This high antibacterial activity of the 3,4-dichloroanilide of 3,4-dichlorobenzenesulfonic acid has also been observed by Chase and Weller.[9] We confirmed (**104** and **105**) their observation that replacement of the sulfonamide proton by an alkyl group destroyed activity. The structural nature of the alkyl side chain also affected activity. Derivatives of cumene were less active than those of *n*-propylbenzene. Similarly the derivatives of *n*-butylbenzene and *sec*-butylbenzene were more active than that of *tert*-butylbenzene. Thus, it appears that a straight chain of three carbon atoms was required for maximum biological activity. As in the case of fatty acid anilides, the activities obtained with sulfonamides from 3,4-dichloroaniline, 2-hydroxy-5-chloroaniline, 2-hydroxy-5-nitroaniline, and 3-nitro-4-chloroaniline were similar.

Additional data on the activity of selected compounds against *S. aureus*, *B. cereus*, *S. faecalis*, and *L. plantarum*, are presented in Table V. These data again demonstrate the importance of the ring substituent, its position, and the side-chain length. For example, an increase in the side chain of 3,5-dichloroanilide (**7**) by one carbon (**12**) broadens the specificity, but a change in the substituent position from the 3,5-dichlorophenyl (**12**) to the 3,4-dichlorophenyl analogue (**11**) lessens its effectiveness against *B. cereus*. While the reason for the effect of substituent position on species specificity is obscure, similar effects have been previously reported.[20]

The most effective of the substituted fatty acid anilides was the *N*-(2-hydroxy-5-nitrophenyl)octanamide (**14**), which was bacteriostatic against all four Gram-positive species at 10 ppm. When the side-chain length was increased to give *N*-(2-hydroxy-5-nitrophenyl)decanamide (**24**), activity against three species was lost. In contrast, when the chain length of an analogous α-alkylacrylic acid, the 2-hydroxy-5-nitroanilide of α-hexylacrylic acid (**42**), was increased, it became effective at a lower concentration (**47**), and ultimately, α-octylacrylic acid derivative (**52**) became bactericidal at 1 ppm. This compound was as effective as hexachlorophene against the four Gram-positive species.

The substituted sulfonanilides (**96–114**) show similar effects of ring substituent and chain length on specificity. The 3,4-dichloroanilide of 3,4-dichlorobenzenesulfonic acid (**96**) was active against all four species, but the 3-nitro-4-chloroanilide (**99**) was active against only two species. The species specificities of the 2-hydroxy-5-nitro- (**113**) and 4-chloro-3-nitroanilides (**114**) of *N*-butylbenzenesulfonic acid were also different.

Because the anilides tested are novel, we can only speculate on their antibacterial mechanisms. Analogies with structurally related compounds of known mechanism may provide some insight. Wilson et al.[21] established that compounds related to those in this study act as uncouplers of oxidative phosphorylation. Halogenated bis(phenols) are also potent uncoupling agents.[19] Lipophilic fatty acids interfere with substrate transport. Some classes of sulfonamides inhibit dihydrofolate reductase, thereby preventing synthesis of folic acid required for cell growth. The variation of activity with chain length is undoubtedly

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Table V. Activity of Selected Compounds against Four Gram-Positive Bacteria

no.[a]	structure	MIC,[b] ppm	effective against					bacteriostatic[c]	bactericidal[c]
			S. aureus	*B. cereus*	*S. faecalis*	*L. planatarum*			
7	3,5-$Cl_2C_6H_3NHCOC_6H_{13}$	10	x			x		+	–
11	3,4-$Cl_2C_6H_3NHCOC_7H_{15}$	10	x	d		x		+	–
12	3,5-$Cl_2C_6H_3NHCOC_7H_{15}$	10	x	x		x		+	–
14	2-OH-5-$NO_2C_6H_3NHCOC_7H_{15}$	10	x	x	x	x		+	–
15	3-NO_2-4-$ClC_6H_3NHCOC_7H_{15}$	10		x		x		–	–
18	2-OH-5-$ClC_6H_3NHCOC_8H_{17}$	>10						–	–
20	3-NO_2-4-$ClC_6H_3NHCOC_8H_{17}$	10		d		x		+	–
24	2-OH-5-$NO_2C_6H_3NHCOC_9H_{19}$	10	x					+	–
35	3,5-$Cl_2C_6H_3NHCOC(=CH_2)$-C_5H_{11}	10	x	d		x		+	–
40	3,4-$Cl_2C_6H_3NHCOC(=CH_2)$-C_6H_{13}	10	x			x		+	–
42	2-OH-5-$NO_2C_6H_3NHCOC(=CH_2)$-C_6H_{13}	10	x	x	x	x		+	–
47	2-OH-5-$NO_2C_6H_3NHCOC(=CH_2)$-C_7H_{15}	1	x	x	x[e]	x		+	–
52	2-OH-5-$NO_2C_6H_3NHCOC(=CH_2)C_8H_{17}$	1	x	x	x	x		+	+
96	3,4-$Cl_2C_6H_3NHSO_2(3,4\text{-}Cl_2C_6H_3)$	10	x	x	x	x		+	–
99	3-NO_2-4-$ClC_6H_3NHSO_2(3,4\text{-}Cl_2C_6H_3)$	10			x	x		+	–
103	3,4-$Cl_2C_6H_3NHSO_2(n\text{-}C_3H_7C_6H_4)$	10			x	x		+	–
106	2-OH-5-$ClC_6H_3NHSO_2(n\text{-}C_4H_9C_6H_4)$	10	x	x	x	x		+	–
113	2-OH-5-$NO_2C_6H_3NHSO_2(n\text{-}C_4H_9C_6H_4)$	10	x	x		x		+	–
114	3-NO_2-4-$ClC_6H_3NHSO_2(n\text{-}C_4H_9C_6H_4)$	10			x	x		–	–
	hexachlorophene control	1	x	x	x	x		+	+

[a] Numbers used in Tables I–IV. [b] An "x" indicates concentration effective against species. [c] Tested against *S. aureus* at 10 ppm. [d] A few resistant colonies grew. [e] *S. faecalis* was inhibited at 10 ppm but not at 1 ppm.

linked to the solubility of the compound in water, the cel wall, the cell membrane, and the cell cytoplasm. A sat isfactory understanding of antibacterial action, whicl would explain all of the above observations at the molec ular level, remains to be developed.

Experimental Section

Materials. 3,5-Dichloroaniline was obtained through the courtesy of the Ishihara Corp.,[22] San Francisco, CA. Heptanoic and nonanoic acids were supplied by the Celanese Co., Inc., Dallas TX. All other reagents were purchased from Aldrich Chemical Co., Milwaukee, WI.

Synthetic Procedures. The synthesis of the fatty acid anilides was described by Bistline et al.[10] The anilides of arylalkanoic acids and of alkylated or chlorinated benzoic acids were prepared in an analogous fashion from the acids, which were converted to acyl halides with oxalyl chloride[23] and then converted to the anilides by reaction with the appropriate substituted aniline in the presence of excess pyridine. Anilides of phenylalkanoic and alkylbenzoic acids were prepared in the same manner. The α-substituted acrylic acids were prepared by the procedure of Serota et al.,[14] converted to the acid chlorides, and reacted with substituted anilines as described above.

***N*-Arylsuccinamic Acids.** A solution of 0.1 mol of succinic anhydride in 50 mL of dioxane was prepared. The addition of 0.1 mol of a substituted aniline was usually accompanied by immediate reaction and precipitation of a crystalline solid. The mixture was heated for 0.5–4 h, poured into 300 mL of cold water, and filtered. The crude product was crystallized from aqueous methanol or ethanol. Neutral equivalents and IR spectra were checked.

3,4-Dichloroanilide of Suberic Acid (84) and Its Methyl Ester (85). Suberic acid (100 g, 0.5 mol) was heated in toluene (250 mL) until completely dissolved. Absolute methanol (100 g) and boron trifluoride–methanol complex (3 g) were added, and the mixture was refluxed for 30 min. After cooling to room temperature, the product was washed three times with 100-mL portions of distilled water. Toluene was distilled off. Vacuum distillation at 0.2 mmHg until the vapor temperature reached 90 °C yielded a distillate (67 g) that was mostly dimethyl suberate. The residue (34 g) was used without further purification. Titration with sodium hydroxide gave a value of 105% as methyl hydrogen suberate, which would indicate the presence of a small amount of unreached suberic acid.

The crude methyl hydrogen suberate (34 g) was dissolved in benzene (125 mL), dimethyl formamide (1.9 mL) was added, and the product was cooled in an ice bath. Oxalyl chloride (25.4 g, 0.2 mol) was added dropwise to the stirred solution, and agitation continued for 4 h at room temperature and then held at 50 °C for 90 min. Benzene was distilled off with a water aspirator (25 mmHg) until the product temperature reached 90 °C. The residue of acyl chloride (37 g) was used without further purification.

3,4-Dichloroaniline (16 g, 0.1 mol) was dissolved in pyridine (80 mL), and a solution of the above acyl halide (18 g, 0.08 mol) in 1,2-dichloroethane (100 mL) was added dropwise with cooling to maintain the temperature at 20 °C. The product was then stirred at room temperature for 1 h and refluxed for 2 h. The warm solution (40 °C) was washed once with distilled water (100 mL) and hydrochloric acid (6 N, 100 mL) and again with water (100 mL). The solution was allowed to crystallize at 10 °C to yield crude 85 (19 g). The mother liquor was saved for the hydrolysis step described below. The crude 85 was recrystallized once more from 1,2-dichloroethane to yield pure 85 (17 g, 63% of theory), mp 112–114 °C, and the mother liquor was combined with the above mother liquor. The IR spectrum showed the characteristic NH absorption at 3360 cm^{-1}, ester C=O at 1720 cm^{-1}, and amide C=O stretching vibration at 1695 cm^{-1}.

The combined mother liquors were evaporated to dryness. The dark residue (12 g) was dissolved in 95% ethanol (30 mL), distilled

(22) Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

(23) Fieser, L. F.; Fieser, M. "Reagents for Organic Synthesis"; Wiley: New York, 1967; p 767.

water (30 mL) and 10% alcoholic potassium hydroxide solution (25 g) were added, and the mixture was refluxed for 30 min. The product was poured into distilled water (200 mL) with vigorous agitation. After cooling and standing at room temperature for 30 min, the cloudy solution was filtered to remove a small amount of solid impurity, the filtrate was acidified with dilute hydrochloric acid, and the precipitate formed was filtered off and dried. The dry product was recrystallized from 1,2-dichloroethane to yield 7 g of 84 melting at 131–132 °C. Its neutral equivalent was within 2% of theory. IR specta showed NH absorption at 3280 cm^{-1}, the carboxylic C=O peak at 1690 cm^{-1}, and the amide C=O absorption at 1660 cm^{-1}. In addition, the broad funnel-shaped band, 3200–2400 cm^{-1}, characteristic of carboxylic acids was apparent.

3,4-Dichloroanilide of *p*-*n*-Propylbenzenesulfonic Acid (103). The synthesis of **103** is typical for this class of compounds. Alkylbenzenesulfonyl chlorides were prepared according to Bistline et al.[24] *n*-Propylbenzene (48 g, 0.4 mol) was dissolved in 1,2-dichloroethane (100 mL), and chlorosulfonic acid (107 g, 0.92 mol) was added dropwise to the stirred solution, which was maintained at 20 °C or below. The product was kept at room temperature for 30 min, poured into a separatory funnel, and allowed to separate overnight. The dark botton layer (31 g), containing mostly sulfuric acid, was discarded. Distilled water (4 mL) was added dropwise to the dichloroethane layer at 20 °C to decompose excess chlorosulfonic acid, and the product was placed again in a separatory funnel. The bottom layer of spent sulfuric acid (24.5 g) was discarded. The organic layer was heated to 50 °C, and solvent was removed by vacuum distillation with a water aspirator. The crude sulfonyl chloride, a yellow oil, weighed 83 g.

Recrystallized 3,4-dichloroaniline (16.2 g, 0.1 mol), was dissolved in pyridine (75 g, 0.95 mol), and the crude *n*-propylbenzenesulfonyl chloride (25 g, 0.114 mol) was added dropwise while the reaction mixture was maintained at 10 °C. After the addition, the reaction mixture was stirred at room temperature for 2 h. The temperature was raised gradually to 90 °C, held there for 30 min, and then lowered to room temperature, and the reaction mixture was slowly added to 400 mL of 6 N HCl with vigorous stirring. The lumpy product initially formed gradually broke up into light brown crystals, which were filtered, washed with water, and dried. The product was crystallized from a mixture of 95% ethanol and water. Evaporation of some of the mother liquor gave a second crop. The combined crops melted at 96–98 °C and gave a yield of 21 g (61% theory). Its IR spectrum showed NH stretching vibrations at 3240 cm^{-1} and S=O stretching vibrations at 1330 and 1160 cm^{-1}.

***N*-Methyl-3,4-dichloroanilide of *n*-Propylbenzenesulfonic Acid (104).** The above prepared **103** (8.6 g, 0.025 mol) was dissolved in absolute ethanol (30 mL), and a 23% methanol solution of sodium methylate (5.44 g, 0.025 mol) was added dropwise. After the solution was stirred at room temperature for 30 min, methyl iodide (3.6 g, 0.0254 mol) was added dropwise. The reaction product was then refluxed for 90 min and allowed to cool to room temperature. The long white needles, mp 112–113 °C, that formed were filtered off. The mother liquor was added to a mixture of distilled water (200 mL) and 50% aqueous sodium hydroxide (1 mL) heated to 50 °C. After the mixture was cooled to room temperature, the precipitate formed was filtered and recrystallized from absolute alcohol to yield a second crop: total yield 6.3 g (70% of theory); the IR spectrum lacked the strong NH absorption at 3240 cm^{-1} but showed the S=O stretching vibrations of the starting sulfonamide **103**.

Microbiological Evaluation. All 116 compounds were initially screened[25] for bacteriostatic activity against *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 8709, *Salmonella typhimurium*, *Salmonella enteritidis*, and *Staphylococcus aureus* ATCC 6538. One percent stock solutions were prepared in 95% ethanol or water. The stock solutions were serially diluted in sterile nutrient agar (pH 6.8) to obtain 1000-, 100-, 10-, and 1-ppm concentration of compound. The agar was poured into sterile Petri dishes, allowed to harden, dried at 37 °C for $^1/_2$ h with covers off, and then inoculated with ten separate drops of a 24-h culture of test microorganism in nutrient broth. The inoculated dishes were incubated for 48 h at 37 °C and examined for growth. Hexachlorophene was used as the control germicidal standard. All tests were run in duplicate. The MIC was determinated to be the lowest concentration at which none of the inocula in either Petri dish grew.

A select group of compounds was also tested against the Gram-positive organisms *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NRRL 3711, *Lactobacillus plantarum* Microlife 804, and *Streptococcus faecalis* JH2-2.[26] Six 15-microliter drops of each species were inoculated onto duplicate plates of nutrient agar containing the test compound at 10, 1.0, and 0.1 ppm. Each drop contained 10^7 vegetative cells, except for the *L. plantarum* inocula which contained 10^6. The plates, along with plates containing hexachlorophene or no compound, were incubated for 5 days at 30 °C and examined for growth. Again, the MIC for each species was determined to be the lowest concentration at which no colonies were formed on either plate.

Distinction between bacteriostatic and bactericidal activity was made by inoculating *S. aureus* at 10^7/mL into 10 mL of nutrient broth containing 10 ppm of the test compound. After the initial A_{660} was read, the tubes were incubated with shaking at 30 °C for 24 h. When bacteriostatic activity (as evidenced by no increase in the A_{660}) was found, the contents of the test tube were centrifuged (10000*g* for 10 min), resuspended in sterile diluent, and streaked onto plates of nutrient agar. The observance of colonies on the plates after 48 h at 30 °C demonstrated that the cells were still viable and, hence, the compound was only bacteriostatic. Plates with no colonies indicated that the compounds had killed the cells and were bactericidal.

Registry No. **1**, 2150-97-2; **2**, 86886-44-4; **3**, 72298-70-5; **4**, 79779-24-1; **5**, 72298-68-1; **6**, 2610-79-9; **7**, 20398-15-6; **8**, 86886-45-5; **9**, 86886-46-6; **10**, 86886-47-7; **11**, 730-25-6; **12**, 20398-46-3; **13**, 72298-71-6; **14**, 72298-65-8; **15**, 72298-69-2; **16**, 733-30-2; **17**, 20398-45-2; **18**, 72298-72-7; **19**, 72298-66-9; **20**, 5754-60-9; **21**, 7239-38-5; **22**, 86886-48-8; **23**, 72298-73-8; **24**, 72298-67-0; **25**, 5540-62-5; **26**, 72298-77-2; **27**, 86886-49-9; **28**, 72298-74-9; **29**, 72298-80-7; **30**, 72298-79-4; **31**, 86886-50-2; **32**, 86886-51-3; **33**, 86886-52-4; **34**, 86886-53-5; **35**, 86886-54-6; **36**, 86886-55-7; **37**, 86886-56-8; **38**, 86886-57-9; **39**, 86886-58-0; **40**, 86886-59-1; **41**, 86886-60-4; **42**, 86886-61-5; **43**, 86886-62-6; **44**, 86886-63-7; **45**, 86886-64-8; **46**, 86886-65-9; **47**, 86886-66-0; **48**, 86886-67-1; **49**, 86886-68-2; **50**, 86886-69-3; **51**, 86886-70-6; **52**, 86886-71-7; **53**, 86886-72-8; **54**, 86886-73-9; **55**, 86886-74-0; **56**, 86886-75-1; **57**, 27816-82-6; **58**, 86886-76-2; **59**, 86886-77-3; **60**, 86886-78-4; **61**, 86886-79-5; **62**, 50916-29-5; **63**, 86886-80-8; **64**, 10286-79-0; **65**, 18094-88-7; **66**, 86886-81-9; **67**, 86886-82-0; **68**, 86886-83-1; **69**, 86886-84-2; **70**, 86886-85-3; **71**, 86886-86-4; **72**, 86886-87-5; **73**, 86886-88-6; **74**, 86886-89-7; **75**, 86886-90-0; **76**, 17722-71-3; **77**, 86886-91-1; **78**, 86886-92-2; **79**, 86886-93-3; **80**, 86886-94-4; **81**, 86886-95-5; **82**, 86886-96-6; **83**, 86886-97-7; **84**, 86886-98-8; **85**, 86886-99-9; **86**, 86887-00-5; **87**, 86887-01-6; **88**, 86887-02-7; **89**, 86887-03-8; **90**, 86887-04-9; **91**, 86887-05-0; **92**, 86887-06-1; **93**, 86887-07-2; **94**, 86887-08-3; **95**, 86887-09-4; **96**, 86887-10-7; **97**, 86887-11-8; **98**, 86887-12-9; **99**, 86887-13-0; **100**, 86887-14-1; **101**, 16964-21-9; **102**, 86887-15-2; **103**, 86887-16-3; **104**, 86887-17-4; **105**, 86887-18-5; **106**, 86887-19-6; **107**, 86887-20-9; **108**, 86887-21-0; **109**, 86887-22-1; **110**, 86887-23-2; **111**, 86887-24-3; **112**, 86887-25-4; **113**, 86887-26-5; **114**, 86887-27-6; **115**, 86887-28-7; **116**, 86887-29-8.

(24) Bistline, R. G., Jr.; Noble, W. R.; Linfield, W. M. *J. Am. Oil Chem. Soc.* **1974**, *51*, 126.

(25) The initial microbiology screening was done at Quality Control Inc., Southampton, PA. Elemental analyses were carried out by Microanalysis Inc., Wilmington, DE.

(26) The technical assistance of Lucy Conway in these microbiological studies is gratefully acknowledged.