Potential Salicylamide Antiplaque Agents: In Vitro Antibacterial Activity against *Actinomyces viscosus*

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A series of 55 salicylamides, including 3,5-dibromo-, 5-*n*-alkyl-, and 5-*n*-acylsalicyloyl derivatives of various anilines, heterocyclic amines, benzylamines, and alkylamines, was synthesized and evaluated for in vitro antibacterial activity against *Actinomyces viscosus*, an adherent oral microorganism implicated in periodontal disease. The in vitro minimum inhibitory concentrations of 15 4′-bromosalicylanilides were found to correlate ($r = 0.92$) with estimated log D values. Several nonhalogenated salicylanilides, such as 5-*n*-hexyl- (**40**) and 5-*n*-decanoyl-4′-nitrosalicylanilide (**47**), were found to exhibit higher levels of in vitro antibacterial activity against a number of *Actinomycetes* than did tribromsalan (**1**) or fluorophene (**2**).

The use of topical applications of antimicrobial agents for the control of oral bacterial plaque, associated with gingivitis and dental caries, has attracted much attention as an alternative to systemic administration.[2] It appears that microbial plaque associated with gingivitis has been described as largely Gram positive and often dominated by members of the genus *Actinomyces*.[3]

Following the development of a quantitative in vitro antiplaque bioassay reflecting oral conditions,[4] representative members of many classes of antimicrobial agents[5-8] were examined with this assay relative to chlorhexidine, an agent with demonstrable clinical effect upon human dental plaque.[9] The salicylanilides were chosen for further development based upon the reported in vitro antiplaque effects of tribromsalan (**1**)[5,10] as well as the caries inhibiting activity of fluorophene (**2**) in the rat.[11,12] Additionally, **1** was a component of an oral preparation found to exhibit clinical effects against plaque formation[13] and gingivitis[14] in man. The only component of this oral preparation found to exhibit in vitro antiplaque effects was **1**.[5] Staining and undesirable organoleptic properties, common to quaternary ammonium antimicrobials, were not reported in this clinical study.

Although structure–antimicrobial activity studies of salicylanilides employing nonplaque-forming microorganisms have been described,[15-17] optimization of activity, among this class of agents, against the suspected periodontopathic adherent species *A. viscosus* may lead to new agents more specifically directed to the control of marginal gingivitis. Since the use of halogenated salicylanilides has been restricted by the FDA,[18] particular interest was directed toward 5-acyl- and 5-alkylsalicyloyl derivatives of anilines, heterocyclic amines, and alkyl- or arylalkylamines representing previously unreported structural modifications of this class of antimicrobial agents.

Chemistry. 5-Acylsalicylic acids were prepared by Friedl–Crafts acylation of methyl salicylate using aluminum chloride catalyst in carbon disulfide, together with the appropriate alkyl acid chloride. 5-Alkylsalicylic acids were obtained from the corresponding 5-acylsalicylic acid esters by Clemmensen reduction employing zinc and hydrochloric acid.

Salicylanilides and salicylamide heterocyclic derivatives were prepared by treatment of the salicylic acids with phosphorus trichloride, followed by reaction with the appropriate amine. The 4′-nitrophenol esters of the salicylic acids were employed in the preparations involving the more basic alkyl and benzylic amines.

Results and Discussion

Table I lists 3,5-dibromo-, 5-alkyl-, and 5-acylsalicylamide derivatives of various anilines, heterocyclic amines, benzylamines, and alkylamines, together with minimum inhibitory concentrations (MIC) against *Actinomyces viscosus* M100-2000. MIC values were determined by standard tube dilution techniques employing Bacto Anaerobe broth with visual evaluation made after 24 and 48 h, as well as plating and reincubation for verification of complete bactericidal action.

Compounds **1–14** were examined in order to determine the effect upon antibacterial activity against *A. viscosus* produced by variation of the amide nitrogen substituent. Although comparable activities were displayed by TBS (**1**), fluorophene (**2**), and the 4′-nitrophenyl derivative **3**, only the more lipophilic heterocyclic derivatives **5**, **6**, and **12** displayed significant activity. The activities of the cyclohexyl derivative **13** and the *p*-chlorobenzyl derivative

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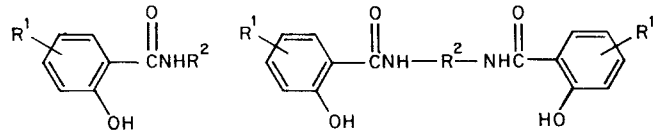
Table I. Salicylamides and Their in Vitro Antibacterial Activity against *Actinomyces viscosus* M-100-2000



1-52

53-55

no.	R^1	R^2	mp, °C	formula[a]	MIC[b]	no.	R^1	R^2	mp, °C	formula[a]	MIC[b]
1	3,5-Br_2	4-BrC_6H_4	225-227[c]	$C_{13}H_8NBr_3O_2$	0.5	26	3-NO_2-5-n-C_6H_{13}	4-BrC_6H_4	153-154	$C_{19}H_{21}N_2BrO_4$	0.40
2	3,5-Br_2	3-$CF_3C_6H_4$	161-163	$C_{14}H_8NBr_2F_3O_2$	1.0	27	5-C_2H_5CO	4-BrC_6H_4	232-233	$C_{16}H_{14}NBrO_3$	13.0
3	3,5-Br_2	4-$NO_2C_6H_4$	300 dec	$C_{13}H_8N_2Br_2O_4$	0.5	28	5-n-$C_5H_{11}CO$	4-BrC_6H_4	185-186	$C_{19}H_{20}NBrO_3$	1.0
4	3,5-Br_2	2-thiazolyl	259-290 dec	$C_{10}H_6N_2Br_2O_2S$	95.0	29	5-n-$C_7H_{15}CO$	4-BrC_6H_4	176-177	$C_{21}H_{24}NBrO_3$	0.5
						30	5-n-$C_9H_{19}CO$	4-BrC_6H_4	169-170	$C_{23}H_{28}NBrO_3$	0.06
5	3,5-Br_2	2-benzothiazolyl	291-292 dec	$C_{14}H_8N_2Br_2O_2S$	1.7	31	5-n-$C_{11}H_{23}CO$	4-BrC_6H_4	163-165	$C_{25}H_{32}NBrO_3$	0.02
						32	5-n-$C_{13}H_{23}CO$	4-BrC_6H_4	156-158	$C_{27}H_{36}NBrO_3$	>5.0
6	3,5-Br_2	2-(4-chlorobenzothiazolyl)	>300	$C_{14}H_7N_2Br_2ClO_2S$	1.7	33	5-CH_3	4-$NO_2C_6H_4$	241-242	$C_{14}H_{12}N_2O_4$	5.0
7	3,5-Br_2	2-(3-methylpyridyl)	253 dec	$C_{13}H_{10}N_2Br_2O_2$	>155.0	34	5-CH_3	4-$CH_3OC_6H_4$	185-186	$C_{15}H_{15}NO_3$	>40.0
8	3,5-Br_2	2-(4,6-dimethylpyridyl)	255-256	$C_{14}H_{12}N_2Br_2O_2$	25.0	35	5-n-C_4H_9	3-$CF_3C_6H_4$	120-121	$C_{18}H_{18}NF_3O_2$	0.5
9	3,5-Br_2	2-(4,6-dimethylpyrimidyl)	237-238 dec	$C_{13}H_{11}N_3Br_2O_2$	200.0	36	5-n-C_6H_{13}	3-$CF_3C_6H_4$	117-118	$C_{20}H_{22}NF_3O_2$	0.5
						37	5-n-C_6H_{13}	2-benzothiazolyl	230-232	$C_{20}H_{22}N_2O_2S$	>20.0
10	3,5-Br_2	2-(5-methyl-1,3,4-thiadiazolyl)	283-285 dec	$C_{10}H_7N_3Br_2O_2S$	100.0	38	5-n-C_6H_{13}	4-$ClC_6H_4CH_2$	122-123	$C_{20}H_{24}NClO_2$	10.0
						39	5-n-C_6H_{13}	3,4-$Cl_2C_6H_3CH_2$	91-92	$C_{20}H_{23}NCl_2O_2$	0.5
11	3,5-Br_2	2-(5-chlorobenzoxazolyl)	289-290 dec	$C_{14}H_7N_2Br_2ClO_3$	>35.0	40	5-n-C_6H_{13}	4-$NO_2C_6H_4$	154-155	$C_{19}H_{22}N_2O_4$	0.05
						41	5-n-C_6H_{13}	3-$NO_2C_6H_4$	167-168	$C_{19}H_{22}N_2O_4$	0.5
12	3,5-Br_2	2-(5-chlorobenzimidazolyl)	>300	$C_{14}H_8N_3Br_2ClO_2$	4.0	42	5-n-C_6H_{13}	4-CH_3-3-$NO_2C_6H_3$	160-161	$C_{20}H_{24}N_2O_4$	>20.0
13	3,5-Br_2	c-$C_6H_{11}CH_2$	130-131	$C_{14}H_{17}NBr_2O_2$	12.0	43	5-n-$C_7H_{15}CO$	2-benzothiazolyl	226-228	$C_{22}H_{24}N_2O_3S$	1.0
14	3,5-Br_2	4-$ClC_6H_4CH_2$	140-142	$C_{14}H_{10}NBr_2ClO_2$	4.0	44	5-n-$C_9H_{19}CO$	2-benzothiazolyl	215-217	$C_{24}H_{28}N_2O_3S$	0.5
15	H	4-BrC_6H_4	171-173[d]	$C_{13}H_{10}NBrO_2$	10.0	45	5-n-$C_{11}H_{23}CO$	2-benzothiazolyl	210-213	$C_{26}H_{32}N_2O_3S$	>20.0
16	5-Br	4-BrC_6H_4	243-244[e]	$C_{13}H_9NBr_2O_2$	0.5	46	5-n-$C_7H_{15}CO$	4-$NO_2C_6H_4$	173-174	$C_{21}H_{24}N_2O_5$	0.10
17	5-OCH_3	4-BrC_6H_4	214-215	$C_{14}H_{12}NBrO_3$	>25.0	47	5-n-$C_9H_{19}CO$	4-$NO_2C_6H_4$	141-143	$C_{23}H_{28}N_2O_5$	0.05
18	5-NO_2	4-BrC_6H_4	245-246[f]	$C_{13}H_9N_2BrO_4$	3.0	48	5-n-$C_{11}H_{23}CO$	4-$NO_2C_6H_4$	131-132	$C_{25}H_{32}N_2O_5$	0.05
19	5-CH_3	4-BrC_6H_4	210-211	$C_{14}H_{12}NBrO_2$	2.5	49	5-n-$C_7H_{15}CO$	3-$CF_3C_6H_4$	127-130	$C_{22}H_{24}NF_3O_3$	0.5
20	5-n-C_3H_7	4-BrC_6H_4	162-163	$C_{16}H_{16}NBrO_2$	1.5	50	5-n-$C_{11}H_{23}CO$	3-$CF_3C_6H_4$	115-117	$C_{26}H_{32}NF_3O_3$	0.5
21	5-n-C_4H_9	4-BrC_6H_4	153-154	$C_{17}H_{18}NBrO_2$	0.1	51	5-n-$C_9H_{19}CO$	3,4-$Cl_2C_6H_3CH_2$	128-129	$C_{24}H_{29}NCl_2O_3$	0.5
22	5-n-C_6H_{13}	4-BrC_6H_4	140-141	$C_{19}H_{22}NBrO_2$	0.01	52	5-n-$C_{11}H_{23}CO$	3,4-$Cl_2C_6H_3CH_2$	119-120	$C_{26}H_{33}NCl_2O_3$	5.0
23	5-n-C_8H_{17}	4-BrC_6H_4	134-135	$C_{21}H_{26}NBrO_2$	0.20	53	H	-$(CH_2)_6$-	141-142	$C_{20}H_{24}N_2O_4$	>20.0
24	5-n-$C_{10}H_{21}$	4-BrC_6H_4	129-130	$C_{23}H_{30}NBrO_2$	3.25	54	5-Br	-$(CH_2)_6$-	200-202	$C_{20}H_{22}N_2Br_2O_4$	>20.0
25	3-NO_2-5-CH_3	4-BrC_6H_4	191-192	$C_{14}H_{11}N_2BrO_4$	45.0	55	5-CH_3	-$(CH_2)_6$-	178-179	$C_{22}H_{28}N_2O_4$	>20.0

[a] Elemental analyses obtained for C, H, and N for all compounds were in agreement (0.4%) with theoretical values. [b] MIC values in micrograms per milliliter. [c] Literature[15] mp 225-227 °C. [d] Literature[15] mp 173-175 °C. [e] Literature[15] mp 240-241 °C. [f] Literature[29] mp 242-245 °C.

Table II. Minimum Inhibitory Concentrations and Physiochemical Constants Used for Equation 1

no.	log P^a	p$K_a{}^b$	log D^c	log (1/MIC)^d obsd	log (1/MIC)^d calcd	Δ
1	6.15	5.85	4.78	5.95	5.48	0.47
15	4.13	7.10	3.78	4.47	4.78	−0.31
16	5.26	6.85	4.75	5.87	5.46	0.41
18	4.58	5.80	3.16	5.05	4.37	0.68
19	4.61	7.35	4.38	5.09	5.20	−0.11
20	5.61	7.35[e]	5.38	5.35	5.89	−0.54
21	6.09	7.35[e]	5.88	6.54	6.23	0.30
22	7.11	7.35[e]	6.88	7.58	6.92	0.65
25	5.06	5.60	3.46	3.94	4.56	−0.62
26	7.56	5.60[f]	5.96	6.02	6.29	−0.27
27	4.52	6.10	3.38	4.43	4.51	−0.08
28	6.02	6.10[g]	4.88	5.60	5.55	−0.05
29	7.02	6.10[g]	5.88	5.92	6.24	−0.32
30	8.02	6.10[g]	6.88	6.87	6.92	−0.06
31	9.02	6.10[g]	7.88	7.37	7.61	−0.24

[a] Log P = 3.27 (salicylanilide, ref 26) + 0.86 ($\pi_{4'\text{-Br}}$) + π_{R^1} (phenolic hydrophobicity substituent constants from ref 27). [b] Spectroscopically measured values unless otherwise noted. [c] Calculated at pH 7.2 with the relationship log D = log P + log [1/(1 + $10^{pH - pK_a}$)] reported in ref 19. [d] Molar MIC values. [e] Estimated from compound 19. [f] Estimated from compound 25. [g] Estimated from compound 27.

14 were indicative that a planar conjugated aromatic substituent is not an absolute requirement for activity against *A. viscosus*.

Introduction of a 5-bromo substituent into compound 15 to give the 5,4′-dibromo derivative 16 produced a 20-fold increase in activity, while no further increase in activity was apparent in the 3,5,4′-tribromo derivative 1.

A series of 5-alkyl-4′-bromosalicylanilides, 19–24, exhibited increasing activity as a function of chain length. Among the normal alkyl groups investigated, activity was optimal for the *n*-hexyl derivative 22 and decreased for the longer chain derivatives 23 and 24. Similar results were obtained for a series of 5-acyl-4′-bromosalicylanilides, 27–32, where highest activity was displayed by the *n*-dodecanoyl derivative 31. Compounds 22 and 31 exhibit 25- to 50-fold enhancements of activity over that of the lead compound 1.

In order to elucidate the effects of the salicylyl ring substituents, a series of 15 4′-bromosalicylanilides was examined for quantitative structure–activity relationships (Table II). Only linear relationships were examined by excluding compounds 23, 24, and 32, since the data appeared insufficient to justify investigation of quadratic relationships. Log P values were calculated since the very limited aqueous solubilities mitigated against their measurement. Although regression of log 1/MIC vs. log P values gave only a marginal correlation, r = 0.84, the regression of log D values,[19] calculated (see Table II) at the pH of the growth medium, showed an improved correlation (eq 1).

$$\log (1/\text{MIC}) = 0.69\ (\pm 0.16)\ \underset{(4.30)}{\log D} + 2.18\ (\pm 0.43) \qquad (1)$$

$$n = 15,\ r = 0.920,\ r^2 = 0.846,\ s = 0.43$$

Attempted correlations with log D employing electronic terms such as σ or σ^- and with pK_a resulted in no significant improvement in the correlations, r = 0.921, 0.920, and 0.921, respectively.

It is apparent that the optimal lipophilicity for this series lies in the range of log D = 6–8. The log P values of the two most active compounds, 22 and 31, are considerably greater than the average log P_0 value, 5.9 (±0.4), for nine examples of nonspecific bactericides against several Gram-positive bacteria.[20] The improvement in correlating lipophilicity with activity by correcting for partial ionization and the higher apparently optimal log P values are both anomalies and would suggest that a more lipophilic receptor is involved in the action of these salicylanilides against *A. viscosus* in comparison to the action of phenols on other Gram-positive bacteria.

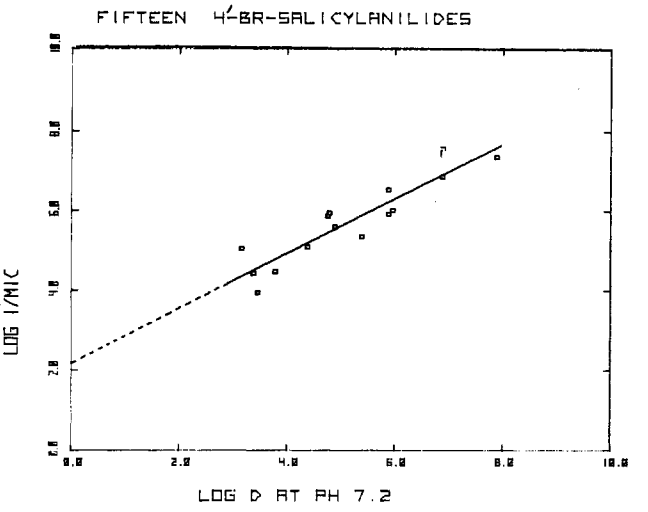


Figure 1. Illustration of the fit of regression eq 1.

The use of log P values corrected for partial ionization in regression analyses assumes only un-ionized species in the receptor compartment and has been criticized[21] for its empirical basis, making interpretations difficult. Hamilton[22] has suggested from a study of *B. megaterium* protoplasts that while 2,4-dinitrophenol caused increased membrane permeability only to hydrogen ion, 3,5,3′,4′-tetrachlorosalicylanilide (TCS) produced specific changes to nitrate ion permeability. Although the activity of salicylanilides as uncouplers of oxidative phosphorylation has been related to lipophilcity and pK_a by Tollenaere,[23] Harold and Baarda[24] have reported that TCS had little effect upon ATP generation or utilization in *Streptococcus faecalis*, an organism lacking cytochrome, but instead inhibited energy-dependent transport of phosphate, alanine, and leucine.

It would thus appear that there is no compelling reason to assume that the intrinsic activities of the salicylanilides in *Actinomycetes*, under anaerobic conditions, should be proportional to their pK_a values. Martin[25] has described

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Table III. In Vitro Minimum Inhibitory Concentrations[a] of Salicylamides against *Actinomycetes*

	A. viscosus	*A. naeslundii*		*A. israelii*		
no.	T14V	12104	8115	10048	12836	28360
1	1.0	1.0	1.0	1.0	0.5	5.0
2	0.5	1.0	0.5	0.5	0.5	0.1
22	0.1	1.0	0.1	0.1	0.5	0.1
31	0.5	0.5	1.0	>5.0	>5.0	0.5
36	0.05	0.05	0.1	0.5	0.5	>5.0
39	0.5	0.5	0.5	0.5	1.0	1.0
40	0.05	0.05	0.05	0.5	0.1	0.1
44	10.0	>10.0	>10.0	5.0	>10.0	>10.0
47	0.05	0.5	0.05	0.05	0.05	0.05
50	0.10	0.10	0.05	0.1	0.5	0.10

[a] MIC in micrograms per milliliter.

nonlinear equations based upon 3- and 4-compartment models which relate lipophilicity, pK_a, and ionization to drug potency. In examining linear equations which result from this nonlinear model when one term dominates, it was found that eq **2** produced a correlation comparable to

$$\log (1/C) = \underset{(8.54)}{0.703\ (\pm 0.082)\ \log P} - \log (1 + K_a/[H^+]) + 2.382\ (\pm 0.510) \quad (2)$$

$$n = 15,\ r = 0.915,\ r^2 = 0.838,\ s = 0.44$$

that of eq **1**. This suggests that the neutral form is the dominant species, since correlations dominated by the ionic species in the nonaqueous and aqueous compartments were less significant, $r = 0.756$ and $0.427i$; $s = 0.720$ and 1.20, respectively. However, if intrinsic activity is related to pK_a, then these data, obtained at a single pH value, may not distinguish between neutral or ionic forms binding to the receptor.

The low activity noted for 4′-methoxy derivative **34** shifted attention to anilide substituents which were electron withdrawing. The 4′-nitro substituent appears to be slightly more effective than 3′-(trifluoromethyl) when comparing **36** vs. **40** and **46** vs. **49**, especially when considering the lower lipophilicity of the nitro derivatives relative to that of **30** and **22**. The lower activity of the 3′-nitro derivatives **41** and **42** could be interpreted either as a steric effect and/or as a resonance effect operative with para substituents.

Significant activity levels, comparable to that of **1**, were shown by the two 3′,4′-dichlorobenzyl derivatives **39** and **51**, even though they are less active than would be predicted by eq 1 (0.017 and 0.02 μg/mL) based upon their estimated log *D* values (7.35 and 7.35, respectively). The activity of these arylalkyl amides and that of the alkyl amide **13** suggested the synthesis of the alkyl-bridged disalicylamidohexamethylene derivatives **53** and **54**, none of which displayed activity below their limits of solubility.

Several of the more active compounds (**40**, **46**, **47**, and **49**) were reexamined by diluting and plating their serially diluted solutions after the incubation period. These compounds were found to be bactericidal at the MIC levels listed in Table I.

A number of the more active compounds were examined for their inhbitory effect upon other *Actinomycetes* (Table III). Although the two 3,5-dibromo derivatives **1** and **2** were effective against all strains and species, several of the 5-alkyl and 5-acyl derivatives (**22**, **40**, **47**, and **50**) exhibited better overall spectra of activity against these *Actinomycetes*. The activity of the 2′-benzothiazolyl derivative **44**, on the other hand, was quite poor against these additional bacteria.

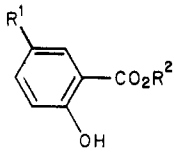
Although a number of 4′-bromoanilides of the 5′-alkyl- and 5-acylsalicylic acids were among the more acitve compounds tested, the poor solubility of these derivatives in aqueous and alcoholic solutions may limit their userfulness. The 3′-(trifluoromethyl)anilides, 4′-nitroanilides, and 3′,4′-dichlorobenzyl derivatives of the 5-alkyl- and 5-acylsalicylic acids were found to be quite soluble in ethanol. The weaker acidity of the 5-alkyl derivatives limits their concentrations in aqueous solution of pH 7.4 to below 20 μg/mL unless substantial amounts of cosolvents are employed.

In conclusion, it has been demonstrated that high levels of antibacterial activity against *Actinomycetes* can be obtained among 5-alkyl- and 5-acylsalicylanilides. Among these derivatives, a number, including nonhalogenated compounds, appeared more active in vitro than 3,5,4′-tribromosalicylanilide. These compounds represent excellent candidates for further evaluations against other adherent and nonadherent oral microorganisms.

Experimental Section

Melting points were determined with a Fisher-Johns hot stage apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA, and determined values are within 0.4% of theoretical values. Infrared spectra (KBr disks) were obtained with a Perkin-Elmer 727B spectrophotometer and a Nicolet 7199 FT interferometer. ^1H NMR spectra were recorded with Varian T-60A and FT-80 spectrometers using 1% (v/v) Me_4Si as an internal standard. UV spectra were recorded

Table IV. Salicylic Acid Derivatives



no.	R^1	R^2	mp, °C	% yield[a]	formula[b]	no.	R^1	R^2	mp, °C	% yield[a]	formula[b]
56	COC_2H_5	CH_3	60-61	96	$C_{11}H_{12}O_4$	65	COC_7H_{15}	H	113-114	93	$C_{15}H_{20}O_4$
57	COC_3H_7	CH_3	70.5-71.5	91	$C_{12}H_{14}O_4$	66	COC_9H_{19}	H	118-119[c]	91	$C_{17}H_{24}O_4$
58	COC_5H_{11}	CH_3	58-60	77	$C_{14}H_{18}O_4$	67	$COC_{11}H_{23}$	H	119-120	94	$C_{19}H_{28}O_4$
59	COC_7H_{15}	CH_3	57-58	85	$C_{16}H_{22}O_4$	68	$COC_{13}H_{27}$	H	117-118	90	$C_{21}H_{32}O_4$
60	COC_9H_{19}	CH_3	63-64	86	$C_{18}H_{26}O_4$	69	n-C_3H_7	H	99-100	53	$C_{10}H_{12}O_3$
61	$COC_{11}H_{23}$	CH_3	70-72	82	$C_{20}H_{30}O_4$	70	n-C_4H_9	H	89-90	51	$C_{11}H_{14}O_3$
62	$COC_{13}H_{27}$	CH_3	69-70	70	$C_{22}H_{34}O_4$	71	n-C_6H_{13}	H	83-84	82	$C_{13}H_{18}O_3$
63	COC_2H_5	H	176-177	99	$C_{10}H_{10}O_4$	72	n-C_8H_{17}	H	72-73[d]	42	$C_{15}H_{22}O_3$
64	COC_5H_{11}	H	116-117	92	$C_{13}H_{16}O_4$	73	n-$C_{10}H_{21}$	H	89-90	31	$C_{17}H_{26}O_3$

[a] Average yields for: Friedel-Crafts reaction, **56-62**; saponification, **63-68**; Clemenson reduction and saponification, **69-73**. [b] Elemental analyses obtained for C and H in agreement with theoretical values. [c] Literature[30] mp 117 °C. [d] Literature[31] mp 72-73 °C.

with a Varian-Cary 118 spectrophotometer. Measurements of pH were made with an Orion 901 microprocesser ion analyzer.

Methyl 5-*n*-Hexanoylsalicylate (58). The following experiment illustrates the general procedure used to prepare the methyl 5-acylsalicylates in Table IV. To a mixture of anhydrous aluminum chloride (60 g, 0.45 mol) in CS_2 (150 mL) maintained at 5–10 °C was added a solution of methyl salicylate (23 g, 0.15 mol) and *n*-hexanoyl chloride (40.4 g, 0.3 mol) in CS_2 (50 mL). During the addition, the reaction mixture was stirred with a mechanical overhead stirrer and the addition rate was adjusted to prevent refluxing of solvent. Following the addition the reaction mixture was stirred for 12 h and then poured into ca. 500 mL of crushed ice containing 25 mL of concentrated hydrochloric acid. The resulting slurry was extracted thrice with Et_2O and the combined Et_2O extracts (500 mL) were washed with saturated sodium chloride solution (100 mL) and dried ($MgSO_4$). The semisolid residue obtained after evaporation of solvent under reduced pressure was recrystallized from petroleum ether (bp 40–60 °C) to give 28.9 g (77%) of **58**: mp 58–60 °C; ^1H NMR ($CDCl_3$) δ 0.70–1.80 [9, m, $(CH_2)_3CH_3$], 2.95 (2, t, $COCH_2$), 4.00 (3, s, OCH_3), 7.0 (1, d, $J_{3,4}$ = 8 Hz, 3-H), 8.0 (1, d × d, $J_{3,4}$ = 8, $J_{4,6}$ = 2 Hz, 4-H), 8.5 (1, d, $J_{4,6}$ = 2 Hz, 6-H).

5-*n*-Hexanoylsalicylic Acid (64). The following experiment illustrates the saponification of the methyl 5-acylsalicylates to the corresponding acids in Table IV. To a solution of **56** (28.9 g, 0.116 mol) in EtOH (150 mL) was added 25 g of NaOH in 300 mL of H_2O. The resulting slurry was heated for 6 h on a steam bath to give a clear solution, which was cooled to 5 °C and acidified (pH 1) with concentrated hydrochloric acid. The resulting precipitate was filtered and recrystallized from EtOH/H_2O to give 25 g (92%) of **64**, mp 116–117 °C.

5-*n*-Hexylsalicylic Acid (71). The following experiment illustrates the preparation of the 5-*n*-alkylsalicylic acids in Table IV. Mossy Zn (24 g, 0.37 mol) was amalgamated by shaking with a solution of 2.4 g of $HgCl_2$ and 1 mL of concentrated hydrochloric acid in H_2O (40 mL). After decantation, **58** (12 g, 0.048 mol), H_2O (15 mL), and concentrated hydrochloric acid (35 mL) were added to the Zn, and then the mixture was heated to reflux for 20 h. Concentrated hydrochloric acid (10 mL) was added periodically (6 h) during the reaction period. The cooled mixture was extracted with Et_2O (150 mL). The residue obtained by evaporation of Et_2O from the extract was dissolved in EtOH (50 mL) and added to a solution of 10 g of NaOH in H_2O (100 mL). The resulting slurry was heated (12 h) on a steam bath. Acidification (pH 1) of the resulting clear solution, cooled to 5 °C, gave a precipitate, which was filtered and recrystallized (EtOH/H_2O) to give 8.7 g (82%) of **71**: mp 83–84 °C; ^1H NMR ($CDCl_3$) δ 0.70–1.80 [11, m, $(CH_2)_4CH_3$], 2.55 (2, t, $ArCH_2$), 6.7 (1, d, 3-H), 7.1 (1, d × d, 4-H), 7.5 (1, d, 6-H).

4'-Bromo-5-*n*-hexylsalicylanilide (22). The following experiment illustrates the preparation of the salicylanilides in Table I. A solution of **71** (1 g, 4.5 mmol) and PCl_3 (0.31 g, 2.25 mmol) in chlorobenzene (20 mL) was refluxed for 15 min and then cooled to 40 °C. 4-Bromoaniline (0.77 g, 4.5 mmol) in chlorobenzene (5 mL) was added and the mixture was refluxed for 3 h. Solvent was removed under reduced pressure and the residue was recrystallized from EtOH to give 0.97 g (57%) of **22** as white crystals: mp 140–141 °C; ^1H NMR (CF_3CO_2H) δ 0.90–1.8 [11, m, $(CH_2)_4CH_3$], 2.50 (2, t, $ArCH_2$), 6.95 (1, d, 3-H), 7.3 (1, d × d, 4-H), 7.5 (1, d, 6-H), 7.6–7.9 (4, m, C_6H_4Br); IR (KBr) 3295, 2950, 1620 cm^{-1}.

***N,N'*-Disalicyloyl-1,6-diaminohexane (53).** The following experiment illustrates the preparation of *N*-alkyl- and *N*-benzylsalicylamides in Table I. A mixture of $POCl_3$ (4 g, 26 mmol), salicylic acid (6.9 g, 50 mmol), and *p*-nitrophenol (6.95 g, 50 mmol) was heated to 95 °C for 2 h. A solution of the cooled residue in chlorobenzene (150 mL) was washed (3 times) with saturated $NaHCO_3$ solution. 1,6-Hexanediamine (1.16 g, 20 mmol) and Et_3N (1.4 g, 15 mmol) were added to the dried ($MgSO_4$) chlorobenzene solution. After the solution was refluxed for 12 h, solvent was removed under reduced pressure and the residue was triturated with 6 N hydrochloric acid (50 mL). The precipitate was filtered and recrystallized from EtOH to give 4.5 g (51%) of **53**: mp 141–142 °C; ^1H NMR (CF_3CO_2H) δ 0.95–1.70 [8, m, $(CH_2)_4$], 3.35 (4, t, NCH_2), 6.55–7.60 (8, m, C_6H_4); IR (KBr) 1640 cm^{-1}.

Determination of pK_a for Salicylanilides. The spectroscopic method described by Albert and Serjeant[28] was employed using 0.01 M phosphate buffers without cosolvents. The limited aqueous solubility of most of the salicylanilides necessitated concentrations below 10^{-4} M and reliable measurements could be obtained only at pH values giving >60% ionization. Analytical wavelengths represented longest wavelength λ_{max} at pH 12 and ranged from 285 to 360 nm. The average pK_a values determined in triplate at three pH values fell within the range ±0.10.

Microbiological Methods. The cultures used were *Actinomyces viscosus* strains M-100 and T14V, *Actinomyces naeslundii* strains ATCC 12104 and ATCC 8115, and *Actinomyces israelii* ATCC 10048, ATCC 12836, and ATCC 28360. The cultures of *A. viscosus* were obtained from Dr. Howard Bladen, National Institute of Dental Research, Bethesda, MD. Stock solutions of test compounds were prepared in EtOH or Me_2SO and diluted 100-fold or more with growth medium to give threefold dilutions (1 mL) in Bacto Anerobe broth. Following inoculation with 50 μL of 10^8 cfu/mL of log phase growth inoculum, tubes were incubated at 37 °C in an anaerobic chamber containing an atmosphere of 5% CO_2–10% H_2–85% Ar. MIC values were determined visually after 24 h. Tubes which were cloudy at the higher test concentrations were plated on Wilkins–Chalgren agar plates and incubated for 72 h to determine if the precipitate was viable cells or insoluble test compound. MIC values shown in Table I are the results of two or more determinations run in triplicate.

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