17542-47-1; $PhCH_2NH(CH_2)_3Cl$, 42245-33-0; $PhCH_2N(Me)(CH_2)_3Cl$, 3161-52-2; $PhCH_2N(Me)(CH_2)_4Cl$, 98901-98-5; $PhCH_2N(Me)(CH_2)_2NHMe$, 102-11-4; $PhCH_2NH(CH_2)_3NH_2$, 13910-48-0; $PhCH_2N(Me)(CH_2)_3NHBu$-*i*, 98901-99-6; $PhCH_2N(Me)(CH_2)_4NHMe$, 98902-00-2; $PhCH_2N(Me)(CH_2)_2N(Me)COPh$, 98902-01-3; $PhCH_2NH(CH_2)_3N(Me)COC_4H_3O$-2, 98902-02-4; $PhCH_2NH(CH_2)_3NHCOPh$, 98902-03-5; $PhCH_2N(Me)(CH_2)_3N(Me)COTHF$-2, 98902-04-6; $PhCH_2N(Me)(CH_2)_3N(Bu$-*i*$)COPh$, 98902-05-7; $PhCH_2N(Me)(CH_2)_4N(Me)COPh$, 98902-06-8; $PhCH_2N(Me)(CH_2)_2N(Me)COph\cdot HCl$, 98902-07-9; $PhCH_2NH(CH_2)_3N(Me)COC_4H_3O$-2·HCl, 98902-08-0; $PhCH_2NH(CH_2)_3NHCOPh\cdot HCl$, 98902-09-1; $PhCH_2N(Me)(CH_2)_3N(Me)COTHF$-2·HCl, 98902-10-4; $PhCH_2N(Me)(CH_2)_3N(Bu$-*i*$)COPh\cdot HCl$, 98902-11-5; $PhCH_2N(Me)(CH_2)_4N(Me)COPh\cdot HCl$, 98921-39-2; $NC(CH_2)_2NHMe$, 693-05-0; $NC(CH_2)_2N(Me)COPh$, 23873-66-7; *c*-$PrCON(Me)(CH_2)_2CN$, 98902-16-0; *c*-$C_5H_9CON(Me)(CH_2)_2CN$, 72104-46-2; $NC(CH_2)_2N(Me)COTHF$-2, 72104-44-0; $NC(CH_2)_2N(Me)COR$ (R = 1,4-benzodioxan-2-yl), 79959-06-1; tetrahydro-2-furancarboxylic acid, 16874-33-2; tetrahydro-3-furoic acid, 89364-31-8; 3-methylbenzoyl chloride, 1711-06-4; 3-methoxybenzoyl chloride, 1711-05-3; 3,5-dimethoxybenzoyl chloride, 17213-57-9; 3,4,5-trimethoxybenzoyl chloride, 4521-61-3; 3-pyridinecarbonyl chloride, 10400-19-8; 2-furancarbonyl chloride, 527-69-5; 2-(methylthio)-1,3,4-oxadiazole-5-carbonyl chloride, 62373-33-5; cyclopropanecarbonyl chloride, 4023-34-1; cyclopentanecarbonyl chloride, 4524-93-0; 1,4-benzodioxane-2-carbonyl chloride, 3663-81-8; tetrahydro-2-furancarbonyl chloride, 52449-98-6.

5-(Alkylsulfonyl)salicylanilides as Potential Dental Antiplaque Agents

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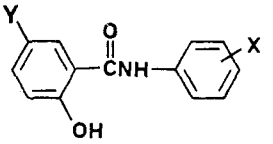
A series of 22 5-(alkylsulfonyl)salicylanilides was synthesized and evaluated for in vitro antibacterial and antiplaque activity against *Actinomyces viscosus* and *Streptococcus mutans*, adherent microorganisms implicated in periodontal disease and dental caries. The minimum inhibitory concentrations of 25 salicylanilides (including 5-acyl-, 5-alkyl-, and 5-(alkylsulfonyl)-4′-bromo- and -4′-(trifluoromethyl)salicylanilides) were found to correlate ($r = 0.94$) with estimated log *D* values. Several salicylanilides, such as 5-(decylsulfonyl)- and 5-(dodecylsulfonyl)-4′-(trifluoromethyl)salicylanilides (**15** and **19**) were found to exhibit high levels of in vitro antibacterial and antiplaque activity against *A. viscosus* and *S. mutans*.

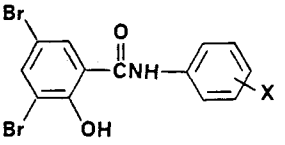
A number of 5-acyl- and 5-alkylsalicylanilides (**1a** and **1b**) have been reported to exhibit high levels of antibacterial activity against *Actinomycetes*,[1] adherent bacteria associated with marginal inflammatory gingivitis.[2] The development of **1a** and **1b** was based upon the antibacterial properties of 3,4′,5-tribromosalicylanilide (tribromsalan, TBS, **2a**), as well as the caries-inhibiting activity of fluorophene (**2b**) in the rat.[3-6] Salicylanilides, such as **1a**, were found to exhibit significant in vitro antiplaque activity in a quantitative antiplaque bioassay reflecting oral conditions.[7-9] TBS (**2a**) was a component of an oral preparation found to exhibit clinical effects against plaque formation and gingivitis in man.[10,11] Since usage of halogenated salicylanilides such as **2a** and **2b** has been restricted by the FDA,[12] due to photoallergic effects observed only with halogenated derivatives,[13] new nonhalogenated salicylanilides were sought with antimicrobial properties optimized against oral bacteria associated with gingivitis, periodontal disease, and caries.

Several 5-acyl derivatives, **1a**, appear to be more effective against *Actinomyces viscosus* in vitro than TBS.[1] One derivative of **1a**, Y = *n*-decanoyl, X = 4′-nitro (**1d**), has been reported to be more effective than TBS in inhibiting the development of gingivitis in Beagle dogs, when employed in an oral mouthrinse preparation.[14] The salicylanilides have not been reported to display the undesirable organoleptic and staining properties associated with cationic surfactant antimicrobials, and therefore, they represent a promising class of agents for the topical control of the development of caries and periodontal disease.

Since the electron-withdrawing 5-acyl group appeared more effective than the 5-alkyl group in enhancing both antimicrobial properties and solubility of these very lipophilic derivatives,[1] the 5-alkylsulfonyl group was chosen



1a, Y = -CR (O)
b, Y = $-CH_2R$
c, Y = $-SO_2R$



2a, X = 4′Br
b, X = 3′CF_3

as an alternative substituent which would further increase phenol acidity and permit incremental adjustments in

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(1) Coburn, R. A.; Batista, A. J.; Evans, R. T.; Genco, R. J. *J. Med. Chem.* **1981**, *24*, 1245.
(2) Ellen, R. P. In "Host–Parasite Interactions in Periodontal Diseases"; Genco, R. J., Mergenhagen, S. E., Eds.; American Society for Microbiology: Washington, DC, 1982; pp 98–111.
(3) Baker, R. J.; Coburn, R. A.; Genco, R. J.; Evans, R. T *J. Periodont. Res.* **1978**, *13*, 474.
(4) Coburn, R. A.; Baker, P. J.; Evans, R. T.; Genco, R. J.; Fischman, S. J. *J. Med. Chem.* **1978**, *21*, 828.
(5) Muhlmann, H. R. *Helv. Odont. Acta* **1973**, *17*, 99.
(6) Tikus, H. W. *Helv. Odont. Acta* **1973**, *17*, 105.
(7) Evans, R. T.; Baker, P. J.; Coburn, R. A.; Genco, R. J. *J. Dent. Res.* **1977**, *56*, 559.
(8) Evans, R. T.; Baker, P. J.; Coburn, R. A.; Genco, R. J. *J. Periodontol.* **1977**, *48*, 156.

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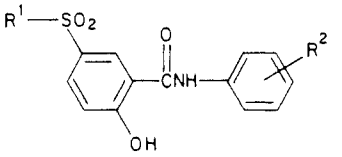
Table I. 5-(*n*-Alkylsulfonyl)salicylanilides and Their in Vitro Activity against *Actinomyces viscous* M100-2000



no.	R^1	R^2	mp, °C	formula[a]	MIC[b]
2a					0.5
5	CH_3	H	230–233	$C_{14}H_{13}NO_4S$	≥1000
6	CH_3	4-Br	268–270	$C_{14}H_{12}BrNO_4S$	≥1000
7	CH_3	4-CF_3	217–219	$C_{15}H_{12}NO_4SF_3$	1000
8	*n*-C_4H_9	4-Br	215–217	$C_{17}H_{19}BrNO_4S$	20
9	*n*-C_4H_9	4-CF_3	239–241	$C_{18}H_{19}NO_4SF_3$	20
10	*n*-C_6H_{13}	4-Br	203–205	$C_{19}H_{22}BrNO_4S$	5
11	*n*-C_6H_{13}	4-CF_3	203–205	$C_{20}H_{22}NO_4SF_3$	5
12	*n*-C_8H_{17}	4-CF_3	173–175	$C_{22}H_{26}NO_4SF_3$	0.5
13	*n*-C_8H_{17}	4-NO_2	167–169	$C_{21}H_{28}N_2O_6S$	1.0
14	*n*-$C_{10}H_{21}$	4-Br	162–163	$C_{21}H_{30}BrNO_4S$	0.5
15	*n*-$C_{10}H_{21}$	4-CF_3	155–157	$C_{24}H_{30}NO_4SF_3$	0.1
16	*n*-$C_{10}H_{21}$	4-NO_2	149–150	$C_{23}H_{30}N_2O_6S$	0.5
17	*n*-$C_{10}H_{21}$	3-CF_3	108–109	$C_{24}H_{30}NO_4SF_3$	0.5
18	*n*-$C_{12}H_{25}$	4-Br	162–163	$C_{25}H_{34}BrNO_4S$	0.5
19	*n*-$C_{12}H_{25}$	4-CF_3	140–142	$C_{26}H_{34}NO_4SF_3$	0.05
20	*n*-$C_{12}H_{25}$	4-NO_2	147–149	$C_{25}H_{34}N_2O_6S$	0.5
21	*n*-$C_{12}H_{25}$	3-CF_3	114–116	$C_{26}H_{34}NO_4SF_3$	0.1
22	*n*-$C_{12}H_{25}$	4-CN	151–152	$C_{26}H_{37}NO_4S$	1
23	*n*-$C_{12}H_{25}$	4-OCH_3	125–126	$C_{26}H_{37}NO_4S$	1000
24	*n*-$C_{14}H_{29}$	4-CF_3	139–140	$C_{28}H_{38}NO_4SF_3$	NE[c]
25	*n*-$C_{14}H_{29}$	4-NO_2	132–134	$C_{27}H_{28}N_2O_6S$	0.5
26	*n*-$C_{14}H_{29}$	3-CF_3	120–122	$C_{28}H_{38}NO_4SF_3$	NE[c]

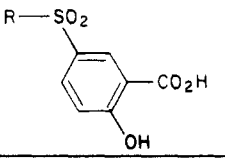
[a] Elemental analyses obtained for C, H, and N for all compounds were in agreement (0.4%) with theoretical values. [b] MIC values in micrograms/milliliter. [c] Not effective.

lipophilicity necessary to optimize antibacterial properties. A series of 5-(alkylsulfonyl)salicylanilides (1c) was prepared and examined for antibacterial effects upon *A. viscosus* in a tube dilution assay. Several derivatives of this class were also evaluated in an in vitro antiplaque assay, employing enamel slabs, against both *A. viscosus* and *Streptococcus mutans*.

Chemistry. The 5-(alkylsulfonyl)salicylanilides shown in Table I were obtained from the corresponding 5-(alkylsulfonyl)salicylic acids. The 5-(alkylsulfonyl)salicylic acids (Table II) were prepared by chlorosulfonation of salicylic acid with chlorosulfuric acid.[15] Reduction of the resulting 5-(chlorosulfonyl)salicylic acid with sodium sulfite in a basic aqueous media, followed by alkylation with a 1-haloalkane in methanol with sodium carbonate, gave the corresponding 5-(alkylsulfonyl)salicylic acid.[16]

The salicylanilides were prepared by two analogous procedures.[17,18] The first involved condensation of the salicylic acid derivative with a substituted aniline in the presence of phosphorus trichloride in dry chlorobenzene. The second method involved conversion of the salicylic acid derivative into its acid chloride with thionyl chloride and a catalytic amount of dimethylformamide in dry toluene, isolation of the acid chloride, and addition of the substituted anilide in dry toluene.

Table II. Properties of 5-(Alkylsulfonyl)salicyclic Acid Derivatives



no.	R	mp, °C	yield, %	formula[a]
27	CH_3	201–202	60	$C_8H_8O_3S$[b]
28	*n*-C_4H_9	120–122	25	$C_{11}H_{14}O_5S$
29	*n*-C_6H_{13}	84–86	45	$C_{13}H_{18}O_5S$
30	*n*-C_8H_{17}	86–88	60	$C_{15}H_{22}O_5S$
31	*n*-$C_{10}H_{21}$	98–99	70	$C_{17}H_{26}O_5S$
32	*n*-$C_{12}H_{25}$	99–100	70	$C_{19}H_{30}O_5S$
33	*n*-$C_{14}H_{29}$	107–108	60	$C_{21}H_{34}O_5S$[b]

[a] Elemental analyses obtained for C, H, and N for all compounds (except where indicated) were in agreement (0.4%) with theoretical values. [b] Employed without further purification.

Results and Discussion

Table I lists the 5-(alkylsulfonyl)salicylanilide derivatives together with the minimum inhibitory concentrations (MIC) against *Actinomyces viscosus* M100-2000. MIC values were determined by standard tube dilution techniques employing Trypticase soy broth with visual evaluation made after 24 h.

A series of 5-(alkylsulfonyl)-4′-bromosalicylanilides (**6**, **8**, **10**, **14**, and **18**) was examined in order to determine the effect upon antibacterial activity against *A. viscosus* produced by variation of the length of the alkyl chain. Only the more lipophilic compounds **14** and **18** demonstrated

(9) Katz, S.; Park, K. *J. Dent. Res.* **1975**, *54*, 540.
(10) Fischman, S.; Cancro, L.; Pader, M.; Bolton, S. *J. Periodontol.* **1973**, *44*, 100.
(11) Fischman, S.; Cancro, L.; Pader, M.; Bolton, S.; Picozzi, A. *J. Periodontol.* **1973**, *44*, 535.
(12) OTC Topical Antimicrobial Products and Drug and Cosmetic Products, *Fed. Regist.* **1974**, 39(179), 33, 102.
(13) Harber, L. C.; Harris, H.; Baer, R. *J. Invest. Dermatol.* **1966**, *46*, 303.
(14) Ritchey, T. W.; Lamster, J. B.; Picozzi, A. In "The Effect of TBS and AN-10 on Developing Gingivitis in the Beagle"; Annual Session, American Association for Dental Research, March 1983, Cincinnati; abstract in *J. Dent. Res.* **1983**, *62*, 199.
(15) Steward, J. *J. Chem. Soc.* **1922**, *21*, 2555.
(16) Clark, R. D.; Field, L. "Organic Syntheses"; Wiley: New York, 1963; Collect Vol. IV, p 674.
(17) Lange, W. E.; Anderson, J. C. *J. Soc. Cosmet. Chem.* **1966**, *17*, 355.
(18) Schultz, H. W. *J. Pharm. Sci.* **1961**, *50*, 832.

Table III. Minimum Inhibitory Concentrations and Physiochemical Constants of the 5-Alkyl- and 5-Acyl-4′-bromosalicylanilides Used in Equations 1–3

				log (1/MIC)[e]						
					calcd			Δ		
no.[a]	log P[b]	pK_a[c]	log D[d]	obsd	eq 1[f]	eq 2[g]	eq 3[h]	eq 1[f]	eq 2[g]	eq 3[h]
1	6.15	5.85	4.78	5.95	5.48	5.48	5.44	0.47	0.47	0.51
15	4.13	7.10	3.78	4.47	4.78	4.83	4.75	−0.31	−0.37	−0.27
16	5.26	6.85	4.75	5.87	5.46	5.46	5.42	0.41	0.41	0.45
18	4.58	5.80	3.16	5.05	4.37	4.44	4.32	0.68	0.61	0.73
19	4.61	7.35	4.38	5.09	5.20	5.22	5.16	−0.11	−0.13	−0.07
20	5.61	7.35[i]	5.38	5.35	5.89	5.87	5.85	−0.54	−0.52	−0.50
21	6.09	7.35[i]	5.88	6.54	6.23	6.19	6.20	0.31	0.35	0.34
22	7.11	7.35[i]	6.88	7.58	6.92	6.83	6.89	0.66	0.75	0.69
25	5.06	5.60	3.46	3.94	4.56	4.63	4.53	−0.62	−0.69	−0.59
26	7.56	5.60[j]	5.96	6.02	6.29	6.24	6.25	−0.27	−0.22	−0.24
27	4.52	6.10	3.88	4.43	4.51	4.58	4.47	−0.08	−0.15	−0.04
28	6.02	6.10[k]	4.88	5.60	5.55	5.55	5.51	−0.05	−0.05	−0.09
29	7.02	6.10[k]	5.88	5.92	6.24	6.17	6.20	−0.32	−0.27	−0.28
30	8.02	6.10[k]	6.88	6.87	6.92	6.83	6.89	−0.05	0.04	−0.02
31	9.02	6.10[k]	7.88	7.37	7.61	7.47	7.58	−0.24	−0.10	−0.21

[a] Compound numbers and values are taken from Table II in ref 1. [b] Log P = 3.27 (salicylanilide, ref 19) + 0.86 (π, 4′-Br) + π, R′ (phenolic hydrophobicity substituent constants from ref 20). [c] Spectroscopically measured values unless otherwise noted, ref 21. [d] Calculated at pH 7.2 with the relationship log D = log P + log [1/(1 + 10^{pH-pK_a})] reported in ref 22. [e] Molar MIC values. [f] Value obtained in eq 1. [g] Value obtained in eq 2. [h] Value obtained in eq 3. [i] Estimated from compound 19. [j] Estimated from compound 25. [k] Estimated from compound 27.

Table IV. Minimum Inhibitory Concentrations and Physiochemical Constants of the 5-(Alkylsulfonyl)-4′-bromo- and 4′-(Trifluoromethyl)salicylanilides Used in Equations 2 and 3

				log (1/MIC)[d]				
					calcd		Δ	
no.	log P[a]	pK[b]	log D[c]	obsd	eq 2[e]	eq 3[f]	eq 2[e]	eq 3[f]
7	2.52	6.2	1.48	2.56		3.16		−0.60
8	4.12	5.2	2.48	4.31	4.00	3.85	0.31	0.46
9	4.14	6.2[g]	3.10	4.31		4.28		0.03
10	5.20	5.2[h]	3.20	4.94	5.10	5.09	0.15	−0.09
11	5.22	6.2[g]	4.18	4.94		5.03		−0.09
12	6.30	6.2[g]	5.26	5.96		5.77		0.19
14	7.36	5.2[g]	5.36	6.00	5.85	5.84	0.15	0.16
15	7.38	6.2[g]	6.34	6.69		6.52		0.17
18	8.44	5.2[h]	6.49	6.02	6.55	6.59	−0.53	−0.57
19	8.46	6.2[g]	7.42	7.01		7.26		−0.25

[a] Log P = 3.27 (salicylanilide, ref 19) + π (4′-halogen, ref 20) + π (R′, phenolic hydrophobicity substituent constants from ref 20). [b] Spectroscopically measured values unless otherwise noted, ref 21. [c] Calculated at pH 7.2 with the relationship log D = log P [1/(1 + 10^{pH-pK_a})], ref 22. [d] Molar MIC values. [e] Value obtained in eq 2. [f] Value obtained in eq 2. [g] Estimated from compound 7. [h] Estimated from compound 6.

in vitro activity comparable to that displayed by TBS (**2a**). Examination of the structure–antibacterial activity relationships revealed that as the alkyl chain length increased so did the in vitro activity. This was also the observation made for the 5-acyl- and 5-alkylsalicylanilides.[1]

It was reported that, for a series of 14 5-alkyl- and 5-acyl-4′-bromosalicylanilides (Table III), the regression of log 1/MIC vs. log P gave only a marginal correlation, r = 0.84, but the regression of log 1/MIC vs. the log of the apparent lipophilicity, at the pH of the bioassay, showed an improved correlation, r = 0.920 (eq 1).[1]

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

$$n = 15,\ r = 0.920,\ r^2 = 0.846,\ s = 0.43 \qquad (1)$$

In order to determine whether the 5-(alkylsulfonyl)-4′-bromo derivatives were acting a manner similar to the 5-acyl and 5-alkyl-4′-bromo derivatives, a quantitative structure–activity relationship was examined for the 19 4′-bromo derivatives (Tables III and IV). Again, log 1/MIC vs. log P gave only a marginal correlation, r = 0.82, but log 1/MIC vs. log D (eq 2) showed an improved correlation, r = 0.917, which deviated only slightly from the correlation in eq 1. Compound **6**, which exhibited no in vitro activity in the tube dilution assay, was excluded from eq 2 in order to limit the variable range for this linear model. Because of the structural similarity and the correlations between these salicylanilides, it would appear that the 5-alkylsulfonyl derivatives may inhibit *A. viscosus* in a manner similar to the 5-acyl- and 5-alkylsalicylanilides.

$$\log (1/\text{MIC}) = \underset{(4.57)}{0.64\ (\pm 0.14)} \log D + 2.41(\pm 0.38)$$

$$n = 19,\ r = 0.917,\ r^2 = 0.842,\ s = 0.41 \qquad (2)$$

The 4′-trifluoromethyl derivatives of the salicylanilides have been reported to be more active in in vitro antibacterial assays than 4′-bromo derivatives.[6] The 5-(alkylsulfonyl)-4′-(trifluoromethyl) derivatives, as well, exhibited an increase in activity over their analogous 4′-bromo derivatives. The in vitro antibacterial activity increased as a function of increasing alkyl chain length. In fact, the in vitro activities of compounds **15** and **19** were higher than those of TBS or the 5-(alkylsulfonyl)-4′-bromo derivatives. A quantitative structure–activity relationship was examined for 25 salicylanilides which included the 4′-bromo- and 4′-(trifluoromethyl)(alkylsulfonyl)salicylanilides (Tables III and IV). The correlation of log 1/MIC vs. log D showed an increase in the regression coefficient to r = 0.945 (eq 3), indicating a strong relationship between activity and the apparent lipophilicity. Compounds **24** and **26**,

Table V. In Vitro Antiplaque Activity of the Salicylanilides against *Actinomyces viscosus* and *Streptococcus mutans*[a]

	A. viscous[b]		*S. mutans*[c]	
compd	IC_{50},[d] total growth	max % inhib[e]	IC_{50},[d] total growth	max % inhib[e]
2a	0.88	53% at 0.2%	1.2	46% at 0.2%
15	0.09	95% at 0.2%	1.9	76% at 0.2%
16	0.23	93% at 0.2%	0.19	80% at 0.2%
17	0.68	83% at 0.1%	2.3	47% at 0.1%
1d	0.11	96% at 0.1%	23.0	59% at 0.2%

[a] Evans et al., ref 5. [b] Strain M-100-2000. [c] Strain 6715-13 WT. [d] Millimolar concentrations inhibiting growth 50% relative to controls. [e] At indicated w/v concentration.

which exhibited little or no in vitro activity in the tube dilution assay, were excluded from eq 3 to limit the variable range. Attempted correlations with log D employing

$$\log (1/\mathrm{MIC}) = \underset{(6.90)}{0.69\ (\pm 0.10)}\ \log D + 2.14(\pm 0.35)$$

$$n = 25,\ r = 0.945,\ r^2 = 0.894,\ s = 0.41 \qquad (3)$$

electronic terms for the 5-position (Y = CH_2, CO, or SO_2) and the 4'-position (X = Br or CF_3) resulted in no significant improvement in the correlations.

The 4'-nitro derivatives were also evaluated for in vitro activity against *A. viscosus*. Among the derivatives investigated for activity, compounds **16**, **20**, and **25** showed activity comparable to that of TBS. The apparent ineffectiveness of compounds **24** and **26**, in spite of the activity of **25**, is most likely a consequence of the greater insolubility of the former two tetradecyl derivatives in the growth media.

The 5-alkylsulfonyl derivatives were found, in general, to be more acidic than either the 5-acyl- or 5-alkyl derivatives of the salicylanilides (Tables III and IV). This may enhance the ability of these agents to be formulated in aqueous media at physiological pH by increasing the percentage of anionic form present. Compounds **15–17** were tested in a quantitative in vitro antiplaque assay developed by Evans et al.[2] These agents were shown to have significant activity in this assay (Table V). In fact, compounds **15** and **16** were more effective than TBS or the 5-acyl derivative **1d** against both *A. viscosus* and *S. mutans*. This assay demonstrates that these compounds are substantive to pellicle-coated enamel surfaces and inhibit the growth of these microorganisms associated with periodontal disease and caries.

In conclusion, it has been demonstrated that high levels of antibacterial activity against *Actinomycetes* can be obtained among the 5-(alkylsulfonyl)salicylanilides. Among these derivatives, a number appeared to be more active in vitro than TBS in both a tube dilution assay for antibacterial activity and a quantitative in vitro antiplaque assay reflecting oral conditions. These compounds represent excellent candidates for further preclinical evaluations in animal models for the topical control of the development of plaque, caries, and peridontal disease.

Experimental Section

Materials and Methods. Melting points were determined with a Fisher-Johns hot stage apparatus or a Thomas-Hoover melting point 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 7199FT interferometer. ^1H NMR spectra were recorded with a Varian T-60 spectrometer using 1% (v/v) Me_4Si as an internal standard. UV spectra were recorded with a Varian-Cary 118 spectrophotometer. Measurements of pH were made with an Orion 901 microprocesser ion analyzer.

5-(Chlorosulfonyl)salicylic Acid (34). A 250-mL flask was fitted with a drying tube and a magnetic stirrer. Chlorosulfuric acid (125 mL) was placed in the flask and cooled to 0 °C. Salicylic acid (25 g, 0.38 mol) was gradually added to the cold chlorosulfuric acid in small portions. The reaction mixture was heated at 75 °C for 1 h, cooled, and poured carefully onto crushed ice (500 g). The resultant white solid was collected and recrystallized from methylene chloride to give **34**: yield 42.8 g (60%); mp 170–172 °C (lit.[15] mp 169–171 °C).

5-Sulfinylsalicylic Acid (35). In a 500-mL flask, equipped with a magnetic stirrer, sodium sulfite (20 g) in H_2O (100 mL) was stirred with **34** (5 g, 21.2 mmol) gradually added (the reaction mixture was kept basic by periodic addition of 20% aqueous NaOH). The reaction mixture was acidified to pH 2 with 20% sulfuric acid and extracted with diethyl ether (3 × 250 mL). The ether extracts were dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to give **35**: yield 3.6 g (85%); mp 154–157 °C (lit.[15] mp 159 °C).

5-(Alkylsulfonyl)salicylic Acid. General Procedure. A 100-mL three-neck flask was fitted with a 25-mL addition funnel and a condenser with an attached drying tube. A mixture of Na_2CO_3 (1 equiv) and methanol (50 mL) was placed in the flask and **35** (3 equiv) was added. The mixture was stirred at room temperature for 20 min and then the mixture was heated at reflux and a 1-haloalkane (8 equiv) was added from the addition funnel over a period of 1 h. After refluxing for 48 h, the solvent was removed under reduced pressure and the residue was dissolved in 95% EtOH (100 mL). A 10% aqueous NaOH (50 mL) solution was added, and the mixture heated at reflux for 24 h, cooled, and acidified with 20% HCl. The mixture was extracted with diethyl ether (3 × 100 mL), and the ether extracts were dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to give a solid, which was recrystallized from CCl_4 to give the product.

5-(Alkylsulfonyl)salicylanilide (1c). General Procedure. Method A. In a 100-mL flask, fitted with a condenser and drying tube and a magnetic stirrer, an appropriate salicylic acid derivative, phosphorus trichloride, and dry chlorobenzene were heated at reflux for 1 h. The mixture was cooled in an ice bath and an aniline in dry chlorobenzene was added slowly. The mixture was stirred for 30 min at 25 °C and then heated at reflux for 3 h. The mixture was cooled, the solvent removed under pressure, and the residue recrystallized from an appropriate solvent.

Method B. In a 100-mL flask, fitted with a condenser and a drying tube and a magnetic stirrer, an appropriate salicylic acid, thionyl chloride, catalytic amount of dimethylformamide, and dry toluene were heated at 50 °C for 1 h. The mixture was cooled and concentrated under reduced pressure to give an oil. The oil was dissolved in dry toluene and an aniline was added. The mixture was heated at reflux for 2 h, cooled, and concentrated under reduced pressure to give a residue which was recrystallized from an appropriate solvent to give the product.

Determination of pK_a for the Salicylanilides. The spectroscopic method described by Albert and Serjeant[21] was employed with use of 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 260 to 370 nm. The average pK_a values were determined in triplicate as three pH values fell within the range ±0.10.

Biological Assay. Microorganisms and Media. Bacterial cultures used were *Actinomyces viscosus* M100-2000 and *Streptococcus mutans* 6715-13WT and were obtained from Dr. R. T. Evans's laboratory at the Department of Oral Biology, SUNY/Buffalo. All cultures were maintained on trypticase soy broth

(19) Leo, A.; Hanch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525.
(20) Fujita, T.; Iwasa, J.; Hanch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5157.
(21) Albert, A.; Serjeant, E. P. "Ionization Constants of Acids and Bases"; Methuen: London, 1962.
(22) Scherrer, R. A.; Howard, S. M. *J. Med. Chem.* **1977**, *20*, 53.

(TBS) with an excess of calcium carbonate.

In Vitro Antibacterial Assay. Stock solutions of test compounds were prepared at concentrations of 1 mg/100 μL of EtOH or Me_2SO and diluted with growth medium to give threefold dilutions (1 mL) in trypticase soy broth. Test concentrations ranged from 1000 to 0.1 μg/mL. Following inoculation with 50 μL of 10^8 cfu/mL of log phase growth inoculum of *S. mutans*, tubes were incubated at 37 °C in an anaerobic chamber containing an atmosphere of 5% CO_2, 10% H_2, and 85% Ar. Conditions for *A. viscosus* were identical except incubation was aerobic. MIC values were determined visually after incubation 24 h.

In Vitro Antiplaque Assay. Bovine teeth were cut into 4 × 8 mm enamel slabs and sterilized by autoclaving in 0.15 M sodium chloride buffered at a pH of 7.5 with 0.02 M sodium phosphate (PBS). Tooth slabs were coated with sterile human saliva by shaking them in a tight lid dish on a rotating platform at 150 rpm at 37 °C for 1 h. The saliva-coated tooth slabs were rinsed twice in PBS and put in the drug solution for 2 min. The drug solution was removed, and the tooth slabs were rinsed in PBS for 1 min. Each slab was placed in 10 × 75 cotton-stoppered glass culture tubes containing 1 mL of growth medium (supplemented with 1% sucrose) which was inoculated with 50 μL of the plaque-forming organism. Incubation was for 24 h at 37 °C under anaerobic conditions as required. After this period, nonadherent cells were removed by pipetting off the supernatant. Cells adherent to the tooth slabs or to the culture tube were washed twice with 0.5 mL of PBS and the washings combined with previously removed nonadherent cells. In this process, three fractions were obtained: tooth adherent, glass adherent, and nonadherent cells. One mL of 0.1 N NaOH was added to each culture containing tooth slab and glass adherent cells. Cells adherent to tooth slabs were suspended by sonification for 20 s with a sonifier equipped with a microtip. Nonadherent cells were centrifuged at 1500*g* for 20 min at 4 °C. The supernatants were removed with a pipet and the cells were resuspended in 1 mL of PBS. The cells were centrifuged a second time and the pellet resuspended in 1 mL of 0.1 N NaOH. The optical density (OD) of each of the three fractions was read at 540 nm on a Beckman DU-2 spectrophotometer in a cuvette with a 1-cm light path. Cell adherence to tooth slabs and glass is referred to as plaque formation. Controls consisted of tooth slabs immersed for 2 min in sterile distilled water and then incubated in inoculated medium. All experiments were run in triplicate. The combined readings from nonadherent and adherent cells is referred to as total growth. For the compounds reported in this study, effects upon adherence correlated with those upon total growth. Effects upon total growth are reported since these values were more reproducible. Total growth values for the controls represent 100% growth. The IC_{50} values for inhibition of total growth were determined by probit analyses of the inhibition of total growth vs. test concentrations of the compounds. In a typical analysis the standard error in measurement was less than 25% of the mean value obtained in triplicate runs.

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Registry No. **2a**, 87-10-5; **5**, 98688-41-6; **6**, 98688-42-7; **7**, 98688-43-8; **8**, 98688-44-9; **9**, 98688-45-0; **10**, 98688-46-1; **11**, 98703-76-5; **12**, 98688-47-2; **13**, 98688-48-3; **14**, 98688-49-4; **15**, 98688-50-7; **16**, 98688-51-8; **17**, 98688-52-9; **18**, 98688-53-0; **19**, 98688-54-1; **20**, 98688-55-2; **21**, 98688-56-3; **22**, 98688-57-4; **23**, 98688-58-5; **24**, 98688-59-6; **25**, 98688-60-9; **26**, 98688-61-0; **34**, 17243-13-9; **35**, 19479-88-0; salicylic acid, 69-72-7; 5-(methylsulfonyl)salicylic acid, 68029-77-6; 5-(butylsulfonyl)salicylic acid, 80955-64-2; 5-(*n*-hexylsulfonyl)salicylic acid, 98688-62-1; 5-(*n*-octylsulfonyl)salicylic acid, 98688-63-2; 5-(*n*-decylsulfonyl)salicylic acid, 98688-64-3; 5-(*n*-dodecylsulfonyl)salicylic acid, 98688-65-4; aniline, 62-53-3; 4-bromoaniline, 106-40-1; 4-(trifluoromethyl)-aniline, 455-14-1; 4-nitroaniline, 100-01-6; 3-(trifluoromethyl)-aniline, 98-16-8; 5-(*n*-tetradecylsulfonyl)salicylic acid, 98688-66-5; 4-cyanoaniline, 873-74-5; 4-methoxyaniline, 104-94-9.

Synthesis and Structure–Activity Relationships of Antibacterial Phosphonopeptides Incorporating (1-Aminoethyl)phosphonic Acid and (Aminomethyl)phosphonic Acid

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Phosphonodipeptides and phosphonooligopeptides based on L- and D-(1-aminoethyl)phosphonic acids L-Ala(P) and D-Ala(P) and (aminomethyl)phosphonic acid Gly(P) at the acid terminus have been synthesized and investigated as antibacterial agents, which owe their activity to the inhibition of bacterial cell-wall biosynthesis. A method for large-scale synthesis of the potent antibacterial agent L-Ala-L-Ala(P) (1, Alafosfalin) is described. Structure–activity relationships in the dipeptide series have been studied by systematic variation of structure 1. L stereochemistry is generally required for both components. Changes in the L-Ala(P) moiety mostly lead to loss of antibacterial activity, but the phosphonate analogues of L-phenylalanine, L-Phe(P), and L-serine, L-Ser(P), give rise to weakly active L-Ala-L-Phe(P) and L-Ala-L-Ser(P). Replacement of L-Ala in 1 by common and rare amino acids can give rise to more potent in vitro antibacterials such as L-Nva-L-Ala(P) (45). Synthetic variation of these more potent dipeptides leads to decreased activity. Phosphonooligopeptides such as $(L\text{-Ala})_2$-L-Ala(P) have a broader in vitro antibacterial spectrum than their phosphonodipeptide precursor, but this is not expressed in vivo, presumably due to rapid metabolism to 1. Stabilized compounds such as Sar-L-Nva-L-Nva-L-Ala(P) (46) have been developed that are more potent in vivo and have a broader in vivo antibacterial spectrum than the parent phosphonodipeptide.

In earlier publications[1-7] we have described the characteristics and the mechanism of antibacterial action of Alafosfalin (1) (Alafosfalin is a British Approved Name (B.A.N.) description; it corresponds to earlier names: Ro

(1) Allen, J. G.; Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Holmes, S. W.; Lambert, R. W.; Nisbet, L. J.; Ringrose, P. S. *Nature (London)* **1978**, *272*, 56.
(2) Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Lambert, R. W.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1979**, *15*, 677.
(3) Allen, J. G.; Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Holmes, S. W.; Lambert, R. W.; Nisbet, L. J.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1979**, *15*, 684.
(4) Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Lambert, R. W.; Lloyd, W. J.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1979**, *15*, 696.

0022-2623/86/1829-0029$01.50/0