bromide (3a). A 30% HBr solution in AcOH (4 ml) was added to DL-α-(*N*-carbobenzoxy-D-alanylamino)propionhydroxamic acid (**13a**, 0.225 g, 00.73 mmol) in a reaction flask protected with a $CaCl_2$ tube under ice–water cooling. After standing 24 hr, 30 ml of absolute Et_2O was added and the resulting oil was separated by decantation. The oil was washed with Et_2O several times and treated with a small amount of EtOH to give a solid. Recrystallization from EtOH–Et_2O gave a pale yellow solid: 0.15 g (88.3%); mp 200–201°; $[\alpha]_D$ 33.0° (*c* 0.5, H_2O); ir (KBr) 3280, 3400, 1680, 1668 cm^{-1}. Anal. ($C_6H_{14}N_3O_3Br$) C, H, N.

D-α-(D-Alanylamino)propionhydroxamic Acid Hydrobromide (3b). This compound was prepared by the same method described above: yield 72.6%; mp 200–202° dec; $[\alpha]_D$ 27.4° (*c* 0.5, H_2O); ir (KBr) 3300, 3400, 1680 cm^{-1}. Anal. ($C_6H_{14}N_3O_3Br$) C, H, N.

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Synthesis and in Vitro Evaluation of 8-Hydroxyquinoline Analogs as Inhibitors of Dental Plaque†,[1]

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A group of 5-substituted 8-hydroxyquinolines with predicted log *P* values in the 1–4 range has been prepared from either 8-hydroxyquinoline or its appropriate derivative. 5-Formyl-, 5-iodo-, 5-fluoro-, 5-acetyl-, and 5-methoxymethyl-8-hydroxyquinoline in addition to methyl 5-(8-hydroxyquinolyl)acetate and ethyl 5-(8-hydroxyquinolyl)acetate displayed greater in vitro antiplaque activity than 8-hydroxyquinoline.

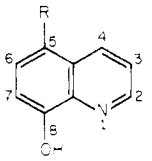
Dental plaque adheres to teeth and soft tissue and consists primarily of oral bacteria and inorganic salts in a matrix of proteins and polysaccharides. The presence of plaque has been linked to gingivitis, periodontal disease, and caries. It is thought that a principle event in plaque formation is the synthesis of polysaccharides by certain oral bacteria. These polysaccharides have been shown to induce precipitation of *Streptococcus mutans* which facilitate colonization of this bacteria and, in turn, serve as a point of attachment for the other components of plaque. Since a bacterial by-product is felt to initiate plaque formation, many antibacterials have been tested for their ability to inhibit the bacteria responsible for the formation of plaque. The most promising development in this area was the discovery that chlorhexidine was a very effective inhibitor of this organism. Clinical studies revealed that this agent caused soreness of the oral mucosa and discoloration of tooth surfaces, the tongue, and gingiva. During an initial screening of antibacterial agents for antiplaque activity, the 8-hydroxyquinolines were shown to have good activity.[1] Utilizing commercially available 8-hydroxyquinolines we recently demonstrated a correlation between antiplaque activity and partition coefficient (log *P*) values.[2] It was observed that only compounds having log *P* values between 1 to 4 displayed antiplaque activity. Based on this information we synthesized a group of 5-substituted 8-hydroxyquinolines with predicted log *P* values in the 1–4 range in an attempt to optimize antiplaque activity.

† This is dedicated to Professor Edward Smissman who will always be remembered by V. D. W. and D. B. M. as an outstanding medicinal chemist, educator, and friend.

Chemistry. 5-Cyanomethyl-8-hydroxyquinoline (**7**) was prepared by an entirely different method than that previously reported by Pujari and Rout.[8] 5-Chloromethyl-8-hydroxyquinoline hydrochloride was allowed to react with sodium cyanide in Me_2SO at 90° for 45 min to give a good yield of 5-cyanomethyl-8-hydroxyquinoline (**7**). Acidic hydrolysis of the cyano compound **7** gave the corresponding acid analog. Methyl 5-(8-hydroxyquinolyl)acetate (**9**) and ethyl 5-(8-hydroxyquinolyl)acetate (**10**) were obtained in good yields by refluxing 5-(8-hydroxyquinolyl)acetic acid with either 3% methanolic HCl or 3% ethanolic HCl. 5-Amino-8-hydroxyquinoline which was used in the synthesis of 5-fluoro-8-hydroxyquinoline (**12**)[10] was obtained by hydrogenation of 5-nitro-8-hydroxyquinoline using PtO_2 as the catalyst and THF as the solvent. The literature method[9] uses ethanol as the solvent but that gave a dark brown or black product which was very difficult to purify. The hydrogenation product was isolated as the dihydrochloride since the free base is very unstable and decomposes on standing (Table I).

Biological Results. Antiplaque activity as displayed by 8-hydroxyquinoline requires that a compound be an antibacterial agent. Therefore the analogs were first evaluated for their in vitro antibacterial activity against *Strept. mutans* 6715, a pure strain of plaque-forming bacteria (see Experimental Section). 8-Hydroxyquinoline and solvent control were tested concurrently. As shown in Table II, except for compounds **2**, **5**, and **8** all com-

Table I. 5-Substituted 8-Hydroxyquinolines



Compd	R	Mp, °C	Recrystn solvent	Yield, %	Formula	Analyses
1	CHO	173-175[a]	EtOH	10.4	$C_{10}H_7NO_2$	C, H, N
2	OH	181-183[b]	C_6H_6	12.6	$C_9H_7NO_2$	C, H, N
3	I	125-127[c]	EtOH-H_2O	26.7	$C_9H_6INO_2$	C, H, N
4	CH_2Cl	275-280[d]	*e*	73.9	*f*	*f*
5	CH_2OH	134-136[g]	C_6H_6	92.1	$C_{10}H_9NO_2$	C, H, N
6	CH_2OCH_3	79-80[h]	MeOH-H_2O	89.9	$C_{11}H_{11}NO_2$	C, H, N
7	CH_2CN	178-180[i]	C_6H_6	61.1	$C_{11}H_8N_2O$	C, H, N
8	CH_2COOH	277-280	MeOH-Et_2O	85.4	$C_{11}H_9NO_3 \cdot HCl$	C, H, N
9	CH_2COOCH_3	95-96	MeOH-H_2O	84.8	$C_{12}H_{11}NO_3$	C, H, N
10	$CH_2COOC_2H_5$	86-88	EtOH-H_2O	68.9	$C_{13}H_{13}NO_3$	C, H, N
11	NH_2	275[j]	*e*	63.0	*f*	*f*
12	F	109-110[k]	*l*	7.1	C_9H_6FNO	C, H, N
13	$COCH_3$	105-107[m]				

[a] Lit.[3] 173°. [b] Lit.[4] 181-183°. [c] Lit.[5] 125-127°. [d] Lit.[6] 280°. [e] Used without crystallization. [f] Compound was not analyzed but used without further purification. [g] Lit.[6] 138-139°. [h] Lit.[7] 78-80°. [i] Lit.[8] >300°. [j] Lit. partial melting at 245° and complete at 275° of dihydrochloride salt. [k] Lit.[9] 110°. [l] Purified by sublimation. [m] Alfred Bader Chemicals.

Table II. 5-Substituted 8-Hydroxyquinolines. Antibacterial Activity

	% inhibition at					
	10^{-4} *M*		10^{-5} *M*		10^{-6} *M*	
Compd[a]	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
8-HQ[b]	100	100	100	100	0	0
1	100	100	0			
2	0	0				
3	100	100	100	60	0	0
5	0	0				
6	100	80	0			
7	80	20	0			
8	0	0				
9	100	100	60	0	0	0
10	100	100	100	0	0	0
12	100	100	80	0	0	0
13	100	100	100	0	0	0

[a] All compounds were evaluated as their HCl salts in aqueous solutions. [b] 8-HQ = 8-hydroxyquinoline.

pounds inhibited bacterial growth at a concentration of 10^{-4} *M* but not 10^{-5} *M* while 8-hydroxyquinoline showed inhibition of bacterial growth at a concentration of 10^{-5} *M* but not 10^{-6} *M*.

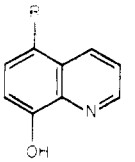
The in vitro antiplaque activity of these compounds was evaluated using the method of Turesky and coworkers[11] which utilizes sterilized extracted human teeth. As shown in Table III, 8-hydroxyquinoline was inactive at a concentration of 10^{-2} *M* while compounds **1**, **3**, **6**, **9**, **10**, **12**, and **13** displayed activity at that concentration. Compounds **5** and **8** were inactive at the highest concentration tested (10^{-1} *M*). In order to look at the relationship between in vitro antiplaque activity and lipophilicity, we determined 1-octanol–water partition coefficients for these compounds. Three important conclusions can be drawn from these data. (1) It is possible to enhance the in vitro antiplaque activity of 8-hydroxyquinoline through proper modification of the phenolic ring system as evidenced by the increased activity of compounds **1**, **3**, **6**, **9**, **10**, **12**, and **13**. (2) A hydroxyl-containing substituent at the 5 position apparently has a detrimental effect on antiplaque activity as is seen for compounds **2**, **5**, and **8**. (3) Inconsistencies between the antiplaque activity and log *P* values of compounds **6**, **7**, and **13** indicate that a single parameter like the log *P* value is not adequate to accurately predict antiplaque activity. For example, the log *P* values of

Table III. 5-Substituted 8-Hydroxyquinolines. Partition Coefficient and Antiplaque Activity

	Log *P* values		% inhibition[a] at			
			10^{-1} *M*		10^{-2} *M*	
Compd[b]	Calcd	Obsd[d]	24 hr	48 hr	24 hr	48 hr
8-HQ[c]	1.96	1.94 (0.034)	100	100	0	0
1	1.58	1.63 (0.007)	100	80	40	40
2	1.15	1.42 (0.081)	60	0	0	0
3	3.39	3.27 (0.050)	100	100	80	20
5	0.81	1.00 (0.009)	0	0		
6	1.46	1.78 (0.070)	80	60	20	20
7	1.39	1.81 (0.083)	80	80	0	0
8	1.41		0	0		
9	1.59	1.59 (0.032)	100	100	20	0
10	2.14	2.26 (0.012)	100	80	20	0
12	2.33	2.30 (0.001)	80	80	80	60
13	1.91	1.80 (0.005)	100	100	60	60

[a] Percentage of teeth which did not show plaque formation after stated incubation period. Subjective estimates were made of plaque formation using a scale from 0 (no growth) to 4 (maximum growth). Because in an overwhelming number of tests growth ratings were either 0 or 4, the findings were expressed as either plaque formation or inhibition. [b] All compounds were evaluated as free bases in Me_2SO solution on five teeth. [c] 8-HQ = 8-hydroxyquinoline. [d] All determinations done in triplicate; values in parentheses indicate standard deviations.

compounds **6**, **7**, and **13** vary by only ±0.02 while their activity at 48 hr ranges from 60 to 100%. On the basis of these findings it would appear that other physicochemical parameters like ionization constants and stability constants also play an important role in determining the antiplaque activity of these compounds.



Compd	R	compd	R
1	CHO	7	CH_2CN
2	OH	8	CH_2CO_2H
3	I	9	$CH_2CO_2CH_3$
4	CH_2Cl	10	$CH_2CO_2C_2H_5$
5	CH_2OH	11	NH_2
6	CH_2OCH_3	12	F
		13	$COCH_3$

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Midwest Microlab Ltd., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Chemistry. Compounds 1–6, 11, and 12 were prepared according to the previously reported methods (see Table I).[3-9]

5-Cyanomethyl-8-hydroxyquinoline (7). To a solution of sodium cyanide (10 g, 0.2 mol) in 150 ml of Me_2SO at 90–100° was slowly added a hot solution of 5-chloromethyl-8-hydroxyquinoline hydrochloride (9.2 g, 0.04 mol) in 100 ml of Me_2SO. The mixture was stirred at 80–90° for 45 min. It was allowed to cool and then cautiously acidified with concentrated HCl in order to decompose the excess NaCN. The brownish solution was then poured onto 500–700 g of crushed ice and neutralized with NaOH. The precipitate was filtered, washed well with water, and dried. Crystallization from benzene gave 4.5 g (61.14%) of **7**: mp 178–180° (lit.[5] mp 300°). Anal. ($C_{11}H_8N_2O$) C, H, N.

5-(8-Hydroxyquinolyl)acetic Acid Hydrochloride (8). A mixture of 5-cyanomethyl-8-hydroxyquinoline (5.52 g, 0.03 mol) and 150 ml of concentrated HCl was refluxed for 3 hr. The reaction mixture was taken to dryness and recrystallized from methanol–ether yielding 6.1 g of **8** (85.4%), mp 277–280°. Anal. ($C_{11}H_9N_3$·HCl) C, H, N.

Methyl 5-(8-Hydroxyquinolyl)acetate (9). A mixture of 5-(8-hydroxyquinolyl)acetic acid hydrochloride (3.0 g, 0.012 mol) and 100 ml of 3% methanolic HCl was refluxed for 3 hr. The mixture was cooled and the methanol removed in vacuo. The residue was dissolved in water and neutralized to give a white precipitate which on crystallization from aqueous methanol gave 2.3 g (84.8%) of **9**, mp 95–96°. Anal. ($C_{12}H_{11}NO_3$) C, H, N.

Ethyl 5-(8-Hydroxyquinolyl)acetate (10). A mixture of 5-(8-hydroxyquinolyl)acetic acid hydrochloride (3.0 g, 0.012 mol) and 100 ml of 3% ethanolic HCl was refluxed for 3 hr. The mixture was cooled and ethanol was removed in vacuo. The residue was dissolved in water and neutralized to give a buff precipitate which on crystallization from aqueous ethanol gave 2.0 g (68.9%): mp 86–88°. Anal. ($C_{13}H_{13}NO_3$) C, H, N.

Partition Coefficients. The compounds were partitioned between 1-octanol saturated with water and distilled water saturated with octanol. Usually 50–150-ml portions of octanol and water were used. In partitioning these compounds, gentle shaking for 90 min was carried out at room temperature (25 ± 5°). The volume ratio of the two phases and the amount of sample were chosen so that, in most cases, the absorbance of the sample from the water layer after partitioning had a value between 0.2 and 0.9 using a 1-cm cell. Only the concentration of the sample in the water layer was determined and that in the octanol was obtained by difference. Analyses of the concentrations of the partitioned substances were made using a Beckmann DB-G spectrophotometer. The partition coefficient was calculated as $P = C_{octanol}/C_{H_2O}$. Each determination was done in at least triplicate using different amounts of sample and the average value for log P has been reported. The partition coefficient of compound 8 was not determined since it exists as a zwitterion.

Antibacterial Activity. To 7.85 ml of sterile trypticase broth, 1 ml of an aqueous solution of test compound and 1 ml of 50% sterile sucrose solution were added. The media was inoculated with 0.15 ml of a 24-hr culture of *Strept. mutans* 6715, a pure strain of plaque-forming bacteria isolated at and made available to us by the National Institute of Dental Research. This mixture was incubated under anaerobic conditions (BBL-Gaspak, BBL, Division of Bioquest, Cockeyville, Md.) at 37°. Bacterial growth was determined after 24 and 48 hr.

Assay for in Vitro Antiplaque Activity. Sterilized extracted human teeth were immersed in Me_2SO solutions of the test compounds for two 1-min periods, each of which was followed by a 1-min exposure to air. These treated teeth were washed with 250 ml of distilled water for 5 min and then incubated at 37° under anaerobic conditions in a sucrose-containing trypticase broth with *Strept. mutans* 6715. The teeth were suspended in test tubes on orthodontic wire (Rocky Mount, 0.71 mm in diameter) threaded through a hole in the root so that the entire tooth was completely immersed. After 24 and 48 hr subjective estimates were made of the adherent microbial growth on the test tube walls, wires, and the teeth and of nonadherent growth in the broth using a scale of 0 (no growth) to 4 (maximum growth). The total microbial accumulation was considered as in vitro plaque. For our results, growth ratings of 0 or 1 have been scored as plaque inhibition. Each compound was evaluated on five teeth with percent inhibition values being reported. This indicates the percentage of teeth which did not show plaque formation after the stated incubation period. The solvent served as the control.

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