From these results, it is evident that the chloroacetyl ester of ajmaline (I) is more effectively hydrolyzed than the acetyl ester (II) by both the atria and ventricles. Also, the apparent affinity for the enzyme is at least 10 times higher for the chloroacetyl than for the acetyl ester, thus substantiating its *in vivo* effect. Further *in vivo* work is being conducted to determine: *(a)* the levels of ajmaline produced at the site of action after administration of I and II and *(b)* the levels reaching the site of action after administration of ajmaline itself.

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Synthesis and *In Vitro* Evaluation of 8-Hydroxyquinolines as Dental Plaque Inhibitors

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Abstract ☐ Some 4- and 5-substituted 8-hydroxyquinolines, with predicted log *P* values in the range of 1.48–2.90, were synthesized by modified Skraup reactions or thermal cyclization. These hydroxyquinolines include the 5-methyl, 4,5-dimethyl, 4-methyl, 5-hydroxy-4-methyl, 5-methoxy, 5-methoxy-4-methyl, 4-hydroxy, 4-chloro, 4-amino, and 5-amino analogs. Partition coefficients, antibacterial activity, and antiplaque activity were determined. Four analogs showed *in vitro* antiplaque activity. None of the derivatives with ionizable functions was active.

Keyphrases ☐ 8-Hydroxyquinoline analogs—synthesis and antibacterial and antiplaque activities ☐ Antibacterial activity—8-hydroxyquinoline analogs ☐ Antiplaque activity—8-hydroxyquinoline analogs

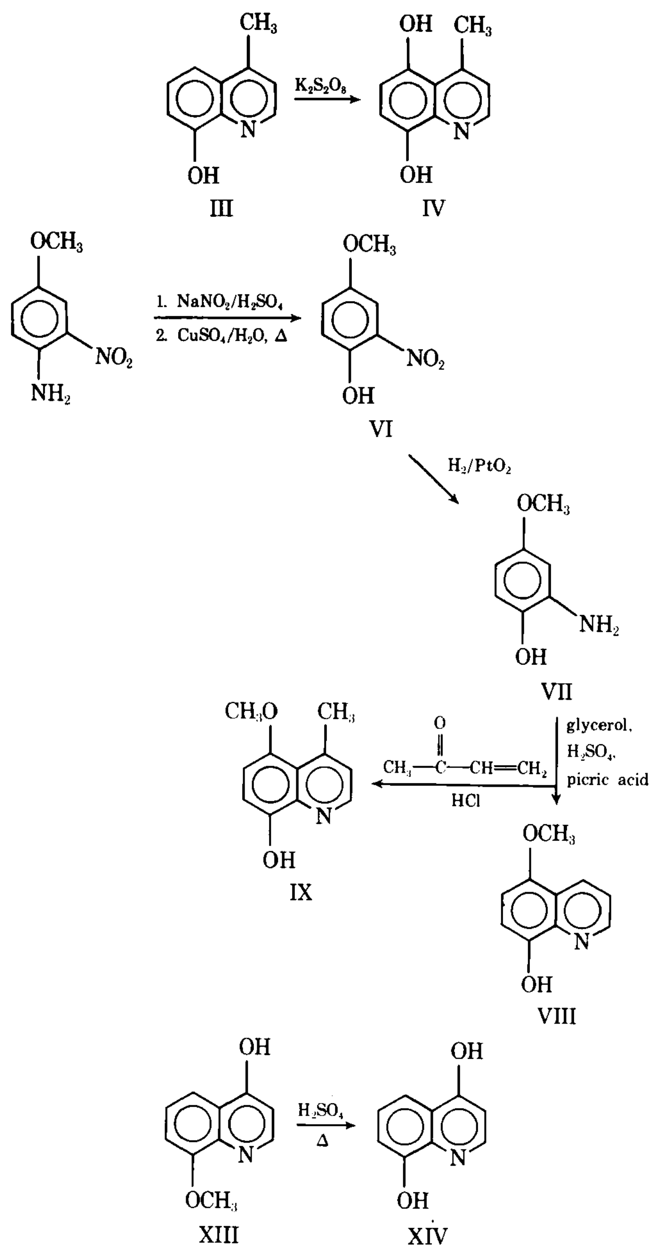
If dental plaque is to be controlled, it must be actively removed or its formation must be prevented. Mechanical cleansing is the principal means of removing plaque and its effectiveness is limited. Since dental plaque consists primarily of bacteria and their metabolites, consideration has been given to controlling plaque formation by use of antibacterial agents administered in dentifrices, mouthwashes, troches, or other appropriate vehicles. The present level of knowledge indicates that bacterial plaque is the direct cause of gingivitis and marginal periodontal disease and that without bacterial plaque there will be a great reduction in the occurrence of caries and peridontitis.

Previous research (1) showed that 8-hydroxyquinoline and some of its analogs inhibit *in vitro* plaque formation. It has been suggested that the antibacterial activity of the 8-hydroxyquinolines and their ability to chelate with trace ions on the surface of teeth, with subsequent slow release, should result in good long-term inhibition of dental plaque. Plaque inhibition by 8-hydroxyquinoline has been correlated favorably with the lipid–water partition coefficients. Analogs with log *P* values in the 1–4 range have been shown to have good antiplaque activity.

In an attempt to optimize antiplaque activity and to investigate other physicochemical factors that influence activity in addition to the partition coefficient, the following compounds with calculated log *P* values in the 1–4 range were synthesized and tested: 5-methyl- (I), 4,5-dimethyl- (II), 4-methyl- (III), 5-hydroxy-4-methyl- (IV), 5-methoxy- (VIII), 5-methoxy-4-methyl- (IX), 4-hydroxy- (XIV), 4-chloro- (XV), 4-amino- (XVII), and 5-amino-8-hydroxyquinoline (XVIII).

Compounds I–III, XV, XVII, and XVIII were prepared by standard methods (Table I). Compounds IV, VIII, and IX are unreported derivatives of 8-hydroxyquinoline, while XIV was previously prepared (10) but by a different method than is presented here. Intermediates VI and VII are known compounds (11, 12), but a different method for their preparation is presented in Scheme I.

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 *Streptococcus mutans* (No. 6715), a pure strain of plaque-forming bacteria. The antiplaque activity of the analogs was evaluated using an *in vitro* screening procedure (2).



Scheme I

EXPERIMENTAL[1]

5,8-Dihydroxy-4-methylquinoline (IV)—4-Methyl-8-hydroxyquinoline (3.18 g, 0.02 mole), sodium hydroxide (4.00 g), and water (120 ml) were stirred and treated dropwise with a solution of potassium persulfate (5.40 g, 0.02 mole) in 100 ml of water. The temperature was maintained between 5 and 10° during the addition. The reaction mixture was stirred for 30 min, refrigerated for 4 days, adjusted to pH 6 with 10% HCl, and filtered. After filtration, the filtrate was extracted with ether (3 × 30 ml).

The aqueous solution was treated with 50 ml of concentrated hydrochloric acid and allowed to stand refrigerated for 4 days. The red crystals which formed were filtered and heated with 30 ml of concentrated hydrochloric acid on a steam bath for 1 hr and then cooled to give 0.4 g (9.5%) of the IV-HCl. Isolation of the free base (neutralization with 20% NaOH) and crystallization from benzene gave the pure compound (IV), mp 294–296°.

4-Methoxy-2-nitrophenol (VI)—A suspension of 22.00 g (0.13 mole) of 4-methoxy-2-nitroaniline in 150 ml of 50% H_2SO_4 was cooled, and 150 ml of ice was added. After a homogeneous mixture and a temperature between 0 and 5° were obtained, a solution of sodium nitrite (26 g + 60 ml of water) was added to the bottom of the mixture while the temperature was maintained. The presence of excess nitrous acid was determined using starch iodide paper. Stirring was continued for 10 min after the completion of the reaction, and the product (V) was used immediately.

A solution containing 350 g of cupric sulfate pentahydrate in 1 liter of water was heated to boiling. The diazonium solution (V) was added slowly as the phenolic product was removed *via* steam distillation. The distillate was filtered, and the precipitate was dissolved in 5% NaOH, filtered, and extracted with ether. The aqueous layer was then acidified with 5% HCl, and the precipitate was recrystallized from petroleum ether, yielding 4.88 g (22.1%) (VI), mp 81–82° [lit. (11) mp 80°].

4-Methoxy-2-aminophenol (VII)—A solution of 5.00 g (0.03 mole) of VI in 150 ml of 95% ethanol and 150 mg of platinum oxide were hydrogenated (120 psi of H_2) for 30 min. The catalyst was filtered off, and the solvent was evaporated to give 3.60 g (95%) of a dark crystalline material (VII), mp 129–132° [lit. (12) mp 135°]. This compound was used without further purification.

5-Methoxy-8-hydroxyquinoline (VIII)—To 4.30 g (0.046 mole) of glycerol were added 2.0 g (0.01 mole) of VII and 0.45 g (0.002 mole) of picric acid. The temperature was raised to 110–120° as 3.50 g (0.04 mole) of concentrated sulfuric acid was added dropwise. The reaction mixture was then stirred and heated to 120–140° for 4 hr. After cooling, 72 ml of water was added and the pH was adjusted to 3 with 5% NaOH.

The product was obtained by steam distillation of the reaction mixture, followed by neutralization with 5 N ammonium hydroxide and extraction with ether. Recrystallization from hexane yielded 0.70 g (28%) of VIII, mp 83–85°.

4-Methyl-5-methoxy-8-hydroxyquinoline (IX)—To 100 ml (3.33 mole) of concentrated hydrochloric acid was added 28.0 g (0.20 mole) of VII. The temperature was raised to 100°, and 35.0 g (0.50 mole) of methyl vinyl ketone was added slowly. The reaction mixture was then stirred and heated for 5 hr at 100–120°. After cooling, the pH was made slightly alkaline with 10% NaOH and the mixture was steam distilled. The product was recrystallized from hexane to yield 5.40 g (14.3%) of IX, mp 130–132°.

4,8-Dihydroxyquinoline (XIV)—A solution of 4-hydroxy-8-methoxyquinoline (XIII) (1.50 g, 0.009 mole), 9.80 ml of concentrated sulfuric acid, and 5.8 ml of water was refluxed for 5.5 hr. Then the solution was poured over crushed ice and neutralized with concentrated ammonium hydroxide to precipitate the product. Recrystallization from water yielded 0.80 g (58%) of XIV, mp 316–318° [lit. (10) mp 315–319°].

Partition Coefficients[2]—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 the absorbance of the sample from the water layer after partitioning usually 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; that in the octanol was obtained by difference.

Analyses of the concentrations of the partitioned substances were made by spectrophotometry[3]. The partition coefficient was calculated as $P = C_{\text{octanol}}/C_{\text{water}}$. Each determination was done in at least triplicate, using different amounts of sample; the average value for log P has been reported. The partition coefficient of XVIII could not be determined since the free base was not stable.

Antibacterial Activity—To 7.85 ml of sterile trypticase broth, 1 ml of an aqueous solution of the hydrochloride salt of the test compound and 1 ml of 50% sterile sucrose solution were added. The medium was inoculated with 0.15 ml of a 24-hr culture of *S. mutans*[4] (No. 6715), a pure strain of plaque-forming bacteria. This

[1] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. 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.

[2] In calculating partition coefficients, a least-squares computer program was used both to produce the equation for the standards and to determine concentrations from experimental values.

[3] Beckman DB-G spectrophotometer.

[4] Isolated at, and made available by, the National Institute of Dental Research.

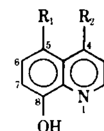


Table I—Physical Data for the 8-Hydroxyquinoline Analogs

Compound	R_1	R_2	Yield, %	Melting Point	Formula	Analysis, % Calc.	Analysis, % Found	Reference
I	CH_3	H	62.5	118–120°	$C_{10}H_9NO$	C 75.38 H 5.65 N 8.79	75.27 5.79 8.79	3
II	CH_3	CH_3	12.8	153–154°	$C_{11}H_{11}NO$	C 76.27 H 6.40 N 8.09	75.98 6.53 8.03	4
III	H	CH_3	10.4	137–139°	$C_{10}H_9NO$	C 75.38 H 5.65 N 8.79	75.24 5.73 8.70	5
IV	OH	CH_3	9.5	294–296°	$C_{10}H_9NO_2$	C 68.56 H 5.18 N 7.99	68.40 5.06 7.69	—
VIII	OCH_3	H	28.0	83–85°	$C_{10}H_9NO_2$	C 68.56 H 5.18 N 8.00	68.29 5.31 8.14	—
IX	OCH_3	CH_3	14.3	131–133°	$C_{11}H_{11}NO_2$	C 69.82 H 5.86 N 7.40	69.92 5.72 7.18	—
XIV	H	OH	58.0	316–318°	$C_9H_7NO_2$	C 67.07 H 4.37 N 8.69	66.93 4.50 8.70	6, 10
XV	H	Cl	64.0	142–143°	C_9H_6ClNO	C 60.18 H 3.37 N 7.80	60.38 3.45 7.87	6, 7, 8
XVII	H	NH_2	67.0	207–208°	$C_9H_8N_2O$	C 67.06 H 5.00 N 17.38	67.17 5.07 17.09	9
XVIII	NH_2	H	87.0	245–265°	$C_9H_8N_2O \cdot 2HCl$	C 46.36 H 4.32 N 12.02	45.39 4.47 11.46	—[a]

[a] J. N. Sane, Northeastern University, Department of Medicinal Chemistry, Boston, Mass., personal communication.

Table II—Partition Coefficient and Antibacterial and Antiplaque Activities for 4- and 5-Substituted 8-Hydroxyquinolines

		Inhibition, %					
		Antibacterial[b]					
		10^{-4} *M*		10^{-5} *M*		Antiplaque[c], 10^{-1} *M*	
Compound	log *P*[a]	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
8-Hydroxy-quinoline	1.94 (0.034)[d]	100	100	100	100	100	100
I	2.37 (0.020)	100	100	20	0	80	40
II	2.71 (0.045)	100	100	80	40	100	60
III	2.36 (0.058)	100	100	100	70	80	40
IV	1.59 (0.103)	0	0	—	—	0	0
VIII	2.06 (0.081)	100	100	0	0	0	0
IX	2.75 (0.168)	100	0	—	—	100	80
XIV	0.62 (0.038)	0	0	—	—	0	0
XV	2.67 (0.050)	0	0	—	—	0	0
XVII	–0.11 (0.037)	0	0	—	—	0	0
XVIII	—	0	0	—	—	0	0

[a] All compounds were evaluated in triplicate. [b] All compounds were evaluated as their hydrochloride salts in aqueous solutions. [c] All compounds were evaluated as free bases (except XVIII) in dimethyl sulfoxide solution on five teeth. Values show percentage of teeth that did not show plaque formation after stated incubation period. [d] All determinations were done in triplicate; values in parentheses indicate standard deviations.

mixture was incubated under anaerobic conditions[5] at 37°. Bacterial growth was determined after 24 and 48 hr.

Assay for *In Vitro* Antiplaque Activity—Sterilized extracted human teeth were immersed in dimethyl sulfoxide 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 5% sucrose-containing trypticase broth with *S. mutans*. The teeth were suspended in test tubes on orthodontic wire[6] (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 adherent microbial growth on the test tube walls, wire, and 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 the results, growth ratings of 0 or 1

[5] BBL-Gaspak, Division of Bioquest, Cockeyville, Md.

[6] Rocky Mount.

were scored as plaque inhibition. Each compound was evaluated on five teeth, with percent inhibition values being reported. This method indicates the percentage of teeth that did not show plaque formation after the stated incubation period. The solvent served as the control.

RESULTS AND CONCLUSIONS

In the group of compounds tested, I–III and VIII showed maximal activity at 10^{-4} *M* after 24 and 48 hr while IX showed a shorter duration of activity. Only III showed equivalent activity to that of 8-hydroxyquinoline at 10^{-5} *M* after 24 hr. At 10^{-5} *M*, all of the active, new agents showed decreased duration of action or decreased activity. The antiplaque studies showed that II and IX had 24-hr activity equal to 8-hydroxyquinoline at 10^{-1} *M* while I and III had only 80% of the activity of the parent compound. Compounds IV, XIV, XVI, XVII, and XVIII showed neither antibacterial nor antiplaque activity.

Analogs with ionizable functions in the 4- or 5-position of 8-hydroxyquinoline showed no antibacterial or antiplaque activity. The inactivity may be due to either the test compounds inability to cross the bacterial membrane or their inability to chelate properly as required for biological activity. These data, as well as previous studies in this laboratory (1), indicate that a single parameter such as log *P* is not adequate to predict antiplaque activity accurately.

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Influence of Monovalent and Divalent Electrolytes on Sorption of Neomycin Sulfate to Attapulgite and Montmorillonite Clays

JAMES W. McGINITY ˣ and JOHN A. HILL

Abstract □ Langmuir isotherms for the adsorption of neomycin sulfate to clays such as attapulgite, bentonite, and magnesium aluminum silicate were constructed. Monovalent and divalent cations were investigated for their influence on the formation of neomycin–clay adsorbates and the resulting equilibrium concentration in a neomycin solution. Divalent magnesium ions were more effective in displacing the antibiotic from each clay than were monovalent sodium ions. Ions present in the GI fluid might increase the bioavailability of neomycin from such neomycin–clay adsorbates.

Keyphrases □ Neomycin sulfate—sorption to attapulgite and montmorillonite clays, influence of monovalent and divalent electrolytes, Langmuir isotherms □ Attapulgite clay—sorption of neomycin sulfate, influence of monovalent and divalent electrolytes, Langmuir isotherms □ Montmorillonite clay—sorption of neomycin sulfate, influence of monovalent and divalent electrolytes, Langmuir isotherms

The adsorptive capacities of magnesium aluminum silicate (1–3), bentonite (2–4), and attapulgite (5–8) for various medicinals have been studied. The ionic incompatibility of neomycin sulfate with these clays was well documented in studies that demonstrated the strong affinity of this cationic antibiotic for the negatively charged clays, with a resultant reduction in the bioactivity of neomycin *in vitro* (2, 3, 9).

Wai and Banker (10) studied the adsorption of cationic drugs to montmorillonite clays and concluded that the mechanism of binding was ion exchange. This mechanism was verified in a recent investigation[1]. These studies, which also encompassed the influence of mono- and divalent electrolytes on the adsorption of amphetamine sulfate to montmorillonite, showed that the electrolytic cations had a significant effect on displacing the drug from the clay.

One objective of the present studies was to evaluate the influence of sodium and magnesium ions on the desorption of neomycin from attapulgite and montmorillonite clays. Another objective was to pro-

[1] Unpublished data.