# Synthesis, Physicochemical Parameters, and *In Vitro* Evaluation of $N^1$-*p*-Chlorophenyl-$N^5$-alkylbiguanides

VICTOR D. WARNER ˣ, DONALD M. LYNCH, and ROSS S. AJEMIAN

**Abstract** □ A series of $N^1$-*p*-chlorophenyl-$N^5$-alkylbiguanides were synthesized as potential inhibitors of dental plaque. Partition coefficients and pKa values were determined by standard methods. Biological activity was evaluated against *Streptococcus mutans*, a pure strain of plaque-forming bacteria. All compounds were compared to chlorhexidine acetate.

**Keyphrases** □ Biguanides, substituted—synthesized, partition coefficients and pKa values determined, ability to inhibit dental plaque screened □ Partition coefficients—determined for series of substituted biguanides □ pKa values—determined for series of substituted biguanides □ Dental plaque inhibitors, potential—series of substituted biguanides synthesized and screened □ Plaque inhibitors, potential—series of substituted biguanides synthesized and screened □ Structure–activity relationships—series of substituted biguanides synthesized and screened for ability to inhibit dental plaque

The accumulation of dental deposits such as plaque shows strong positive correlation with caries formation and periodontal disease (1). Mechanical means for removing plaque are of limited effectiveness, since it can only be removed by vigorous use of abrasives. Chlorhexidine [1,6-bis-($N^5$-*p*-chlorophenyl-$N^1$-biguanido)hexane] (I), an antibacterial bisbiguanide, has shown promise as an effective inhibitor of dental plaque (2).

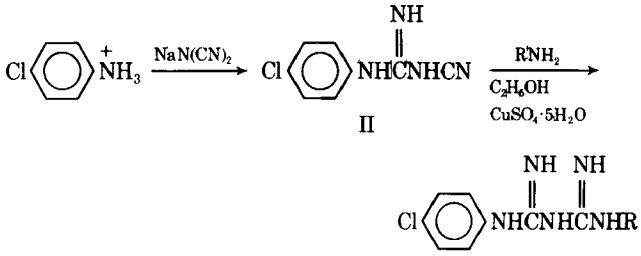
The monobiguanide chloroguanide, III (R = isopropyl), produces no inhibition of plaque growth (3). This observation, coupled with an earlier report that a molecule carrying only one biguanide residue was only weakly antibacterial (4), indicated that two biguanide residues may be required in this type of molecule for antiplaque activity. In an attempt to test this hypothesis and determine physicochemical properties that may be correlated to antiplaque activity, Compounds IV–X were prepared and compared to chlorhexidine acetate.

## EXPERIMENTAL

**Synthesis**[1]—The synthetic approach involved the method of Curd and Rose (5) as outlined in Scheme I.

N¹-p-*Chlorophenyl*-N³-*cyanoguanidine* (*II*)—*p*-Chloroaniline hydrochloride (27.33 g, 0.162 mole) and sodium dicyanamide (15.14 g, 0.17 mole) were dissolved in water (175 ml) and stirred at 25° for 12 hr. The reaction mixture was then filtered. The tan residue was recrystallized from ethanol, yielding white needles (25.18 g, 79.9%), mp 202–203° [lit. (5) mp 202.5–203°].

N¹-p-*Chlorophenyl*-N⁵-*alkylbiguanides* (*IV–X*)—Compound II (9.73 g, 0.05 mole), the appropriate alkylamine (0.2 mole), ethanol (80 ml), and copper sulfate pentahydrate (6.25 g, 0.025 mole) in water (30 ml) were refluxed with stirring for 16 hr. A semisolid precipitate of the copper–biguanide complex formed upon the addition of water (250 ml) to the purple solution. The copper complex was destroyed upon addition of acetic acid (15 g, 0.25 mole). Copper was removed by bubbling gaseous hydrogen sulfide through the solution and the resulting copper sulfide was removed by vacuum filtration. Adjustment of the filtrate pH to 8 using concentrated ammonium hydroxide and evaporation *in vacuo* to about one-third its volume resulted in precipitation of the alkylbiguanide acetate. The crude biguanide acetate was then recrystallized using the appropriate solvent. Details are given in Table I.




III: R = isopropyl
IV: R = methyl
V: R = ethyl
VI: R = *n*-propyl
VII: R = *n*-butyl
VIII: R = *n*-hexyl
IX: R = *n*-octyl
X: R = *n*-dodecyl

*Scheme I*

**Determination of pKa**—The pKa's of IV–VII were determined in duplicate by potentiometric titration. A water-jacketed 200-ml beaker connected to a circulating water bath held the temperature of the titrating vessel at 25°. Nitrogen was bubbled through the solution to be titrated, and the buret (10 ml) was fitted with a soda lime drying tube to exclude atmospheric carbon dioxide. At least 15 0.25-ml aliquots of carbonate-free 0.02 *N* KOH were added to 100 ml of a $10^{-3}$ *M* solution of the alkylbiguanide in deionized distilled water. For each addition of titrant, the pH was measured[2] and the pKa was calculated using the method of Albert and Serjeant (7).

Solubility problems for VIII–X and chlorhexidine acetate[3] were encountered. Therefore, a $10^{-3}$ *M* solution of IV–VII and chlorhexidine in water–ethanol (3:1) was used to determine relative pKa values (Table II).

**Partition Coefficients**—Compounds IV–VI were partitioned between a 0.05 *M* phosphate buffer (pH 11.5) saturated with octanol and octanol saturated with phosphate buffer. Usually, 50–150-ml portions of octanol and buffer were used. In partitioning these compounds, gentle shaking for 90 min was carried out at room temperature (25 ± 5°). The volume ratio of these two phases and the amount of sample were chosen so that the absorbance[4] of the sample from the buffered layer after partitioning had a value between 0.2 and 0.9, using a 1-cm cell and buffer solution as a blank. By working at a fixed pH and knowing the pKa for these compounds, the partition coefficient ($P$) of the free base could be determined using (8):

$$P = \frac{C_{\text{octanol}}}{C_{\text{buffer}}(1 - \alpha)} \qquad \text{(Eq. 1)}$$

where $\alpha$ = degree of ionization.

The higher homologs and chlorhexidine were insoluble at this pH, and their partition coefficients as free bases could not be experimentally determined. However, due to the additive nature of $\pi$ (9), the log $P$ of each homolog could be calculated by the addition of 0.5 for each methylene unit. Since chlorhexidine and V–X were highly lipid soluble, their protonated forms could be partitioned. To do 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. NMR (Varian Associates model T-60 spectrometer) and IR (Perkin-Elmer model 700 spectrometer) spectral data were in accord with the assigned structures.

[2] Orion Research Ionanalizer model 801 digital pH meter with full range Corning combination electrode.

[3] Ayerst Laboratories, Inc.

[4] Spectra of all compounds were recorded at 253 nm using a Beckman DB-G spectrophotometer.

Table I—$N^1$-*p*-Chlorophenyl-$N^5$- alkylbiguanide Acetates

| Compound | R | Yield, % | Melting Point (Solvent) | Formula | Analysis, % Calc. | Found |
|---|---|---|---|---|---|---|
| IV | Methyl | 22 | 149° (ether) | $C_{11}H_{16}ClN_5O_2$ | C 46.24 | 46.40 |
| | | | | | H 5.65 | 5.41 |
| | | | | | N 24.51 | 24.64 |
| V | Ethyl | 42 | 161°[a] (ether) | $C_{12}H_{18}ClN_5O_2$ | C 48.08 | 48.08 |
| | | | | | H 6.05 | 5.86 |
| | | | | | N 23.36 | 23.15 |
| VI | *n*-Propyl | 31 | 165–166°[b] (acetone–ethanol) | $C_{13}H_{20}ClN_5O_2$ | C 49.76 | 49.86 |
| | | | | | H 6.42 | 6.48 |
| | | | | | N 22.32 | 22.43 |
| VII | *n*-Butyl | 59 | 158–159°[c] (acetone–ethanol) | $C_{14}H_{22}ClN_5O_2$ | C 51.30 | 51.18 |
| | | | | | H 6.78 | 6.71 |
| | | | | | N 21.36 | 21.52 |
| VIII | *n*-Hexyl | 35 | 137°[d] (ethanol–water) | $C_{16}H_{26}ClN_5O_2$ | C 53.99 | 53.73 |
| | | | | | H 7.36 | 7.38 |
| | | | | | N 19.68 | 19.63 |
| IX | *n*-Octyl | 24 | 132°[e] (ethanol–water) | $C_{18}H_{30}ClN_5O_2$ | C 56.42 | 56.25 |
| | | | | | H 7.88 | 7.95 |
| | | | | | N 18.28 | 18.33 |
| X | *n*-Dodecyl | 57 | 169° (ethanol–water) | $C_{22}H_{38}ClN_5O_2$ | C 60.04 | 59.78 |
| | | | | | H 8.70 | 8.82 |
| | | | | | N 15.92 | 15.93 |

[a] Lit. (5) mp 160–161°. [b] Lit. (5) mp 164–165°, (6) 165–166°. [c] Lit. (5) mp 158°. [d] Lit. (6) mp 136–137°. [e] Lit. (6) mp 133.5–134°.

Table II—Physicochemical Parameters of $N^1$-*p*-Chlorophenyl-$N^5$-alkylbiguanides

| Compound | Log *P*[a] Free Base[c] | Log *P*[a] Acetate[d] | pKa[b] Water | pKa[b] Water–Ethanol (3:1) |
|---|---|---|---|---|
| IV | 1.58 (0.030) | −0.754[e] | 10.95 ± 0.04 | 10.60 ± 0.04 |
| V | 2.14 (0.030) | −0.254 (0.043) | 11.01 ± 0.03 | 10.82 ± 0.06 |
| VI | 2.63 (0.029) | 0.208 (0.023) | 11.02 ± 0.06 | 10.78 ± 0.06 |
| VII | 3.13[e] | 0.783 (0.007) | 10.83 ± 0.05 | 10.78 ± 0.04 |
| VIII | 4.13[e] | 1.85 (0.013) | — | — |
| IX | 5.13[e] | 2.85[e] | — | — |
| X | 7.13[e] | 4.85[e] | — | — |
| Chlorhexidine | 4.87[e] | 0.081 (0.011) | — | 10.78 ± 0.05 |

[a] All determinations were done in triplicate using different amounts of samples, and the average value for log *P* was determined. Values in parentheses indicate standard deviations. [b] All determinations were done in duplicate. [c] Aqueous phase, 0.05 *M* phosphate buffer (pH 11.5). [d] Aqueous phase, 0.05 *M* acetate buffer (pH 5.0). [e] Calculated log *P*.

Table III—Comparison of Log *P* and *In Vitro* Antibacterial Activity of $N^1$-*p*-Chlorophenyl-$N^5$-alkylbiguanides

| Compound | Log *P* | Inhibition, % $10^{-3}$ *M*, 24 hr | $10^{-4}$ *M*, 24 hr | $10^{-5}$ *M* 24 hr | $10^{-5}$ *M* 48 hr | $10^{-6}$ *M*, 24 hr |
|---|---|---|---|---|---|---|
| IV | 1.58 | 100 | 0 | — | — | — |
| V | 2.14 | 100 | 0 | — | — | — |
| VI | 2.63 | 100 | 0 | — | — | — |
| VII | 3.13 | — | 100 | 0 | — | — |
| VIII | 4.13 | — | 100 | 100 | 0 | 0 |
| IX | 5.13 | — | 100 | 100 | 100 | 0 |
| X | 7.13 | — | 100 | 100 | 0 | 0 |
| Chlorhexidine | 4.87 | — | 100 | 100 | 100 | 0 |

Compounds V–VIII and chlorhexidine were partitioned as their acetate salts between a 0.05 *M* acetate buffer (pH 5) and octanol.

The log *P* values for compounds determined either in both buffer systems or in one buffer system and the calculated log *P* of its conjugate differed by an average value of 2.35 ± 0.07. This average difference between the log *P* of the free base and its salt enabled the assignment of a $\pi$ value of −2.35 for the protonation of nitrogen in this series. Since chlorhexidine is a dibasic compound, the log *P* of the free base could be calculated from the experimentally determined log *P* of the diacetate salt, yielding a value of 4.87 (Table II).

**Antibacterial Activity**—To 8.85 ml of sterile trypticase broth, 0.1 ml of an ethanolic solution 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 *Streptococcus mutans* (No. 6715), a pure strain of plaque-forming bacteria[5]. This mixture was incubated under anaerobic conditions[6] at 37°. After 24 and 48 hr, subjective estimates were made of adherent microbial growth on the test tube walls 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.

[5] Isolated and made available by the National Institute of Dental Research.
[6] BBL-Gaspack, BBL, Division of Bioquest, Cockeysville, Md.

For the results, growth ratings of 0 or 1 were scored as inhibition. Each compound was evaluated on five tubes, with percent inhibition values being reported. This procedure indicates the percentage of tubes that did not show plaque formation after the stated incubation period. The solvent served as the control (Table III).

## RESULTS AND CONCLUSION

The biological results for VII–X (Table III), when compared to chlorhexidine acetate, indicate that two biguanide residues are not required for activity. The length of the alkyl chain need only be extended to achieve the higher lipid solubility required for activity. The two most active compounds tested, IX and chlorhexidine, have log *P* values near 5, suggesting an optimal log *P* for this series. This information will enable prediction, prior to synthesis, of the expected relative activity of biguanides based on lipophilic considerations.

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[x] To whom inquiries should be directed.

# Calibration of a Horizontal Pendulum-Type Tablet Breaking Strength Tester

**FRANK W. GOODHART [x], J. RONALD DRAPER, MICHAEL J. KILLEEN, and FRED C. NINGER**

**Abstract** □ A rapid, simplified method of checking the calibration of a new motorized pendulum type of tablet hardness tester and the accuracy and precision of three testers were determined. Two force gauges were calibrated, and both gauges were used by two operators to determine the accuracy of the new testers. This calibration method using the force gauge was reliable for checking the accuracy. The results show the various new testers to be in calibration and to give reproducible results.

**Keyphrases** □ Tablet hardness testers—motorized pendulum type, method for checking calibration and determining accuracy and precision, three testers compared □ Hardness testers, tablet—motorized pendulum type, method for checking calibration and determining accuracy and precision, three testers compared □ Strength, tablet—testers, motorized pendulum type, method for checking calibration and determining accuracy and precision, three testers compared

Certain deficiencies in the more popular tablet hardness testers have been reported previously (1–3). A new type of tablet hardness tester[1], which operates on the principle of a pendulum being displaced by consistent loading obtained by an electric motor-driven anvil, was made available in 1970 (4). The operation and merits of this tester were reported previously (1). Among its advantages compared to the more typical pneumatic type were: (*a*) more uniform force application, (*b*) less maintenance, and (*c*) the need for fewer calibration checks.

Since that report was completed, a new tablet breaking strength tester[2] became available. Its operating principle is the same as the original but the load scale readout is horizontal rather than circular. The overall size of the new tester is larger, and there are two anvil opening settings, 15 and 35 mm, to accommodate small and large size tablets. Also, the maximum scale values have been increased to 20 kg and 28 Strong-Cobb units to allow for the breakage of harder tablets such as troches and mints. A schematic diagram is shown in Fig. 1.

At the time the new tester was marketed, the manufacturer did not offer a method for checking the calibration. A persistent problem in the use of tablet breaking strength testers has been the lack of a suitable calibration checking device. The objectives of this study were to develop a rapid method for checking the calibration of the horizontal pendulum-type instrument and to determine the accuracy and reproducibility of this new tester.

## EXPERIMENTAL

Two force gauges[3] (range of 0–25 kg in 0.25-kg increments) were calibrated against a tension compression testing machine[4] using the

[1] Heberlein.

[2] Heberlein model 2E, Dr. K. Scheuniger & Co., Zurich, Switzerland.

[3] Model U, W. C. Dillon & Co., Van Nuys, CA 92407

[4] Instron Corp., Canton, Mass.