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Stereochemical Evaluation of the Relative Activities of the Cinchona Alkaloids against *Plasmodium falciparum*

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Quinine and quinidine were over 100 times more active than 9-epiquinine and 9-epiquinidine against chloroquine-sensitive *Plasmodium falciparum* and over 10 times more active against chloroquine-resistant *P. falciparum*. Since the only structural difference between quinine, quinidine, 9-epiquinine, and 9-epiquinidine is their three-dimensional configuration, the three-dimensional structures of these four alkaloids were examined in order to explain the large difference in relative activities between the 9-epi alkaloids and quinine and quinidine. The crystal structure of 9-epiquinidine hydrochloride monohydrate was determined by X-ray diffraction and was compared with the crystal structures of quinine, quinidine sulfate dihydrate, and 9-epiquinine hydrochloride dihydrate. The crystallographic parameters for 9-epiquinidine hydrochloride monohydrate were as follows: chemical formula, $C_{20}H_{25}N_2O_2^+ \cdot Cl^- \cdot H_2O$; M_r, 378.9; symmetry of unit cell, orthorhombic; space group, $P2_12_12_1$; parameters of unit cell, *a* was 7.042 ± 0.001 Å (1 Å = 0.1 nm), *b* was 9.082 ± 0.001 Å, *c* was 31.007 ± 0.005 Å; the volume of unit cell was 1,983.1 ± 0.6 Å^3; number of molecules per unit cell was 4; the calculated density was 1.27 g cm^{-3}; the source of radiation was Cu Kα (λ = 1.54178 Å); μ (absorption coefficient) was 18.82 cm^{-1}; *F*(000) (sum of atomic scattering factors at zero scattering angle) was 808; room temperature was used; final *R* (residual index) was 5.72% for 1,501 reflections with $|F_o| > 3\sigma$ (*F*). The intramolecular distance from N-1 to O-12 in 9-epiquinidine and 9-epiquinine, although shorter than the corresponding distance in quinine and quinidine, was similar to those of other active amino alcohol antimalarial agents. In all four alkaloids, both the hydroxyl and amine groups formed intermolecular hydrogen bonds, showing the potential for forming hydrogen bonds with cellular constituents. However, the positioning of the N$^+$-1—H-N1 and O-12—H-O12 groups relative to each other was quite different in the 9-epi alkaloids versus quinidine. This difference in positioning may determine the relative strengths of the formation of hydrogen bonds with cellular constituents important to antimalarial activity and, therefore, may determine the relative strength of antimalarial activity.

The goal of this study was to establish the relative in vitro antimalarial activities of quinine, quinidine, 9-epiquinine, 9-epiquinidine, dihydroquinine, and dihydroquinidine (Fig. 1) against the human parasite *Plasmodium falciparum* and to determine why the configuration of the 9-epi alkaloids greatly reduces their antimalarial activity. 9-Epiquinine and 9-epiquinidine have been labeled inactive since the 1930s because of their poor activity in avian malaria assays (2, 24). These studies were performed prior to the development of in vitro human malaria assay systems and prior to the observation of chloroquine-resistant malaria. Consequently, these studies may not relate to present-day strains of human-infecting *P. falciparum*. Once the relative antimalarial activities of the cinchona alkaloids against *P. falciparum* were established in our in vitro assay system, these data along with the three-dimensional structures of the cinchona alkaloids were used to define the relationship of conformation to antimalarial activity. These three-dimensional parameters are being defined so that they can be incorporated into the design of new antimalarial agents.

The only difference in the chemical structures of quinine, quinidine, 9-epiquinine, and 9-epiquinidine is the geometry of the 9-hydroxyl group and the quinuclidine ring system. Since the conformation of the hydroxyl and amine groups must therefore be important to relative antimalarial activity, a comparison of the antimalarial activities and conformations of these four alkaloids will identify the orientation of the hydroxyl and amine groups which yields the highest antimalarial activity. This orientation is illustrated in our report.

MATERIALS AND METHODS

Chemicals. Crystals of 9-epiquinidine hydrochloride dihydrate and 9-epiquinine hydrochloride monohydrate were a generous gift of K. Barry Sharpless of the Chemistry Department, Massachusetts Institute of Technology. Quinine sulfate dihydrate and quinidine were obtained from the National Institute of Science and Technology (Gaithersburg, Md.) and Aldrich Chemical Co. (St. Louis, Mo.), respectively. Dihydroquinine and dihydroquinidine were obtained from the Walter Reed Army Institute of Research inventory. The degrees of purity of all six alkaloids were established by high-performance liquid chromatography using UV detection at 254 nm. Quinidine, quinine, epiquinine, epiquinidine, dihydroquinidine, and dihydroquinine eluted at 5.0, 5.7, 6.4, 6.7, 7.6, and 8.8 min, respectively, through an octyl Ultrasphere column (4.6 by 150 mm; particle diameter, 5 μm) (Beckman, Fullerton, Calif.) using a mobile phase of water–acetonitrile–1 M monobasic sodium dihydrogen phosphate–1 M sodium perchlorate (83.5:12.5:2:1). The pH was adjusted to 1.7 by the addition of phosphoric acid, and flow was maintained at 1.5 ml/min (4). 9-Epiquinidine and 9-epiqui-

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nine eluted at 8.3 and 9.4 min, respectively, through a Waters C-18 Radial Pak column (internal diameter, 8 mm; particle diameter, 5 μm) (Millipore Corp., Milford, Mass.), using a mobile phase of methanol–PIC B-5 (50:50) at 1 ml/min.

Antimalarial activity. The assays were conducted in vitro by using a modified version of the semiautomated microdilution technique of Desjardins et al. (5) and Milhous et al. (14) with two *P. falciparum* clones from Indochina (W-2) and Sierra Leone (D-6). The clones were derived by single-erythrocyte micromanipulation (15) from patient isolates obtained from the Centers for Disease Control (Atlanta, Ga.) in 1980 and 1982, respectively, and represent infections acquired in Vietnam or Sierra Leone. The Indochina clone is resistant to the antimalarial agents chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the Sierra Leone clone is resistant to mefloquine but sensitive to chloroquine, quinine, sulfadoxine, and pyrimethamine (13). These clones were maintained in a 5-ml suspension of human type A+ erythrocytes (6% hematocrit) containing less than 2% parasitized cells in RPMI 1640 culture medium (GIBCO, Grand Island, N.Y.) with 32 mM $NaHCO_3$, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), and 10% (vol/vol) heat-inactivated human plasma at 37°C in sealed 50-ml culture flasks under a 5% O_2–5% CO_2–90% N_2 atmosphere.

The cinchona alkaloids were dissolved in dimethyl sulfoxide and diluted 400-fold in RPMI 1640 culture medium with 10% heat-inactivated human plasma. The alkaloids were subsequently further diluted into microtiter wells using the Cetus Pro/Pette (Perkin-Elmer Corp., Norwalk, Conn.) over ranges of 34 to 25,000 ng/ml for the 9-epi alkaloids and 0.34 to 250 ng/ml for the other four alkaloids. The alkaloids were incubated with parasite inocula (0.5% parasitemia and a 1% hematocrit) for 24 h at 37°C prior to the addition of 0.37 μCi of [G-^{3}H]hypoxanthine monochloride (17.2 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.). After further incubation for 18 h at 37°C, particulate matter was harvested from each microtiter well with an automated cell harvester (MACH II; TOMTEC, Orange, Conn.) onto filter paper disks (no. 934-AH; Whatman Inc., Clifton, N.J.). The dried disks were counted in a scintillation spectrometer (LKB 1205 Betaplate; LKB Wallac, Gaithersburg, Md.). Concentration-response data were analyzed by nonlinear regression, and the 50% inhibitory concentrations (IC_{50}s) of each alkaloid were calculated.

X-ray crystallography. Diffraction data on 9-epiquinidine hydrochloride monohydrate were collected from a colorless prism (0.38 by 0.52 mm; 0.17 mm thick) in the θ/2θ mode (23) with a maximum value of 112° for 2θ on the four-circle diffractometer (R3m/micro Nicolet; Siemens, Madison, Wis.) equipped with a graphite monochromator. The X-ray source was Cu Kα radiation (50 kV, 40 mA). The indices ranged from −8 to 0 for *h*, −10 to 0 for *k*, and 0 to 34 for *l*. The total number of independent reflections was 1,567. The standard reflections 2,0,0, 0,6,0, and 0,0,16 were monitored after every 97 intensity measurements. The standards remained constant within 1.5%. The lattice parameters were based on 25 centered reflections with 2θ values between 31 and 61°. The data were corrected for Lorentz and polarization effects, but no correction for absorption was used.

The structure was solved routinely by direct phase determination (9). All of the nonhydrogen atoms except the vinyl group were found in the first *E* map. The vinyl group and the hydrogen atoms attached to the oxygen and nitrogen atoms were found in the difference maps. The difference map is an electron density map of the difference between the observed magnitudes of the reflections and the magnitude calculated from the atoms already positioned in the structure. The resulting electron density represents the location of atoms which were not yet identified. Least-square refinement was performed using 1,501 reflections with $|F_o| > 3\sigma(F)$ (*Rmerge* = 0.007), where F_o is the observed structure factor and F is the structure factor. Coordinates for all atoms except the hydrogen atoms on the carbon atoms were refined (on F) with a blocked cascade program in the SHELXTL system (22). The hydrogen atoms bonded to the carbon atoms were placed in idealized positions and allowed to ride with the carbon atoms. Anisotropic thermal parameters for the C, N, O, and Cl atoms and isotropic thermal parameters for the H atoms bonded to the N and O atoms were refined for a total of 252 parameters. The final values for R (residual index) and wR (a weighted correlation factor) were 5.72 and 6.01%, respectively; w was calculated from the following equation: $w = 1/[\sigma^2(|F|) + 0.0005\ (F_o)^2]$. The coordinates listed in Table 3 represent the correct absolute configuration, since the mirror image of this structure gave a higher final R value of 6.85%. The final difference in electron density was $|\rho|_{max}$ equal to 0.44 and $|\rho|_{min}$ equal to −0.25 e Å^{-3}. The goodness-of-fit value S was equal to 2.7. The maximum change in a parameter divided by the estimated standard deviation of a parameter $(\Delta/\sigma)_{max}$ was equal to 0.39. Atomic scattering factors were those incorporated in SHELXTL (22). Crystallographic parameters are further defined in reference 23.

Figures 2 to 4 were drawn on an Evans and Sutherland PS390 graphics system (Salt Lake City, Utah) with SYBYL software (Tripos, St. Louis, Mo.) and a Hewlett-Packard 7550A Graphics Plotter (Rockville, Md.) using the experimentally determined coordinates from Table 3.

TABLE 1. Antimalarial activities of the cinchona alkaloids

Cinchona alkaloid	IC_{50} (nM) (mean ± SEM)	
	Chloroquine-sensitive clone D-6 (n = 5)	Chloroquine-resistant clone W-2 (n = 8)
Quinine	29.3 ± 9.5	103.2 ± 23.9
Dihydroquinine	21.3 ± 7.2	151.7 ± 45.8[a]
Quinidine	13.4 ± 4.6	43.7 ± 11.5
Dihydroquinidine	10.4 ± 3.8	74.1 ± 18.0[a]
9-Epiquinine	3,471 ± 797	1,179 ± 279
9-Epiquinidine	2,700 ± 704	1,024 ± 229

[a] n = 5.

RESULTS

Antimalarial activities of the cinchona alkaloids. The in vitro *P. falciparum* assay (Table 1) demonstrated that 9-epiquinine and 9-epiquinidine (the 8*S*,9*S* and 8*R*,9*R* alkaloids) are substantially less active than quinine and dihydroquinine (the 8*S*,9*R* alkaloids) and quinidine and dihydroquinidine (the 8*R*,9*S* alkaloids) for both the chloroquine-sensitive and chloroquine-resistant clones. On the basis of IC_{50}s, 9-epiquinine was 143 times weaker than quinine and 334 times weaker than quinidine against the chloroquine-sensitive D-6 clone (Table 2). 9-Epiquinidine was 107 times weaker than quinine and 248 times weaker than quinidine against the chloroquine-sensitive D-6 clone. The 9-epi alkaloids were also substantially less active than quinine and quinidine against the chloroquine-resistant W-2 clone, but the difference in relative activity was smaller. 9-Epiquinine

TABLE 2. Relative antimalarial activities of the cinchona alkaloids

Cinchona alkaloids	Ratio of IC_{50}[a] (mean ± SD)	
	Chloroquine-sensitive clone D-6 (n = 5)	Chloroquine-resistant clone W-2 (n = 8)
Quinine and quinidine	2.30 ± 0.21	2.82 ± 1.38
9-Epiquinine and 9-epiquinidine	1.35 ± 0.23	1.19 ± 0.28
Dihydroquinine and dihydroquinidine	2.07 ± 0.65	1.88 ± 0.24[b]
Dihydroquinine and quinine	0.73 ± 0.09	1.17 ± 0.08[b]
Dihydroquinidine and quinidine	0.88 ± 0.25	1.28 ± 0.35[b]
9-Epiquinine and quinine	143 ± 37	13.6 ± 3.2
9-Epiquinidine and quinine	107 ± 25	11.3 ± 4.8
9-Epiquinine and quinidine	334 ± 99	31.3 ± 9.5
9-Epiquinidine and quinidine	248 ± 65	26.5 ± 7.3

[a] The IC_{50} of the first alkaloid was divided by the IC_{50} of the second alkaloid for each individual experiment. The ratios obtained from individual experiments were then averaged and reported in this table.
[b] n = 5.

was 13.6 and 31.3 times less active than quinine and quinidine, respectively, against the chloroquine-resistant W-2 clone. 9-Epiquinidine was 11.3 and 26.5 times less active than quinine and quinidine, respectively. Quinidine and dihydroquinidine (the 8*R*,9*S* conformation) were 1.9 to 2.8 times more active than quinine and dihydroquinine (the 8*S*,9*R* conformation) against both clones. However, 9-epiquinine and 9-epiquinidine were almost equally active against both clones. Saturation of the 10,11-vinyl group did not produce much change in antimalarial activity.

A much larger variation in the actual IC_{50}s of the individual alkaloids was observed from week to week than is shown in the ratios reported in Table 2. No matter what the absolute IC_{50}s were, the ratios were quite constant from experiment to experiment. This result demonstrates that the ratio values reported in Table 2 are valid over a range of IC_{50}s. The large variation in IC_{50}s probably stems from the technical difficulties of having precisely the same culture and parasitemia conditions from experiment to experiment.

The degrees of purity of the alkaloids as measured by high-performance liquid chromatography were 98.6% for quinine, 97.0% for quinidine, and over 99% for their dihydro

TABLE 3. Fractional coordinates and thermal parameters U_{eq}[a] for 9-epiquinidine hydrochloride monohydrate

Atom	Fractional coordinate			U_{eq}[b] (Å^2)
	x	y	z	
N-1	0.3003 (0.0005)	0.6358 (0.0004)	0.7990 (0.0001)	0.043 (0.001)
C-2	0.1488 (0.0006)	0.5439 (0.0005)	0.8203 (0.0005)	0.048 (0.001)
C-3	0.2391 (0.0007)	0.4397 (0.0005)	0.8527 (0.0002)	0.057 (0.002)
C-4	0.4437 (0.0008)	0.4924 (0.0005)	0.8604 (0.0002)	0.055 (0.002)
C-5	0.5572 (0.0007)	0.4703 (0.0006)	0.8201 (0.0002)	0.068 (0.002)
C-6	0.4539 (0.0007)	0.5354 (0.0006)	0.7812 (0.0002)	0.057 (0.002)
C-7	0.4371 (0.0007)	0.6572 (0.0005)	0.8709 (0.0001)	0.052 (0.001)
C-8	0.3922 (0.0006)	0.7422 (0.0005)	0.8298 (0.0001)	0.038 (0.001)
C-9	0.2712 (0.0006)	0.8793 (0.0005)	0.8347 (0.0001)	0.039 (0.001)
C-10	0.1291 (0.0011)	0.4221 (0.0009)	0.8922 (0.0003)	0.095 (0.003)
C-11	−0.0194 (0.0015)	0.4614 (0.0014)	0.9035 (0.003)	0.148 (0.005)
O-12	0.2665 (0.0005)	0.9440 (0.0004)	0.7929 (0.0001)	0.052 (0.001)
N-13	0.5541 (0.0007)	1.1646 (0.0004)	0.9269 (0.0001)	0.067 (0.002)
C-14	0.6180 (0.0008)	1.1398 (0.0005)	0.8881 (0.0002)	0.058 (0.002)
C-15	0.5299 (0.0007)	1.0506 (0.0005)	0.8583 (0.0002)	0.050 (0.002)
C-16	0.3602 (0.0007)	0.9816 (0.0005)	0.8681 (0.0001)	0.043 (0.001)
C-17	0.2845 (0.0007)	1.0020 (0.0005)	0.9100 (0.0001)	0.046 (0.001)
C-18	0.1135 (0.0007)	0.9387 (0.0006)	0.9250 (0.0002)	0.054 (0.002)
C-19	0.0508 (0.0009)	0.9681 (0.0006)	0.9658 (0.0002)	0.069 (0.002)
C-20	0.1560 (0.0011)	1.0668 (0.0007)	0.9930 (0.0002)	0.081 (0.002)
C-21	0.3114 (0.0011)	1.1275 (0.0007)	0.9792 (0.0002)	0.073 (0.002)
C-22	0.3857 (0.0008)	1.0992 (0.0005)	0.9379 (0.0001)	0.053 (0.002)
O-23	−0.1080 (0.0007)	0.9149 (0.0006)	0.9851 (0.0002)	0.104 (0.002)
C-24	−0.2163 (0.0012)	0.8337 (0.0010)	0.9607 (0.0002)	0.104 (0.003)
Cl	−0.0652 (0.0002)	1.1677 (0.0001)	0.7876 (0.0001)	0.056 (0.001)
W-1[c]	0.1697 (0.0005)	1.3111 (0.0004)	0.7095 (0.0001)	0.074 (0.001)
H-N1[d]	0.2642 (0.0073)	0.6749 (0.0055)	0.7744 (0.0014)	0.066 (0.014)
H-O12[d]	0.1832 (0.0111)	1.0189 (0.0094)	0.7895 (0.0022)	0.136 (0.026)
H-W1A[d,e]	0.1601 (0.0088)	1.4256 (0.0070)	0.7102 (0.0017)	0.096 (0.017)
H-W1B[d,e]	0.0835 (0.0104)	1.2760 (0.0113)	0.7473 (0.0030)	0.182 (0.033)

[a] Values in parentheses are estimated standard deviations.
[b] $U_{eq} = (\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a}_i \mathbf{a}_j)/3$, where i and j are the summation indices, a is the length of the basis vector of the reciprocal lattice, and $\mathbf{a}$ is the basis vector of the direct lattice.
[c] Oxygen atom of water molecule.
[d] Atom refined isotropically.
[e] Hydrogen atom of water molecule.

TABLE 4. Bond lengths for 9-epiquinidine

Bond	Bond length (Å)[a]	Bond	Bond length (Å)[a]
N-1—C-2	1.508 (0.006)	N-1—C-6	1.519 (0.006)
N-1—C-8	1.506 (0.005)	C-2—C-3	1.519 (0.007)
C-3—C-4	1.537 (0.007)	C-3—C-10	1.457 (0.009)
C-4—C-5	1.497 (0.007)	C-4—C-7	1.532 (0.006)
C-5—C-6	1.530 (0.007)	C-7—C-8	1.524 (0.006)
C-8—C-9	1.516 (0.006)	C-9—O-12	1.423 (0.005)
C-9—C-16	1.525 (0.006)	C-10—C-11	1.160 (0.014)
N-13—C-14	1.303 (0.007)	N-13—C-22	1.369 (0.007)
C-14—C-15	1.377 (0.007)	C-15—C-16	1.382 (0.007)
C-16—C-17	1.417 (0.006)	C-17—C-18	1.413 (0.007)
C-17—C-22	1.426 (0.007)	C-18—C-19	1.367 (0.007)
C-19—C-20	1.437 (0.009)	C-19—O-23	1.358 (0.008)
C-20—C-21	1.298 (0.010)	C-21—C-22	1.408 (0.007)
O-23—C-24	1.303 (0.009)		

[a] Values in parentheses are estimated standard deviations.

analogs. The major contaminant in the quinine and quinidine samples coeluted with their respective dihydro analogs. The 9-epi alkaloids were essentially 100% pure, as no other peaks were observed in the chromatogram.

X-ray crystal structure of 9-epiquinidine. Fractional coordinates and thermal parameters (U_{eq} values) for the nonhydrogen atoms and fractional coordinates for the hydrogen atoms attached to N-1 and O-12 are listed in Table 3, bond lengths are listed in Table 4, and bond angles are listed in Table 5. The bond length of the hydrogen atoms attached to the carbon atoms was kept fixed at 0.96 Å (1 Å = 0.1 nm) throughout the refinement procedure. Selected torsion angles are listed in Table 6, and the numbering scheme for 9-epiquinidine is shown in Fig. 1.

9-Epiquinidine crystallized as a tertiary amine hydrochloride salt with one water molecule per 9-epiquinidine molecule. The bicyclo rings of the quinuclidine moiety assumed a boat conformation, which is the typical conformation of the cinchona alkaloids (Fig. 2). The boats had a slight twist such that the values of the torsion angles of C-3—C-4···N^+-1—C-2, C-5—C-4···N^+-1—C-6, and C-7—C-4···N^+-1—C-8 were 8.6 ± 0.6, 9.4 ± 0.6, and 12.8 ± 0.6°, respectively. This twist was more pronounced in 9-epiquinidine than in 9-epiquinine, where the methylene groups of the quinuclidine ring system nearly eclipsed each other (11). The vinyl group attached to C-3 and the aryl group attached to C-8 were both *endo* with respect to the boat defined by N^+-1—C-2—C-3—C-4—C-7—C-8, which positioned the vinyl group pointing toward the methoxyl group. In 9-epiquinine, the aryl group was in the *exo* position, which positioned the aryl group pointing away from the methoxyl group. Like 9-epiquinine, the N^+-1 atom was *anti* to the quinoline ring with the torsion angle of N^+-1—C-8—C-9—C-16 equal to 176.5 ± 0.6°. Also like 9-epiquinine, H-8 and H-9 were *anti* to one another with the H-8—C-8—C-9—H-9 torsion angle equal to 179.4 ± 0.6° and with H-9 pointing toward the methoxyl group. The solution nuclear magnetic resonance spectrum of 9-epiquinidine (6) also places atom H-9 pointing toward the methoxyl group. The methoxyl group was approximately 4° out of the plane of the quinoline ring and pointed toward the quinuclidine ring system.

TABLE 5. Bond angles for 9-epiquinidine

Bond	Bond angle (°)[a]	Bond	Bond angle (°)[a]
C-2—N-1—C-6	109.3 (0.3)	C-2—N-1—C-8	112.4 (0.3)
C-6—N-1—C-8	108.0 (0.3)	N-1—C-2—C-3	109.9 (0.4)
C-2—C-3—C-4	107.5 (0.4)	C-2—C-3—C-10	113.6 (0.5)
C-4—C-3—C-10	113.7 (0.5)	C-3—C-4—C-5	109.2 (0.4)
C-3—C-4—C-7	108.0 (0.4)	C-5—C-4—C-7	108.9 (0.4)
C-4—C-5—C-6	110.7 (0.4)	N-1—C-6—C-5	106.5 (0.4)
C-4—C-7—C-8	108.9 (0.4)	N-1—C-8—C-7	107.2 (0.3)
N-1—C-8—C-9	110.4 (0.3)	C-7—C-8—C-9	116.7 (0.3)
C-8—C-9—O-12	105.1 (0.3)	C-8—C-9—C-16	109.7 (0.4)
O-12—C-9—C-16	112.1 (0.4)	C-3—C-10—C-11	134.4 (0.8)
C-14—N-13—C-22	117.0 (0.4)	N-13—C-14—C-15	124.4 (0.5)
C-14—C-15—C-16	120.6 (0.5)	C-9—C-16—C-15	118.9 (0.4)
C-9—C-16—C-17	123.2 (0.4)	C-15—C-16—C-17	117.8 (0.4)
C-16—C-17—C-18	124.7 (0.4)	C-16—C-17—C-22	116.6 (0.4)
C-18—C-17—C-22	118.5 (0.4)	C-17—C-18—C-19	120.1 (0.5)
C-18—C-19—C-20	120.0 (0.5)	C-18—C-19—O-23	127.2 (0.5)
C-20—C-19—O-23	112.8 (0.5)	C-19—C-20—C-21	120.3 (0.5)
C-20—C-21—C-22	122.5 (0.6)	N-13—C-22—C-17	123.4 (0.4)
N-13—C-22—C-21	118.0 (0.5)	C-17—C-22—C-21	118.6 (0.5)
C-19—O-23—C-24	115.3 (0.5)		

[a] Values in parentheses are estimated standard deviations.

The vinyl group was not positioned in the crystal in precisely the same orientation from molecule to molecule, as indicated by the large thermal parameters listed in Table 3 for atoms C-10 and C-11. This large motion caused the refined C-10—C-11 bond length to appear to be too short and the C-3—C-10—C-11 bond angle about the vinyl group to be somewhat distorted.

9-Epiquinidine hydrochloride monohydrate was packed such that hydrophilic channels ran parallel to the *b* axis (Fig. 3). The channels contain chains of chloride ions hydrogen bonded to two water molecules. Each chloride ion also participated in two hydrogen bonds with the hydroxyl and amine groups of two separate 9-epiquinidine molecules (Table 7). In Fig. 3, two of the hydrogen bonds to the chloride ions, one from a water molecule and one from H-O12, are nearly on top of each other and almost parallel to the *b* axis. No intramolecular hydrogen bond between the hydroxyl and amine groups of 9-epiquinidine was observed.

Comparison of the three-dimensional structures of the cinchona alkaloids. The only structural difference between the active *erythro* cinchona alkaloids, quinine and quinidine, and the relatively inactive *threo* cinchona alkaloids, 9-epiquinine and 9-epiquinidine, is the conformation about atoms C-8 and C-9. This conformational difference results in a difference in the positioning of the amine and hydroxyl groups relative to each other. This conformational difference could manifest itself in three possible ways: preference by the alkaloid for forming intramolecular versus intermolecular hydrogen bonds, distance between the aliphatic nitrogen atom and the hydroxyl oxygen atom, and direction of the N—H and O—H bonds relative to each other. Each of these factors could influence antimalarial activity by influencing the direction and strength of hydrogen bond formation between the cinchona alkaloid and cellular constituents important to antimalarial activity. However, an examination of the crystal structures of the *erythro* and *threo* alkaloids indicates that the third factor is the predominant factor influencing antimalarial activity.

The cinchona alkaloids were examined for each of these factors. First, when crystallized as salts, both the *threo* and *erythro* cinchona alkaloids formed intermolecular hydrogen bonds with their amine and hydroxyl groups, showing that both the *erythro* and *threo* cinchona alkaloids should be able to form intermolecular hydrogen bonds with cellular constituents. Second, the separation of the aliphatic nitrogen atom and hydroxyl oxygen atom in 9-epiquinine and 9-epiquini-

TABLE 6. Selected torsion angles for 9-epiquinidine

Bond	Torsion angle (°)[a]	Bond	Torsion angle (°)[a]
C-6—N-1—C-2—C-3	52.1	C-8—N-1—C-2—C-3	−67.8
C-2—N-1—C-6—C-5	−69.4	C-8—N-1—C-6—C-5	53.2
C-2—N-1—C-8—C-7	46.0	C-2—N-1—C-8—C-9	−82.1
C-6—N-1—C-8—C-7	−74.7	C-6—N-1—C-8—C-9	−157.2
N-1—C-2—C-3—C-4	14.3	C-2—C-3—C-4—C5	−67.6
C-2—C-3—C-4—C-7	50.7	C-4—C-5—C-6—N-1	15.4
C-4—C-7—C-8—N-1	21.0	C-4—C-7—C-8—C-9	145.3
N-1—C-8—C-9—O-12	−62.8	N-1—C-8—C-9—C-16	176.5
C-7—C-8—C-9—O-12	174.6	C-7—C-8—C-9—C-16	53.9
C-8—C-9—C-16—C-17	−106.7	O-12—C-9—C-16—C-17	137.0
C-14—C-15—C-16—C-9	−177.8	O-23—C-19—C-20—C-21	−179.7
C-20—C-19—O-23—C-24	175.0		

[a] Estimated standard deviations for the torsion angles are near 0.6°.

dine was similar to those in active antimalarial agents. The intramolecular distance from N^+-1 to O-12 of 2.816 ± 0.006 Å in 9-epiquinidine was essentially equal to the 2.806 ± 0.007 Å distance found in 9-epiquinine (11). This distance was slightly shorter than the distance from the aliphatic nitrogen to hydroxyl oxygen of 2.84 to 3.22 Å exhibited by the active cinchona alkaloids (1, 3, 7, 8, 12, 14, 16–19, 21), but equivalent to the 2.73- to 2.85-Å distance from the aliphatic nitrogen to hydroxyl oxygen found in salt and free base forms of the antimalarial agent mefloquine (10), a synthetic amino alcohol antimalarial agent in which the quinuclidine ring of quinine or quinidine is replaced with a piperidine ring. Neither of these factors apparently account for the difference in antimalarial activity between the *threo* alkaloids 9-epiquinine and 9-epiquinidine and the *erythro* alkaloids quinine and quinidine.

The third factor appears to be causing the difference in antimalarial activity. The O-12—C-9···N-1—H-N1 torsion angles are −0.2, −15.4, −117.7, and 97.0° for 9-epiquinine (11), 9-epiquinidine, quinine (21), and quinidine (8), respectively. The torsion angles have an estimated standard deviation of approximately 3.5°. Since quinine was crystallized as a free base, the torsion angle for quinine is calculated by assuming that converting quinine to the salt form does not change the overall conformation of the molecule. Thus, the *threo* alkaloids have a torsion angle approximately 90° different from the torsion angle in the *erythro* alkaloids. The different torsion angles force the N—H and O—H bonds of the *erythro* and *threo* alkaloids to be oriented differently with respect to each other, as illustrated in Fig. 4. The result of these differences is that the *threo* alkaloids cannot form intermolecular hydrogen bonds in the same directions as the *erythro* alkaloids.

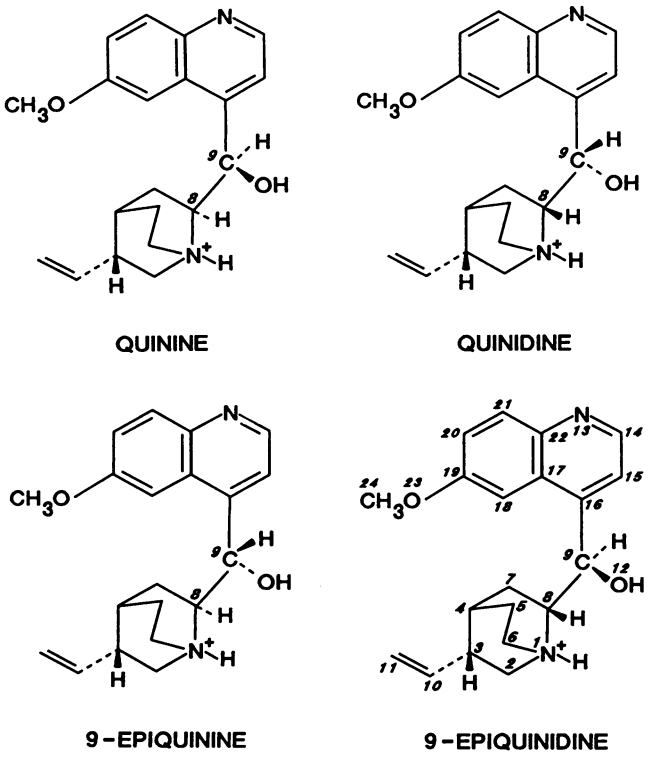

FIG. 1. Chemical structures of quinine (8*S*,9*R*), quinidine (8*R*,9*S*), 9-epiquinine (8*S*,9*S*), and 9-epiquinidine (8*R*,9*R*) portrayed in salt form. The numbering scheme is shown in italics. Dihydroquinine (8*S*,9*R*) and dihydroquinidine (8*R*,9*S*) are saturated at the 10,11-vinyl group. In each molecule, the quinoline ring is the unsaturated bicyclo fused ring system, and the quinuclidine ring system is the saturated bicyclo ring system.

DISCUSSION

The results from the in vitro *P. falciparum* assay confirm the stereospecificity of antimalarial activities of the cinchona

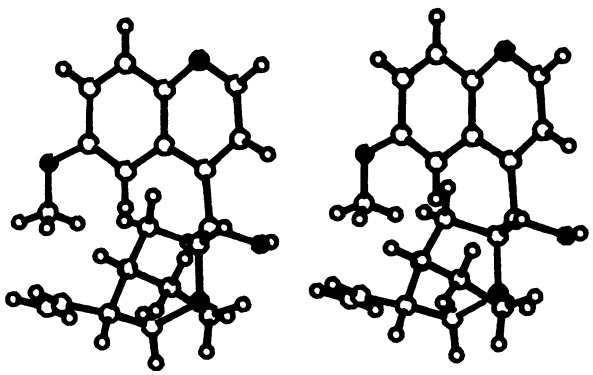

FIG. 2. Stereodrawing of 9-epiquinidine. The oxygen and nitrogen atoms are colored solid black. The diagram can be viewed in three dimensions with the aid of a stereoviewer (Hubbard Scientific Co., Northbrook, Ill.) or by holding the drawing steady approximately 45 cm from your eyes and allowing your eye muscles to relax until the center image comes into focus.

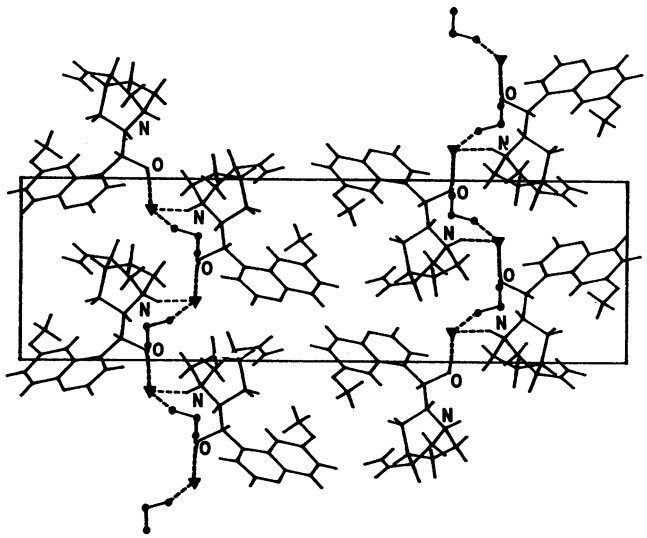

FIG. 3. Packing diagram of 9-epiquinidine hydrochloride monohydrate viewed down the *a* axis. The *b* axis is vertical, and the *c* axis is horizontal. The box represents the smallest repeating unit in the crystal. The dashed lines represent hydrogen bonds. The triangles indicate the location of the chloride ions, and the atoms of the water molecules are indicated by the small solid circles. The N-1 and O-12 atoms of each molecule are labeled.

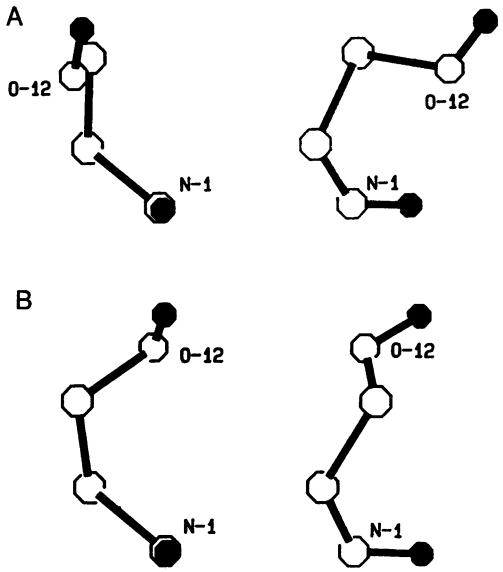

FIG. 4. (A) Views at right angles of the H-N1—N^+-1—C-8—C-9—O-12—H-O12 segment of 9-epiquinidine hydrochloride monohydrate. (B) Views at right angles of the same segment of quinidine sulfate dihydrate (8). The C-8, N-1, and H-N1 atoms of both molecules were oriented as similarly as possible. In all drawings, the hydrogen atoms are colored solid black.

alkaloids. For the Indochina W-2 and Sierra Leone D-6 clones, the *threo* alkaloids 9-epiquinine and 9-epiquinidine were substantially less active than the *erythro* alkaloids quinine and quinidine. Even among the *erythro* alkaloids, the 8*R*,9*S* alkaloids quinidine and dihydroquinidine were more active than the 8*S*,9*R* alkaloids quinine and dihydroquinine. This result is consistent with published data showing that quinidine is 2.5 times more effective in vitro than quinine and that cinchonine (the demethoxyl analog of quinidine) is 2.8 times more effective in vitro than cinchonidine (the demethoxyl analog of quinine) against the Papua New Guinea *P. falciparum* FCQ-27/PNG strain (26). Clinically, quinidine was twice as potent as quinine against induced McClendon *P. falciparum* infections (25) and more effective than quinine against Thai *P. falciparum* (20, 27). The poor antimalarial activity demonstrated by the 9-epi alkaloids against *P. falciparum* confirms the avian model prediction (2, 24) that the 9-epi alkaloids are poor candidates for clinical use in human malaria. Interestingly, 9-epiquinine and 9-epiquinidine are more active against the chloroquine-resistant clone than against the chloroquine-sensitive clone, which is opposite to the sensitivity of the two clones to quinine and quinidine (Table 1). The antimalarial activities of dihydroquinine and dihydroquinidine are very similar to those of quinine and quinidine, respectively, showing that saturation of the vinyl group does not inhibit antimalarial activity.

Thus, the preferred orientation of the amine and hydroxyl groups for the highest antimalarial activity among the cinchona alkaloids appears to be the 8*R*,9*S* conformation of quinidine. The conformation of the amine and hydroxyl groups of quinidine in its sulfate salt form (8) is illustrated in Fig. 4B. The N—H and the O—H bonds are positioned at an angle of 13° from each other in contrast to the 67° angle found in both 9-epiquinine hydrochloride dihydrate (11) and 9-epiquinidine hydrochloride monohydrate. Since 9-epiquinine and 9-epiquinidine, the *threo* cinchona alkaloids, cannot form hydrogen bonds to cellular constituents in the same direction as the *erythro* cinchona alkaloids quinine and quinidine, the strength of these interactions may be weakened, resulting in the weak antimalarial activities of 9-epiquinine and 9-epiquinidine.

TABLE 7. Hydrogen bond distances and angles in 9-epiquinidine hydrochloride monohydrate

Donor atom	Hydrogen atom	Acceptor atom	Distance (Å)[a]			Donor-hydrogen-acceptor angle (°)[b]	Symmetry equivalent operations to obtain donor
			Donor-acceptor	Hydrogen-donor	Hydrogen-acceptor		
N-1	H-N1	Cl	3.167	0.88	2.38	149	$-x, 0.5 + y, 1.5 - z$
O-12	H-O12	Cl	3.100	0.90	2.21	167	x, y, x
W-1[c]	H-W1A	Cl	3.323	1.04	2.30	166	$-x, -0.5 + y, 1.5 - z$
W-1[c]	H-W1B	Cl	3.209	1.36	1.90	159	x, y, z

[a] Estimated standard deviations for the donor-acceptor, hydrogen-donor, and hydrogen-acceptor distances are near 0.006, 0.07, and 0.07 Å, respectively.
[b] Estimated standard deviations for the donor-hydrogen-acceptor angles are near 2°.
[c] W-1 is the oxygen atom of the water molecule, and H-W1A and H-W1B are the hydrogen atoms of the water molecule.

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