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WR 194,965 phosphate, a new antimalarial agent containing a biphenyl ring structure active against chloroquine-resistant Plasmodium falciparum, crystallized in ionic form with a positive charge on the quaternary nitrogen atom. The oxygen and nitrogen atoms of WR 194,965 were hydrogen bonded to the same phosphate group. The nitrogen atom was also hydrogen bonded to a second phosphate group. The phosphate ions formed discrete clusters of four phosphate moieties. The phosphate clusters contained fourfold inversion symmetry. The intramolecular N-O distances in the active cinchona alkaloids and may be important for activity. A comparison of the crystalline structure of WR 194,965 with those of mefloquine and quinidine sulfate demonstrated that the regions of the three molecules in the vicinity of the aliphatic nitrogen atom and the oxygen atom superimpose. Much of the remainder of the WR 194,965 molecule spatially overlapped with the combined three-dimensional space defined by quinidine and mefloquine. The crystallographic parameters were: $C_{21}H_{19}ClNO^{+}\cdot H_2PO^{-}_4$; M_r = 443.9; symmetry of unit cell, tetragonal; space group, I41/a; parameters of unit cell, a = b = 24.305 ± 0.002

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Å, c = 17.556 ± 0.003 Å; V (volume of unit cell) = 10370.9 $Å^3$; Z (number of molecules per unit cell) = 16, D_x (calculated density) = 1.137 g cm^{-3}; source of radiation, CuKα (λ = 1.54178 Å); μ (absorption coefficient) = 21.3 cm^{-1}; F(000) (sum of atomic scattering factors at zero scattering angle) = 3,440; room temperature; final R = 8.2% for 2,508 reflections with $|Fo| > 3\sigma$.

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Crystal and Molecular Structure of the Antimalarial Agent 4-(*tert*-Butyl)-2-(*tert*-Butylaminomethyl)-6-(4-Chlorophenyl)Phenol Dihydrogen Phosphate (WR 194,965 Phosphate)

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WR 194,965 phosphate, a new antimalarial agent containing a biphenyl ring structure active against chloroquine-resistant *Plasmodium falciparum*, crystallized in ionic form with a positive charge on the quaternary nitrogen atom. The oxygen and nitrogen atoms of WR 194,965 were hydrogen bonded to the same phosphate group. The nitrogen atom was also hydrogen bonded to a second phosphate group. The phosphate ions formed discrete clusters of four phosphate moieties. The phosphate clusters contained fourfold inversion symmetry. The intramolecular N-O distance in WR 194,965 of 3.073 Å (1 Å = 0.1 nm) was close to the reported values for N-O distances in the active cinchona alkaloids and may be important for activity. A comparison of the crystalline structure of WR 194,965 with those of mefloquine and quinidine sulfate demonstrated that the regions of the three molecules in the vicinity of the aliphatic nitrogen atom and the oxygen atom superimpose. Much of the remainder of the WR 194,965 molecule spatially overlapped with the combined three-dimensional space defined by quinidine and mefloquine. The crystallographic parameters were: $C_{21}H_{29}ClNO^+ \cdot H_2PO_4^-$; M_r = 443.9; symmetry of unit cell, tetragonal; space group, $I4_1/a$; parameters of unit cell, $a = b = 24.305 \pm 0.002$ Å, $c = 17.556 \pm 0.003$ Å; V (volume of unit cell) = 10370.9 Å^3; Z (number of molecules per unit cell) = 16; D_x (calculated density) = 1.137 g cm^{-3}; source of radiation, CuKα (λ = 1.54178 Å); μ (absorption coefficient) = 21.3 cm^{-1}; F(000) (sum of atomic scattering factors at zero scattering angle) = 3,440; room temperature; final R = 8.2% for 2,508 reflections with $|F_o| > 3\sigma$.

Although there are four *Plasmodium* species that affect humans, most of the mortality, an estimated 1,000,000 to 2,000,000 deaths per year, is attributed to *Plasmodium falciparum*. Chloroquine-resistant *P. falciparum*, first reported in the Far East and South America in the 1960s, is now prevalent in all major equatorial continents. WR 194,965 phosphate (Fig. 1) represents the first compound containing a biphenyl ring structure to be developed as an antimalarial agent for treating chloroquine-resistant *P. falciparum*. WR 194,965 hydrochloride has a 90% curative dose (CD_{90}) of 27 mg (base) per kg in the *Aotes* monkey infected with the multidrug resistant strain, *P. falciparum* Vietnam Smith (15). CD_{90} is a function of total dose rather than of dosage regimen, and the CD_{90} of WR 194,965 is almost identical to the CD_{90} of chloroquine against the susceptible strain and compares favorably with the CD_{90} of mefloquine against the resistant strain (16). WR 194,965 phosphate, when orally administered three times to six human subjects infected with the Vietnam Smith strain at a dose of 750 mg every 12 h, cured four of the six patients (5). Although two subjects recrudesced (at 29 and 40 days posttreatment) and were successfully cured with mefloquine, in vitro tests demonstrated that the parasites from these patients were sensitive towards WR 194,965.

The mechanisms of activity of the amino alcohol antimalarial agents, such as quinine and mefloquine, and the dialkylaminoalkylamino antimalarial agents, such as chloroquine, and of the resistance of *Plasmodium* strains to these agents have yet to be fully elucidated. The importance of three-dimensional structure to antimalarial activity is exemplified by the cinchona alkaloids, in which the asymmetric centers at the two carbon atoms of the side chain adjacent to the quinoline ring must be in the *erythro* conformation for the alkaloid to exhibit activity (19). Other features relating amino alcohol structure to activity, such as the number of carbon atoms allowable between a hydroxyl group and an aliphatic nitrogen atom, have been recognized through extensive synthesis and testing programs over the last 45 years. These structural requirements suggest the possibility of a receptor which triggers the antimalarial action of these compounds. Further evidence for a receptor or an intracellular effector for antimalarial action is the recent work of Geary et al. (6) which demonstrated no significant difference in the rate of chloroquine accumulation or in the steady-state intracellular chloroquine concentration between sensitive and resistant strains of *P. falciparum*. However, a detailed structure of a possible receptor for these antimalarial agents has yet to emerge.

The cinchona alkaloids (and/or analogs) and mefloquine, both active to various extents against resistant strains of *P. falciparum*, have been studied by X-ray crystallography (2, 3, 9–14, 18), yielding information concerning the three-dimensional requirements of an active antimalarial agent. WR 194,965, which also possesses antimalarial activity against resistant strains of *P. falciparum*, represents a structure type different from either the cinchona alkaloids or mefloquine. To provide a more complete picture of the three-dimensional structural requirements for antimalarial activity, the three-dimensional structure of WR 194,965 phosphate was established and compared with those of quinidine and mefloquine.

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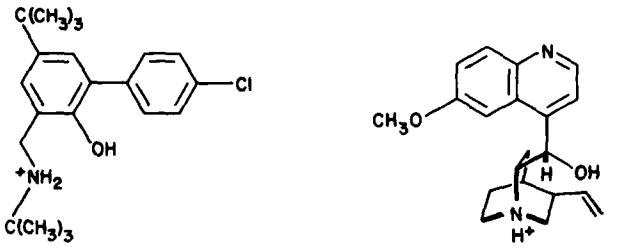

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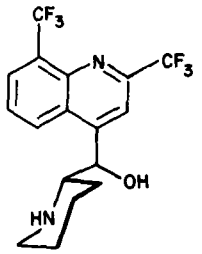

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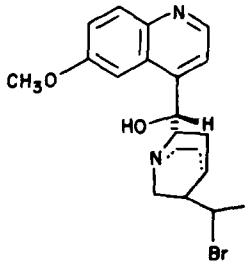

IV

FIG. 1. Chemical structures of WR 194,965 (I), quinidine (II), mefloquine (III), and 10-bromo-10,11-dihydroepiquinidine (IV).

MATERIALS AND METHODS

4-(*tert*-Butyl)-2-(*tert*-butylaminoethyl)-6-(4-chlorophenyl) phenol phosphate (WR 194,965 phosphate) was synthesized on contract for the Walter Reed Army Institute of Research by Cordova Chemical Co. (Sacramento, Calif.) and was crystallized from methanol-ethyl acetate. Diffraction data were collected from a clear octahedral-shaped crystal (maximum width, 0.07 mm) in the θ-2θ mode to a maximum 2θ value of 115° on a four-circle diffractometer (R3M Nicolet; Nicolet, Madison, Wis.) with a graphite monochrometer. The X-ray source was CuKα radiation (50 kV, 40 mA). The ranges of indices were h, $0 \rightarrow 26$; k, $0 \rightarrow 26$; and l, $0 \rightarrow 19$. The total number of independent reflections was 3,564. The standard reflections 12,0,0, 0,12,0, and 0,0,4 were monitored after every 60 intensity measurements. The standards remained constant within 5.2%. The lattice parameters were based on 19 centered reflections with 2θ values between 25° and 30°. The data were corrected for Lorentz and polarization effects, but no correction for absorption or extinction was used.

The structure was solved routinely by direct phase determination (8). All but one of the nonhydrogen atoms were found in the first E map. The next cycle produced the remaining nonhydrogen atom. All of the hydrogen atoms were found in difference maps. There were no other significant peaks around the atoms to which hydrogen atoms were bonded. Least-square refinement was performed by using 2,508 reflections with $|F_0| > 3\sigma(F_0)$. The hydrogen atoms attached to carbon atoms were placed in idealized positions. Coordinates for the nonhydrogen atoms and the five hydrogen atoms attached to oxygen and nitrogen atoms were refined by least squares (on F) with a blocked cascade program in the SHELXTL system (17). Anisotropic thermal parameters for the C, Cl, N, O, and P atoms and isotropic thermal parameters for the hydrogen atoms were refined for a total of 298 parameters. The parameters for the five hydrogen atoms subjected to least-squares refinement remained stable, although, of course, their standard deviations were 5 to 10 times larger than those for the C or O atoms. Additional peaks, occurring in the difference maps near the fourfold screw axis, must have belonged to atoms in the solvent molecules. However, the relatively low weight of the peaks and their positional relationship with respect to each other indicated molecules disordered along a solvent channel. The type of solvent molecule and the disorder in the solvent could not be elucidated. Such a disorder in a discrete part of a crystal usually leads to a higher R factor than would be expected otherwise, since a few low index reflections cannot be fitted well. The final values for R and wR (a weighted correlation factor) were 8.2 and 7.6%, respectively; w was calculated from the following: $w = 1/(\sigma^2 [|F_0| + (0.0004)(F_0)^2])$. The final difference in electron density was $|\rho| < 0.81$ eÅ^{-3} (1 Å = 0.1 nm). Atomic scattering factors were those incorporated in SHELXTL (17).

RESULTS

Coordinates and thermal parameters (U_{eq}) for the nonhydrogen atoms are listed in Table 1, coordinates for the hydrogen atoms are listed in Table 2, and bond lengths and angles are listed in Tables 3 and 4. The bond length of the hydrogen atoms attached to the carbon atoms was kept fixed at 0.96 Å throughout the refinement procedure. The torsion angles are listed in Table 5, and the numbering scheme for the molecule is shown in Fig. 2.

WR 194,965 phosphate crystallized in ionic form with a positive charge on the quaternary nitrogen atom (Fig. 2). The average planes of the two phenyl rings were twisted from each other by 44.8 ± 1°. The phenyl ring composed of C-1 to C-6 deviated significantly from planarity by a few degrees (Table 5). Significant interatomic distances within the WR 194,965 molecule were N-14–O-23, 3.073 Å; O-23–Cl, 6.752 Å; and N-14–Cl, 9.662 Å. The oxygen and nitrogen atoms were hydrogen bonded to the same phosphate group (O-23–O-3P; 3.008 Å) (N-14–O-2P; 2.724 Å) (Table 6). The nitrogen was also hydrogen bonded to a second phosphate group (N-14–O-2P'; 2.972 Å) from a second discrete phosphate cluster. The hydrogen atom in this second hydrogen bond was positioned equidistant between the nitrogen and the phosphate oxygen (see Table 3 and Fig. 3). The O-2 atom of the phosphate group was hydrogen bonded to N-14 of two separate WR 194,965 molecules (Fig. 3).

The phosphorus-to-oxygen bond lengths of the phosphate groups ranged from 1.493 to 1.504 (± 0.003) Å for O-1 and O-2 (P=O bonds) and from 1.569 to 1.571 (± 0.005) Å for O-3 and O-4 (P-OH bonds). These values were very close to the values for imidazolinium dihydrogen orthophosphate, histidinium dihydrogen orthophosphate orthophosphoric acid, and (−)-ephedrine dihydrogen phosphate which range from 1.489 to 1.518 (± 0.003) Å for the P=O bonds and 1.544 to 1.578 (± 0.005) Å for the P-OH bonds (1, 7).

The phosphate ions formed discrete clusters containing four phosphate groups (Fig. 4). Each phosphate cluster had fourfold inversion symmetry. Each phosphate group was hydrogen bonded to two other phosphate groups, to two amine groups on separate WR 194,965 molecules, and to one hydroxyl moiety (Fig. 2 to 5). Four WR 194,965 molecules circle the phosphate clusters (Fig. 5, center top and center bottom). The entire unit of four WR 194,965 molecules

TABLE 1. Fractional coordinates and thermal parameters U_{eq} for nonhydrogen atoms[a]

Atom	Fractional coordinate			U_{eq}[b]
	x	y	z	
P	0.5094 (0.0001)	0.6410 (0.0001)	0.0632 (0.0001)	0.037 (0.001)
O-1	0.5046 (0.0002)	0.6520 (0.0002)	0.1472 (0.0002)	0.039 (0.002)
O-2	0.5085 (0.0002)	0.5827 (0.0002)	0.0362 (0.0003)	0.046 (0.002)
O-3	0.5651 (0.0002)	0.6657 (0.0002)	0.0333 (0.0003)	0.053 (0.002)
O-4	0.4620 (0.0002)	0.6723 (0.0002)	0.0208 (0.0003)	0.060 (0.002)
Cl	1.0944 (0.0002)	0.6616 (0.0001)	0.0038 (0.0002)	0.086 (0.001)
C-1	0.9485 (0.0003)	0.6902 (0.0003)	0.0965 (0.0004)	0.045 (0.003)
C-2	1.0041 (0.0003)	0.6842 (0.0003)	0.0862 (0.0004)	0.050 (0.003)
C-3	1.0239 (0.0003)	0.6698 (0.0003)	0.0168 (0.0004)	0.049 (0.003)
C-4	0.9893 (0.0003)	0.6618 (0.0003)	−0.0433 (0.0004)	0.053 (0.003)
C-5	0.9342 (0.0003)	0.6653 (0.0003)	−0.0321 (0.0004)	0.048 (0.003)
C-6	0.9119 (0.0003)	0.6803 (0.0003)	0.0379 (0.0004)	0.040 (0.003)
C-7	0.8511 (0.0003)	0.6858 (0.0003)	0.0454 (0.0004)	0.044 (0.003)
C-8	0.8205 (0.0003)	0.6629 (0.0003)	0.1051 (0.0004)	0.044 (0.003)
C-9	0.7631 (0.0003)	0.6657 (0.0003)	0.1042 (0.0004)	0.042 (0.003)
C-10	0.7353 (0.0003)	0.6915 (0.0003)	0.0463 (0.0004)	0.046 (0.003)
C-11	0.7641 (0.0003)	0.7160 (0.0003)	−0.0142 (0.0004)	0.047 (0.003)
C-12	0.8220 (0.0003)	0.7113 (0.0003)	−0.0121 (0.0004)	0.046 (0.003)
C-13	0.7320 (0.0003)	0.6390 (0.0003)	0.1688 (0.0004)	0.046 (0.003)
N-14	0.7357 (0.0002)	0.6719 (0.0002)	0.2407 (0.0003)	0.036 (0.002)
C-15	0.7238 (0.0003)	0.6404 (0.0003)	0.3139 (0.0004)	0.044 (0.003)
C-16	0.6707 (0.0003)	0.6084 (0.0003)	0.3057 (0.0005)	0.062 (0.003)
C-17	0.7191 (0.0004)	0.6839 (0.0003)	0.3747 (0.0004)	0.071 (0.004)
C-18	0.7726 (0.0004)	0.6022 (0.0004)	0.3315 (0.0005)	0.072 (0.004)
C-19	0.7348 (0.0003)	0.7449 (0.0004)	−0.0796 (0.0004)	0.057 (0.003)
C-20	0.6811 (0.0004)	0.7732 (0.0004)	−0.0521 (0.0005)	0.099 (0.005)
C-21	0.7184 (0.0005)	0.7010 (0.0004)	−0.1388 (0.0005)	0.121 (0.006)
C-22	0.7692 (0.0004)	0.7912 (0.0005)	−0.1146 (0.0007)	0.147 (0.007)
O-23	0.8437 (0.0003)	0.6349 (0.0002)	0.1653 (0.0003)	0.055 (0.002)

[a] Values in parentheses are estimated standard deviations.
[b] $U_{eq} = (\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j)/3$.

surrounding a phosphate cluster formed the entity which was positioned about the fourfold screw axis. Disordered solvent atoms were found in a solvent channel shown by the open spaces at center right and center left of Fig. 5. These solvent atoms were positioned tightly around a fourfold screw axis and were surrounded by hydrophobic groups. These atoms were not included in the structure factor calculations.

DISCUSSION

The historical precedent for the antimalarial activity of the amino alcohols comes from the cinchona alkaloids. A number of key structural features necessary for antimalarial activity of the four main cinchona alkaloids, quinine, quinidine, cinchonine, and cinchonidine, as well as amino alcohol antimalarial agents in general, have emerged from examination of active and inactive analogs. The compound must have an aromatic nucleus connected to a carbinol group and an aliphatic nitrogen separated by no more than one or two carbon atoms from the carbinol group (19). As demonstrated by the cinchona alkaloids for which the *erythro* forms are active and the *threo* forms are inactive, the hydroxyl group and the aliphatic nitrogen atom must be able to assume the geometry necessary to interact with the intracellular effector. In the quinidine molecule, the carbinol group and the rigidly fixed aliphatic nitrogen atom are separated by N-C-C-O torsion angles of 61° and 81° (9) for the two similar conformations of quinidine sulfate and 76° for quinidine base (10). The N-C-C-O torsion angles of the potent amino

TABLE 2. Fractional coordinates for hydrogen atoms attached to nitrogen (Hn) or oxygen (Ho)

Atom	Fractional coordinate[a]		
	x	y	z
Hn-1	0.7640 (0.0022)	0.6906 (0.0022)	0.2498 (0.0029)
Hn-2	0.7033 (0.0040)	0.7147 (0.0040)	0.2262 (0.0051)
Ho-3	0.5769 (0.0027)	0.6938 (0.0027)	0.0462 (0.0038)
Ho-4	0.4450 (0.0026)	0.6939 (0.0026)	0.0484 (0.0036)
Ho-23	0.8725 (0.0035)	0.6420 (0.0038)	0.1815 (0.0052)

[a] Values in parentheses are estimated standard deviations.

TABLE 3. Bond lengths

Bond	Bond length (Å)[a]	Bond	Bond length (Å)[a]
P–O-1	1.504 (0.005)	P–O-2	1.493 (0.005)
P–O-3	1.571 (0.005)	P–O-4	1.569 (0.005)
C-1–C-2	1.371 (0.010)	Cl–C-3	1.741 (0.007)
C-2–C-3	1.355 (0.011)	C-1–C-6	1.382 (0.010)
C-4–C-5	1.356 (0.010)	C-3–C-4	1.363 (0.011)
C-6–C-7	1.489 (0.010)	C-5–C-6	1.391 (0.010)
C-7–C-12	1.379 (0.010)	C-7–C-8	1.400 (0.010)
C-8–O-23	1.378 (0.009)	C-8–C-9	1.398 (0.010)
C-9–C-13	1.510 (0.010)	C-9–C-10	1.371 (0.010)
C-11–C-12	1.413 (0.010)	C-10–C-11	1.405 (0.010)
C-13–N-14	1.496 (0.009)	C-11–C-19	1.523 (0.010)
C-15–C-16	1.514 (0.010)	N-14–C-15	1.525 (0.009)
C-15–C-18	1.537 (0.011)	C-15–C-17	1.506 (0.011)
C-19–C-21	1.542 (0.013)	C-19–C-20	1.554 (0.012)
C-19–C-22	1.530 (0.014)		

[a] Values in parentheses are estimated standard deviations.

TABLE 4. Bond angles

Bond	Bond angle (°)[a]	Bond	Bond angle (°)[a]
O-1–P–O-2	118.7 (0.3)	O-1–P–O-3	109.0 (0.3)
O-2–P–O-3	105.6 (0.3)	O-1–P–O-4	108.7 (0.3)
O-2–P–O-4	107.5 (0.3)	O-3–P–O-4	106.7 (0.3)
C-1–C-2–C-3	119.8 (0.7)	C-2–C-1–C-6	121.2 (0.7)
Cl–C-3–C-4	119.3 (0.6)	C-3–C-2	119.8 (0.6)
C-3–C-4–C-5	119.2 (0.7)	C-2–C-3–C-4	120.9 (0.7)
C-1–C-6–C-5	116.9 (0.6)	C-4–C-5–C-6	122.0 (0.7)
C-5–C-6–C-7	119.3 (0.6)	C-1–C-6–C-7	123.9 (0.6)
C-6–C-7–C-12	118.9 (0.6)	C-6–C-7–C-8	124.0 (0.6)
C-7–C-8–C-9	120.3 (0.7)	C-8–C-7–C-12	116.9 (0.6)
C-8–C-9–C-13	118.0 (0.6)	C-7–C-8–O-23	123.5 (0.7)
C-9–C-8–O-23	116.2 (0.6)	C-8–C-9–C-10	121.4 (0.7)
C-9–C-10–C-11	120.6 (0.7)	C-10–C-9–C-13	120.5 (0.6)
C-10–C-11–C-19	122.2 (0.6)	C-10–C-11–C-12	116.2 (0.6)
C-7–C-12–C-11	124.5 (0.7)	C-12–C-11–C-19	121.6 (0.6)
C-13–N-14–C-15	115.5 (0.5)	C-9–C-13–N-14	111.8 (0.6)
N-14–C-15–C-16	109.8 (0.6)	N-14–C-15–C-17	105.1 (0.5)
C-16–C-15–C-17	111.3 (0.6)	N-14–C-15–C-18	109.0 (0.6)
C-16–C-15–C-18	111.4 (0.6)	C-17–C-15–C-18	110.0 (0.6)
C-11–C-19–C-20	111.3 (0.6)	C-11–C-19–C-21	108.1 (0.7)
C-20–C-19–C-21	107.4 (0.7)	C-11–C-19–C-22	112.8 (0.7)
C-20–C-19–C-22	104.9 (0.8)	C-21–C-19–C-22	112.3 (0.8)

[a] Values in parentheses are estimated standard deviations.

alcohol mefloquine are 61° and 66° for the two similar crystalline conformations (14). However, the inactive cinchona alkaloid epiquinidine, crystallized as 10-bromo-10,11-dihydroepiquinidine, has an N-C-C-O torsion angle of −45° (3).

WR 194,965 is structurally similar to the amino alcohols containing an alcohol function in the form of a phenol and an aliphatic nitrogen separated from the alcohol function by three carbons rather than the two carbons found in the cinchona alkaloids. The angle between the C-8–O-23 bond and the C-13–N-14 bond in WR 194,965 is 66°. To correlate the three-dimensional geometry of WR 194,965 with the active cinchona alkaloids and the potent mefloquine, the phenol oxygen and aliphatic nitrogen atoms of WR 194,965

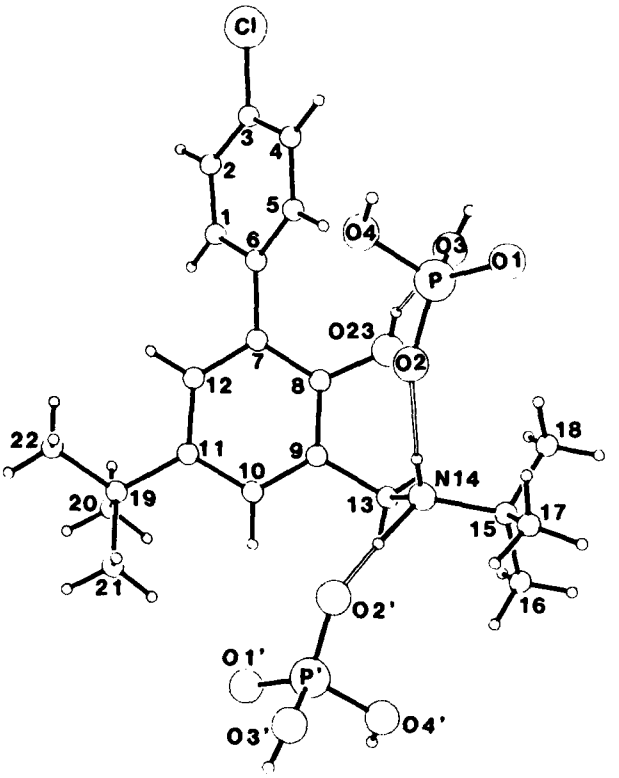

FIG. 2. WR 194,965 numbering scheme. The hydrogen bonding between WR 194,965 and the phosphate groups is designated by the hollow bonds. The sizes of the spheres were arbitrarily chosen to generally correspond to the atomic weights of the atoms.

have been superimposed with the hydroxyl and aliphatic nitrogen atoms from quinidine sulfate (Fig. 1 and 6A) (9) and from mefloquine (Fig. 1 and 6B) (14). As the diagrams demonstrate, the oxygen and nitrogen atoms of the three molecules superimposed well. When examined individually,

TABLE 5. Torsion angles

Bond	Torsion angle (°)[a]	Bond	Torsion angle (°)[a]
C-6–C-1–C-2–C-3	1.8 (1.1)	C-7–C-8–C-9–C-13	178.3 (0.6)
C-2–C-1–C-6–C-5	−1.4 (1.0)	O-23–C-8–C-9–C-10	−178.6 (0.6)
C-2–C-1–C-6–C-7	179.6 (0.7)	O-23–C-8–C-9–C-13	0.7 (1.0)
C-1–C-2–C-3–Cl	−179.4 (0.6)	C-8–C-9–C-10–C-11	−0.2 (1.1)
C-1–C-2–C-3–C4	0.7 (1.1)	C-13–C-9–C-10–C-11	−179.4 (0.6)
Cl–C-3–C-4–C-5	176.5 (0.6)	C-8–C-9–C-13–N-14	74.7 (0.8)
C-2–C-3–C-4–C-5	−3.7 (1.1)	C-10–C-9–C-13–N-14	−106.1 (0.7)
C-3–C-4–C-5–C-6	4.2 (1.1)	C-9–C-10–C-11–C-12	1.4 (1.0)
C-4–C-5–C-6–C-1	−1.7 (1.1)	C-9–C-10–C-11–C-19	179.9 (0.7)
C-4–C-5–C-6–C-7	177.4 (0.7)	C-10–C-11–C-12–C-7	−1.6 (1.1)
C-1–C-6–C-7–C-8	−48.4 (1.1)	C-19–C-11–C-12–C-7	179.9 (0.7)
C-1–C-6–C-7–C-12	136.6 (0.7)	C-10–C-11–C-19–C-20	33.3 (1.0)
C-5–C-6–C-7–C-8	132.6 (0.8)	C-10–C-11–C-19–C-21	−84.4 (0.9)
C-5–C-6–C-7–C-12	−42.4 (1.0)	C-10–C-11–C-19–C-22	150.8 (0.8)
C-6–C-7–C-8–C-9	−174.3 (0.7)	C-12–C-11–C-19–C-20	−148.3 (0.7)
C-6–C-7–C-8–O-23	3.1 (1.1)	C-12–C-11–C-19–C-21	94.0 (0.9)
C-12–C-7–C-8–C-9	0.8 (1.0)	C-12–C-11–C-19–C-22	−30.8 (1.1)
C-12–C-7–C-8–O-23	178.2 (0.7)	C-9–C-13–N-14–C-15	−159.6 (0.6)
C-6–C-7–C-12–C-11	175.9 (0.7)	C-13–N-14–C-15–C-16	−49.3 (0.7)
C-8–C-7–C-12–C-11	0.5 (1.1)	C-13–N-14–C-15–C-17	−169.1 (0.6)
C-7–C-8–C-9–C-10	−1.0 (1.1)	C-13–N-14–C-15–C-18	73.1 (0.7)

[a] Values in parentheses are estimated standard deviations.

TABLE 6. Hydrogen bond distances and angles

Donor atom	Hydrogen atom[a]	Acceptor atom	Donor-acceptor distance (Å)[b]	Hydrogen-acceptor distance (Å)[b]	Donor-hydrogen-acceptor angle (°)[c]	Symmetry equivalent operations to obtain donor
N-14	Hn-1	O-2	3.008	2.17	173	$1/4 + y, 5/4 - x, 1/4 + z$
N-14	Hn-2	O′-2	2.724	1.40	177	$5/4 - y, 1/4 + x, 1/4 - z$
O-23	Ho-23	O-3	2.972	2.32	142	$1/4 + y, 5/4 - x, 1/4 + z$
O-3	Ho-3	O′-1	2.607	1.85	164	$5/4 - y, 1/4 + x, 1/4 - z$
O-4	Ho-4	O′-1	2.712	1.89	174	$-1/4 - y, 5/4 - x, 1/4 - z$

[a] Hn, Hydrogen bonded to nitrogen; Ho, hydrogen bonded to oxygen.
[b] Estimated standard deviations for the donor-acceptor and the hydrogen-acceptor distances are near 0.007 and 0.07 A, respectively.
[c] Estimated standard deviations for the donor-hydrogen-acceptor angle are between 5 and 7°.

much of the WR 194,965 molecule did not superimpose well with the quinidine or mefloquine molecule. The methoxyl group of quinidine did coincide with the *tert*-butyl group of WR 194,965; however, the methoxyl group is not necessary for activity (19).

When all three molecules, WR 194,965, quinidine, and mefloquine, were overlapped, a three-dimensional space became defined. The WR 194,965 molecule fitted more closely to the three-dimensional space defined by both quinidine and mefloquine than by each individually. Although the aromatic ring is necessary for activity, the geometry of the ring apparently need not be as precise. Superposition of WR 194,965 with the inactive epiquinidine molecule on the C-O phenol-alcohol bonds defined a different three-dimensional space (Fig. 1 and 6C), which may not interact with the intracellular effector for antimalarial activity. Instead of being positioned off position 2 of the quinoline ring in a fashion similar to that of the trifluoromethyl group of mefloquine, the chlorine atom of WR 194,965 protruded from the opposite side of the quinoline ring. The quinuclidine moiety in epiquinidine and in quinidine were on the opposite sides of the amino side chain of WR 194,965.

The positioning of the N and OH groups may be the most important spatial feature for defining activity. There was lack of superposition between the active WR 194,965 and the inactive epiquinidine with respect to the N and OH groups (Fig. 6C). In her review, Oleksyn (13) points out that in the crystal structures of the active cinchona alkaloids and/or derivatives the aliphatic N and OH groups are always involved in intermolecular rather than intramolecular hydrogen bonding, as found in the inactive epiquinidine. The intermolecular binding of the WR 194,965 molecule to the phosphate groups and the intermolecular binding of mefloquine molecules to each other (14) corroborates this theory. In addition, the interatomic N-O distances in active cinchona alkaloids range from 2.84 to 3.17 Å, and in the two conformations of mefloquine, the distances are 2.78 Å and 2.85 Å.

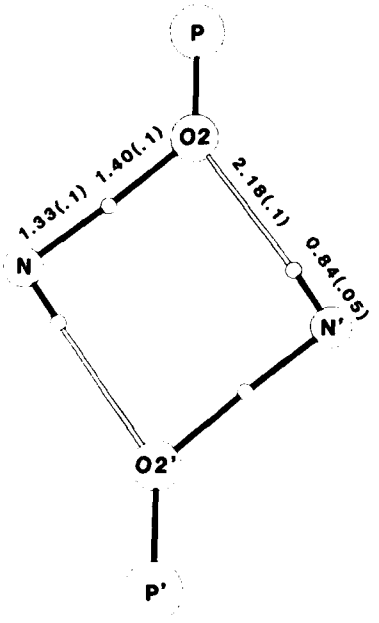

FIG. 3. Symmetrical hydrogen bonding between two WR 194,965 molecules and two phosphate molecules. The sizes of the spheres were arbitrarily chosen to correspond to the atomic weights of the atoms. The hollow bonds represent hydrogen bonds. Selected bond lengths are shown in Ångström units with estimated standard deviations in parentheses.

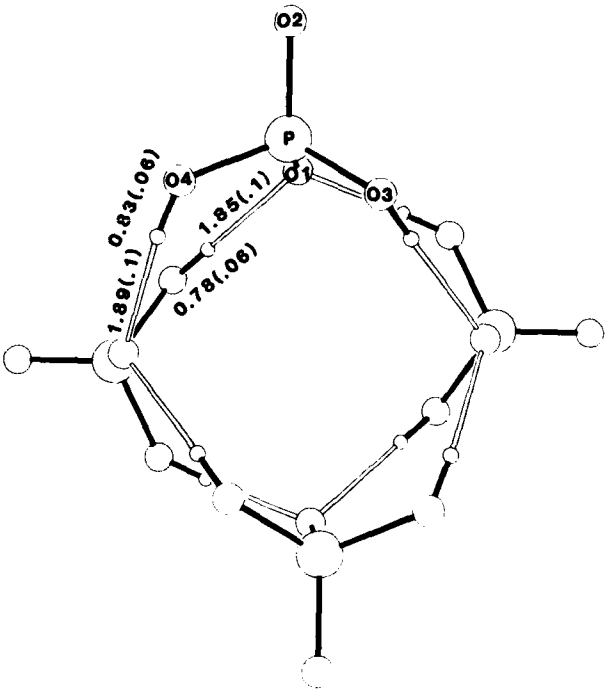

FIG. 4. Discrete phosphate cluster composed of four individual dihydrogen phosphate groups. The cluster contains fourfold inversion symmetry. The sizes of the spheres were arbitrarily chosen to correspond to the atomic weights of the atoms. The hollow bonds represent hydrogen bonds. Selected bond lengths are shown in Angström units with estimated standard deviations in parentheses.

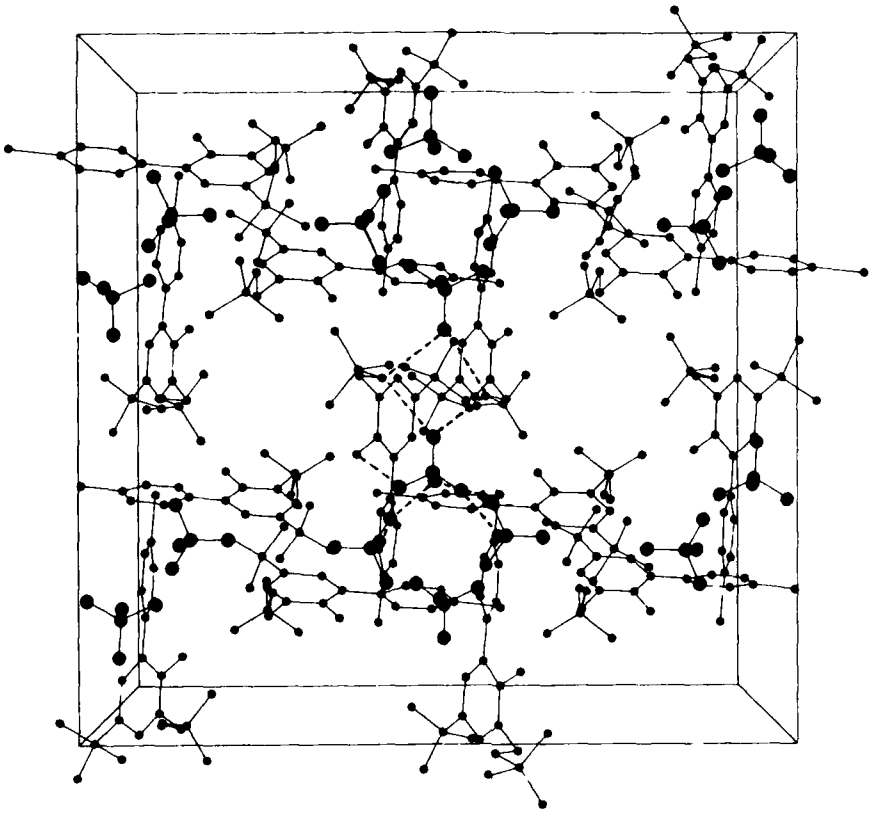

FIG. 5. Packing of WR 194,965 phosphate. Only nonhydrogen atoms are shown. The contents of one unit cell are illustrated with small spheres for the 16 WR 194,965 molecules and large spheres for the 16 phosphate groups. In the center of the diagram, dotted lines have been drawn between atoms to illustrate representative hydrogen bonds.

The N-O distance in quinidine sulfate is 3.09 Å, whereas the N-O distance in the inactive 10-bromo-10,11-dihydroepiquinidine is 2.60 Å with the N and OH groups positioned in the opposite direction with respect to the aromatic rings. The interatomic N-O distance in WR 194,965 at 3.07 Å fits right into the range for the active amino alcohols.

The phosphate groups are noteworthy because of the uniqueness of their three-dimensional structures. Phosphate groups in organic structures usually crystallize in sheets or chains interwoven with hydrogen bonding throughout the three-dimensional structure of the crystal (4). To our knowledge, the WR 194,965 phosphate structure is the first exam-

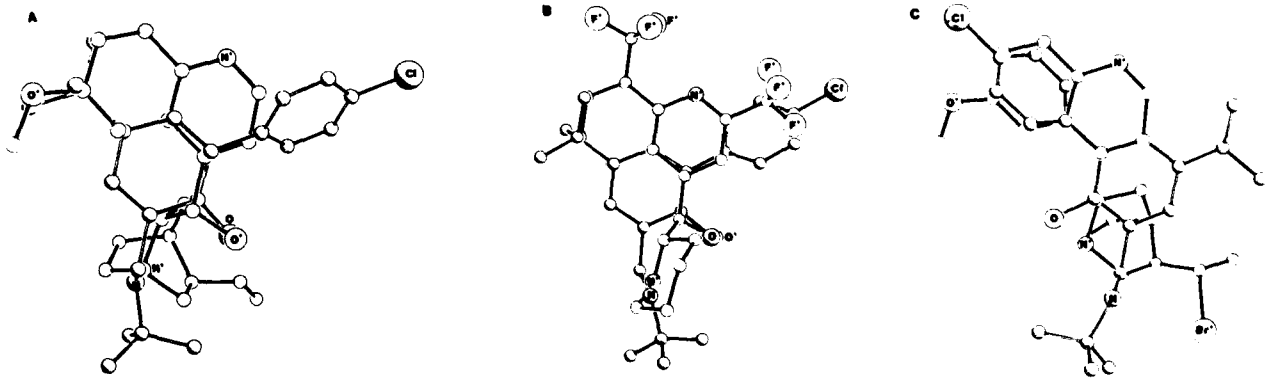

FIG. 6. Superposition of WR 194,965 (hollow bonds) with quinidine (A), mefloquine (B), and 10-bromo-10,11-dihydroepiquinidine (C) (solid bonds). Only nonhydrogen atoms are shown. The heteroatoms of each molecule are labeled without prime marks on the WR 194,965 molecule labels and with prime marks for the other three molecules. (A and B) The aliphatic nitrogen and the phenol-alcohol oxygen were superimposed. (C) The C-O phenol-alcohol bonds were superimposed, causing WR 194,965 to be rotated with respect to Fig.1 and panels A and B. The sizes of the spheres were arbitrarily chosen to generally correspond to the atomic weights of the atoms.

ple in which phosphate groups crystallize in discrete clusters of four $H_2PO_4^-$ moieties in a ring. The phosphate groups were involved in hydrogen bonding to WR 194,965 molecules, but did not form three-dimensional hydrogen bonding networks between phosphate clusters throughout the crystal.

In summary, the three-dimensional structure of WR 194,965 reinforces the importance of the geometry of the N-O portion of the molecule and the possible importance of hydrogen bonding with a receptor. Although the remainder of the WR 194,965 molecule did not superimpose well with either mefloquine or the active cinchona alkaloids on an individual basis, WR 194,965 fitted more closely into the three-dimensional space defined by a combination of mefloquine and quinidine.

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