Anomalous dispersion of sulfur in quinidine sulfate, $(C_{20}H_{25}N_2O_2)_2SO_4\cdot2H_2O$: Implications for structure analysis

(x-ray analysis/tangent formula/Bijvoet differences/hydrogen bonding/packing)

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ABSTRACT   A Patterson-type map computed with Bijvoet differences squared as coefficients, $(|F_h| - |F_{-h}|)^2$, as recommended by Rossmann, readily yielded the position of the S atom. The experiment was performed with Cu Kα radiation which is far from the absorption edge for sulfur. The coordinates of the remainder of the 54 C, N, and O atoms were derived by means of partial structure development by use of the tangent formula. The latter was used only to effect phase extension, not phase refinement. A main purpose of this experiment was to reaffirm, as first shown in the investigation of the protein crambin by Hendrickson and Teeter, that, in the presence of a large number of lighter atoms, sulfur atoms can be located by use of anomalous dispersion at wavelengths far from the absorption edge. The space group is $P2_1$ with $a = 26.718(8)$ Å, $b = 6.987(3)$ Å, $c = 10.857(6)$ Å, and $\beta = 99.51(4)°$ and contains two quinidyl ions, one sulfate ion, and two water molecules per asymmetric unit. The conformations of the two independent quinidyl ions differ mainly in the torsional angle of the bond between the vinyl side chain and the quinuclidine moiety. The R factor is 4.9% for all 2869 data.

The substance of interest in this investigation is the quinidine molecule that occurs as a sulfate salt. It is one of the antimalarial compounds that can be extracted from the bark of the cinchona tree. A main aspect of the analysis is the use, with ease, of the anomalous scattering of Cu Kα radiation from a sulfur atom far from its absorption edge (5.02 Å) to locate the position of the sulfur atom. This study follows the pioneering investigation of the protein crambin by Hendrickson and Teeter (1) in which the structure determination, based solely on data from the native protein, utilized primarily the anomalous dispersion of Cu Kα radiation by three disulfide bridges. In the crystal structure investigated here, there are 54 C, N, and O atoms and 1 S atom in the asymmetric unit.

It would have been possible to have located the S atom by use of the standard Patterson function. Its clearest representation is given by a Harker section of a standard Patterson function that is computed from only the high-angle scattering data because the contribution of the S atom to the scattered intensities relative to the other atoms is greatest at the highest angles. Even in this case, the definition of the appropriate peak, as measured by its enhancement over the next largest ones, is not as great as that obtained by use of only the 69 largest Bijvoet differences forming the coefficients, $(|F_h| - |F_{-h}|)^2$, for a Patterson function suggested by Rossmann (2). In other instances, it can be expected that atomic positions could be obtained from the Bijvoet differences but would not be derivable from a standard Patterson function. Calculation has shown that this is the case for crambin.

The structure of the quinidine molecules was developed from

the known position of the S atom by use of the tangent formula for phase determination. Special considerations were involved in this partial structure development because of special characteristics of space group $P2_1$ and also because the partial structure contained an atom that is heavier than the rest.
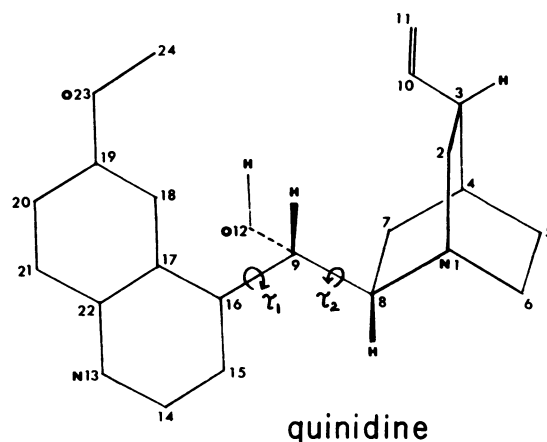
quinidine

The bark of the cinchona tree yields a mixture of alkaloids of which cinchonine, quinidine, cinchonidine, and quinine are active antimalarials whereas their epimers at C-9, epicinchonine, epiquinidine, epicinchonidine, and epiquinine, are inactive. Cinchonine and cinchonidine lack the OCH_3 group shown for quinidine. Cinchonidine and quinine are epimers of cinchonine and quinidine at C-8. Crystal structures have been reported for the free alkaloid cinchonine (3), the salt cinchoninium·CdCl₄·H₂O containing the divalent cation (4), the salt quinidyl 1,1'-dimethylferrocene-3-carboxylic acid containing the monovalent cation (5), and (+)-10-bromo-10,11-dihydro-epiquinidine (6). The absolute configuration was determined or confirmed in the latter three papers. In addition, conformational analyses of cinchona alkaloids by theoretical calculations using the EENY program (7) have been performed (8).

EXPERIMENTAL

A long, clear, prismatic crystal selected from a commercial sample from Aldrich had dimensions of 0.19 and 0.26 mm in cross section and 0.85 mm in length. A Nicolet P3F diffractometer equipped with a graphite monochromator was used with Cu Kα radiation for intensity measurement in the $\theta/2\theta$ scanning mode up to $2\theta_{max} = 112°$. In order to measure the Friedel pairs as closely in time as possible and still make efficient use of angle settings, the reflections were measured in rows for a particular h and k index while all the l and $\bar{l}$ indices were scanned, and then the corresponding row with $\bar{h}$ and $\bar{k}$ held constant was measured while all the l and $\bar{l}$ indices were scanned. The angular

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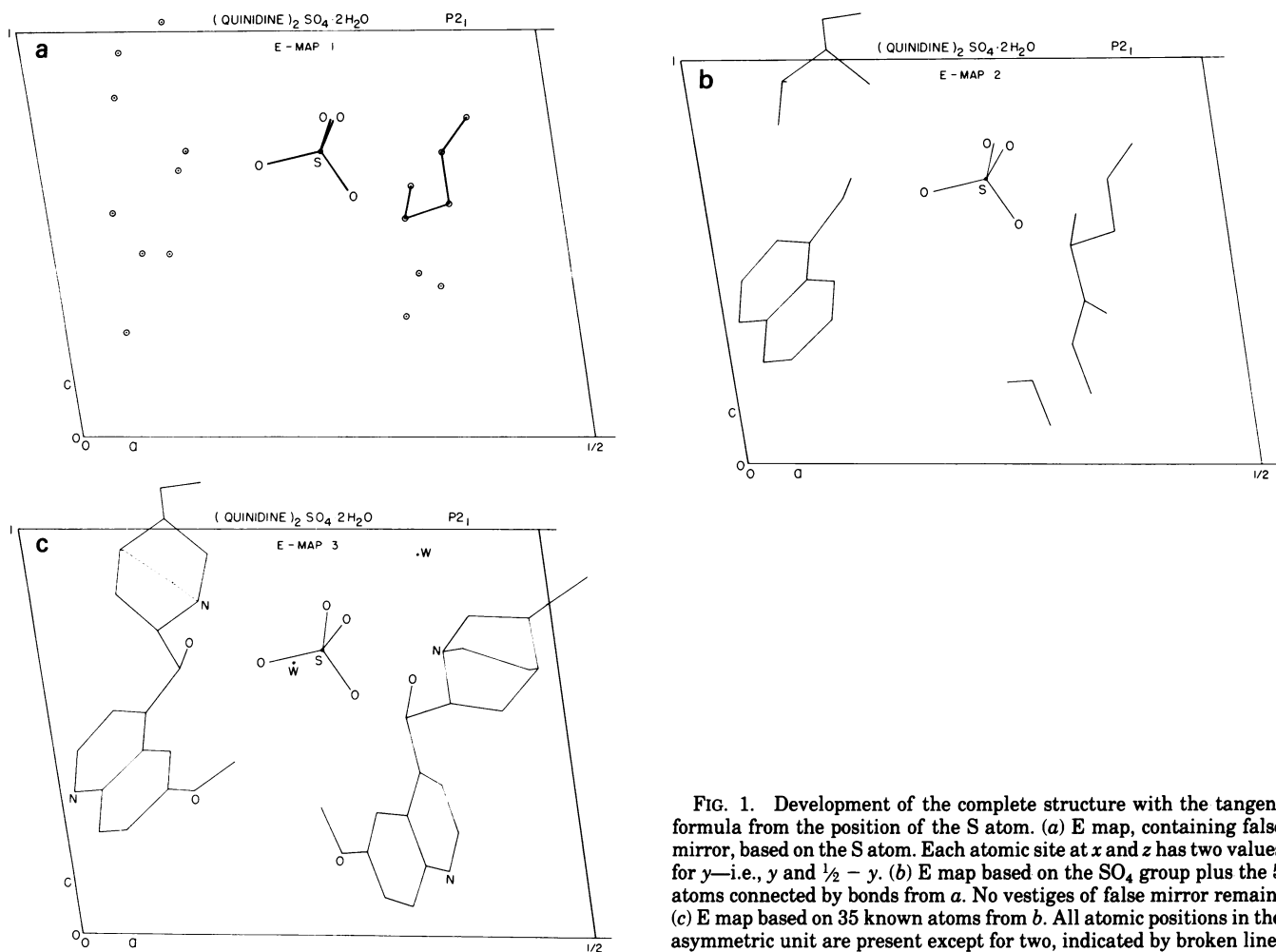*Proc. Natl. Acad. Sci. USA 78 (1981)*   5939

FIG. 1. Development of the complete structure with the tangent formula from the position of the S atom. (*a*) E map, containing false mirror, based on the S atom. Each atomic site at *x* and *z* has two values for *y*—i.e., *y* and ½ − *y*. (*b*) E map based on the SO₄ group plus the 5 atoms connected by bonds from *a*. No vestiges of false mirror remain. (*c*) E map based on 35 known atoms from *b*. All atomic positions in the asymmetric unit are present except for two, indicated by broken line.

settings 2θ, ω, ϕ, and ψ for *hkl* became 2θ, ω, ϕ-180°, and 360° − ψ for $\overline{hkl}$. A total of 2 × 2869 reflections were measured and considered observed (except, of course, the space group extinctions, 0k0 with k odd). It was important to use six reflections for standards, three *hkl* and the corresponding $\overline{hkl}$. The data were corrected for Lorentz and polarization effects but not for absorption. The path lengths for the beam through the crystal

are essentially identical for each Friedel pair and thus any absorption effects would not affect the differences in their intensities to a significant extent.

Crystal Data. $(C_{20}H_{25}N_2O_2)_2SO_4 \cdot 2H_2O$; FW 782.97; space group $P2_1$, $a = 26.718(8)$ Å, $b = 6.987(3)$ Å, $c = 10.857(6)$ Å, $\beta = 99.51(4)°$; V = 1999.2 Å³, Z = 2, $D_x = 1.3000$ g/cm³, $\mu = 1.219/mm$; Cu Kα radiation.
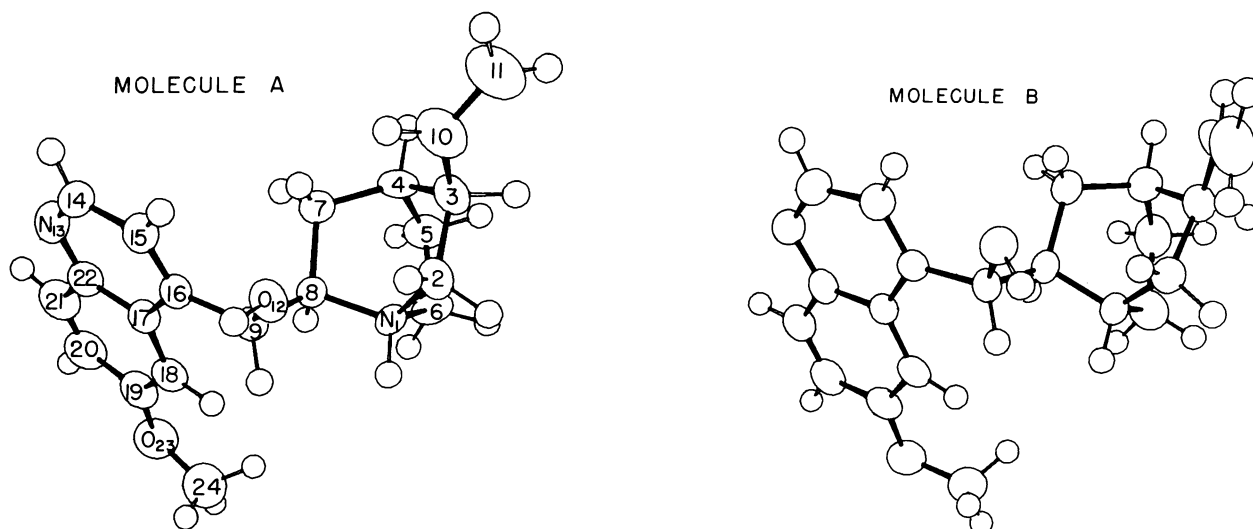

FIG. 2. Conformations of the two independent quinidyl ions, A and B. The main difference in conformation between molecules A and B occurs in the rotation about the C-3—C-10 bond.

Table 1. Fractional coordinates, with estimated SDs in parentheses, and thermal factors expressed as B_{eq}

	x	y	z	B_{eq}*
N-1A	0.3943 (2)	0.4862 (8)	0.2005 (4)	2.5
C-2A	0.4219 (2)	0.6193 (11)	0.2986 (6)	3.6
C-3A	0.4790 (3)	0.6286 (12)	0.2863 (7)	4.0
C-4A	0.4838 (2)	0.5413 (11)	0.1577 (7)	3.7
C-5A	0.4728 (3)	0.3262 (12)	0.1627 (8)	4.2
C-6A	0.4218 (2)	0.2985 (9)	0.2106 (7)	3.6
C-7A	0.4449 (2)	0.6312 (12)	0.0537 (6)	3.7
C-8A	0.3909 (2)	0.5611 (10)	0.0682 (5)	2.6
C-9A	0.3491 (3)	0.7090 (11)	0.0328 (6)	3.0
C-10A	0.4983 (3)	0.8318 (13)	0.3028 (9)	6.1
C-11A	0.5394 (3)	0.8853 (15)	0.3782 (9)	7.1
O-12A	0.3594 (2)	0.8684 (7)	0.1138 (4)	3.3
N-13A	0.3624 (2)	0.8679 (13)	−0.3488 (6)	5.2
C-14A	0.3763 (3)	0.9844 (14)	−0.2529 (7)	5.0
C-15A	0.3710 (3)	0.9355 (12)	−0.1285 (7)	4.0
C-16A	0.3500 (2)	0.7658 (12)	−0.1034 (5)	3.1
C-17A	0.3312 (2)	0.6423 (12)	−0.2060 (6)	3.4
C-18A	0.3044 (2)	0.4661 (12)	−0.1941 (6)	3.6
C-19A	0.2883 (3)	0.3529 (14)	−0.2985 (7)	4.6
C-20A	0.2997 (3)	0.4112 (17)	−0.4181 (7)	5.6
C-21A	0.3234 (3)	0.5750 (18)	−0.4312 (7)	5.9
C-22A	0.3396 (3)	0.6992 (13)	−0.3262 (7)	4.4
O-23A	0.2620 (2)	0.1885 (9)	−0.3000 (5)	5.4
C-24A	0.2445 (3)	0.1278 (15)	−0.1881 (8)	5.9
N-1B	0.1632 (2)	1.1893 (9)	0.3239 (5)	3.2
C-2B	0.1833 (2)	1.0613 (12)	0.4334 (6)	3.6
C-3B	0.1446 (3)	1.0595 (13)	0.5244 (6)	4.0
C-4B	0.0932 (3)	1.1278 (13)	0.4508 (7)	4.0
C-5B	0.0970 (3)	1.3412 (13)	0.4264 (8)	4.9
C-6B	0.1464 (3)	1.3786 (13)	0.3701 (7)	5.0
C-7B	0.0817 (2)	1.0202 (12)	0.3257 (6)	3.8
C-8B	0.1182 (2)	1.0982 (10)	0.2403 (6)	3.0
C-9B	0.1359 (2)	0.9487 (10)	0.1513 (6)	2.9
C-10B	0.1450 (3)	0.8660 (14)	0.5887 (7)	5.6
C-11B	0.1812 (4)	0.7302 (16)	0.5995 (7)	6.0
O-12B	0.1478 (2)	0.7723 (7)	0.2103 (4)	3.3
N-13B	0.0111 (2)	0.8786 (11)	−0.1606 (5)	4.1
C-14B	0.0214 (3)	0.7536 (15)	−0.0701 (7)	4.4
C-15B	0.0613 (2)	0.7685 (13)	0.0325 (6)	3.6
C-16B	0.0929 (2)	0.9250 (10)	0.0394 (6)	2.8
C-17B	0.0842 (2)	1.0651 (10)	−0.0565 (6)	2.9
C-18B	0.1154 (2)	1.2340 (12)	−0.0590 (6)	3.4
C-19B	0.1033 (3)	1.3637 (12)	−0.1552 (6)	4.0
C-20B	0.0595 (3)	1.3349 (14)	−0.2493 (7)	4.5
C-21B	0.0309 (3)	1.1765 (14)	−0.2485 (7)	4.5
C-22B	0.0422 (3)	1.0383 (12)	−0.1543 (6)	3.7
O-23B	0.1295 (2)	1.5275 (9)	−0.1683 (5)	4.9
C-24B	0.1715 (3)	1.5738 (13)	−0.0708 (7)	5.0
S	0.2774 (1)	0.2508	0.2000 (1)	2.7
O-1S	0.2823 (2)	0.1054 (8)	0.2962 (5)	4.5
O-2S	0.3026 (2)	0.4324 (7)	0.2588 (4)	3.1
O-3S	0.3024 (2)	0.1972 (8)	0.0965 (4)	4.4
O-4S	0.2237 (2)	0.2954 (8)	0.1610 (4)	4.2
W-1	0.3840 (4)	0.0383 (12)	0.4270 (7)	5.5
W-2†	0.2436 (5)	0.7463 (28)	0.2674 (15)	6.0
W-3†	0.2450 (5)	0.7606 (28)	0.1911 (10)	3.8

* The estimated SDs for B_{eq} are in the range 0.1–0.2 Å².
† The second water molecule is disordered with an occupancy factor of ≈½ at sites W-2 and W-3.

STRUCTURE DETERMINATION AND REFINEMENT

Comparison of the Bijvoet differences, $|F_h| − |F_{-h}|$, showed that 69 pairs had a difference greater than ±1.4 (an arbitrarily cho-

Table 2. Selected torsion angles

	Angle, °*	
	Molecule A	Molecule B
About the C-9—C-8 bond		
C-16–C-9–C-8–N-1	−179	−158
C-16–C-9–C-8–C-7	58	80
O-12–C-9–C-8–C-7	−62	−42
O-12–C-9–C-8–N-1	61	81
About the C-9—C-16 bond		
O-12–C-9–C-16–C-17	−167	−161
O-12–C-9–C-16–C-15	15	21
C-8–C-9–C-16–C-15	−103	−102
C-8–C-9–C-16–C-17	74	76
About the C-4—N-1 line		
C-3–C-4–N-1–C-2	9	12
C-3–C-4–N-1–C-8	133	134
C-3–C-4–N-1–C-6	−109	−108
C-7–C-4–N-1–C-8	11	13
C-7–C-4–N-1–C-6	129	131
C-7–C-4–N-1–C-2	−113	−109
C-5–C-4–N-1–C-6	10	11
C-5–C-4–N-1–C-2	127	131
C-5–C-4–N-1–C-8	−108	−107
About the C-3—C-10 bond		
C-4–C-3–C-10–C-11	−110	141
C-2–C-3–C-10–C-11	130	18

* The estimated SDs are of the order of 0.7°.

sen value on an absolute scale). A Patterson function calculated with the squares of the 69 differences used as coefficients yielded a Harker section with the strongest peak corresponding to the S—S vector clearly displayed and approximately 75% stronger than the next strongest peak.

Once the position of the S atom was determined, the procedure used for deriving the remainder of the structure involved an application of the tangent formula for partial structure development in space group P2₁ (9, 10). In this procedure, phases computed from the partial structure are not refined by use of the tangent formula if there is a subset of atoms present of at least moderately heavier weight. In the presence of heavier atoms, the contribution of the lighter atoms to the phase values are decreased or even eliminated upon refinement with the tangent formula.

Phases based on a single atom in space group P2₁ will necessarily have values characteristic of a centrosymmetric struc-

Table 3. Hydrogen bonds

Donor	Acceptor	Sym.*	Bond length, Å			D–H–A angle
			D—A	D—H	H—A	
N-1A	O(2S)	a	2.658	1.00	1.65	175°
N-1B	O(4S)	b	2.690	1.03	1.70	160°
O-12A	O(3S)	b	2.746	1.00	1.76	167°
O-12B	W(2)†	a	2.540	1.05	1.50	173°
	W(3)†	a	2.640	—	1.65	155°
W-1	N(13A)	c	2.853	0.82	2.06	163°
W-1	O(1S)	a	2.887	0.94	1.97	166°
W-2†	O(1S)	b	2.712			
W-3†	O(1S)	b	2.780			
W-2†	O(2S)	a	2.711			
W-3†	O(2S)	a	2.791	0.92	1.88	170°

* The symmetry equivalents of the acceptor atoms are as follows: $a \equiv x, y, z$; $b \equiv x, 1 + y, z$; $c \equiv x, -1 + y, 1 + z$.
† The second H₂O molecule is disordered between atomic sites W-2 and W-3, each with ~½ occupancy. The two sites are mutually exclusive.

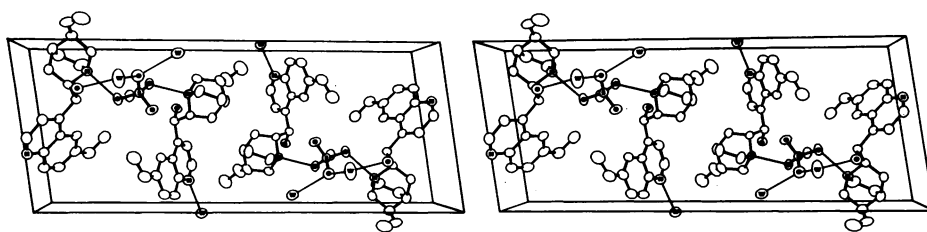Chemistry: Karle and Karle

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FIG. 3. Packing diagram of quinidine sulfate dihydrate. The axial directions are $a \rightarrow$, c ↖, and b directed into the page. Molecules B are at the extreme left and extreme right, whereas molecules A are near the middle of the cell. W-1 is shown near the top and bottom of the cell (along the c direction), W-2 is shown above the sulfate group, and W-3 is shown behind the sulfate group.

ture. If the S atom is fixed at $y = 0.25$, then the phase values will be 0 or π. An E map based on the position of the S atom, computed after phase extension with the tangent formula, has the expected false mirror at $y = 0.25$ (Fig. 1a), where each peak at x and z has two values for y. At this point it is necessary to choose a set of atoms, preferably at y not near 0.25, that will select the enantiomorph and break the mirror symmetry. The four oxygen atoms of the SO_4 group, included as part of the known structure for the second E map, are nearly related by a mirror and thus could not break the false mirror symmetry. Five atoms that could be connected with appropriate bond lengths and bond angles (solid lines, Fig. 1a), and sufficiently far from $y = 0.25$ so that it would be quite certain that they belong to one enantiomorph, were used along with the SO_4 group to obtain initial phases. The tangent formula was used for phase extension only, not refinement. The resulting E map, shown in Fig. 1b, contained no vestiges of the false mirror. The correct value for the y coordinate was selected for each pair of peaks shown in the first E map, and the new map also contained additional atomic positions. The process was repeated again, with 35 atoms as the known structure, and yielded the E-map shown in Fig. 1c which contains all the atomic positions except for two, indicated by a broken line. They were subsequently located in a difference map.

The difference map revealed an additional site for the second water molecule that appears to occupy sites W-2 or W-3 at random with approximately equal frequency.

Alternation of least-squares refinement and examination of difference maps led to the location of all the hydrogen atoms, except some of those on the water molecule occupying sites W-2 and W-3. Refinement with anisotropic thermal factors for the C, N, O, and S atoms and with anomalous dispersion parameters for the S atom was terminated at a conventional R factor of 4.9% for all 2869 data.

The atomic positions and B_{eq} values for the nonhydrogen atoms are listed in Table 1.*

DESCRIPTION OF STRUCTURE

The two molecules of quinidine in the asymmetric unit, in reality quinidyl ions with the proton on N-1, are shown in Fig. 2. They are quite similar in all respects except two: the rotation of the vinyl side chain about C-3—C-10 and the rotation of the hydroxyl hydrogen about C-9—O-12. The latter rotation results from the different modes of hydrogen bonding of the two cations. Thus, in molecule A the hydrogen atom on O-12 is *gauche* with respect to the hydrogen on C-9, whereas in molecule B the two hydrogen atoms are eclipsed. The cause of the rotation about the C-3—C-10 bond where the C-2-C-3-C-10-C-11 torsional angles are 130° and 18° for molecules A and B, respectively, is not immediately apparent. Presumably, the packing arrangement shown in Fig. 3 benefits energetically from the two different conformations of the vinyl side chain.

Bond lengths and bond angles for the two quinidyl cations are similar for molecules A and B in this crystal and also compare closely to those found for cinchonine (3) and the cinchoninium^{2+} cation (4). Moreover, the torsional angles listed in Table 2 show the conformational similarity of molecules A and B. These values can be compared directly with those listed by Oleksyn *et al.* (3) in their table 5 and show that the quinidine and cinchonine molecules, and their cations, have an essentially constant conformation (except for the vinyl side chain) despite the presence of different anions, different hydrogen bonding, and different packing arrangements.

The independent molecules A and B are related approximately in the unit cell by a noncrystallographic 2-fold rotation axis, skewed with respect to the cell edges. The sulfate anion and the three water sites are located between the quinuclidine moieties of the two molecules. The nitrogen atoms in the quinuclidine moieties, N-1A and N-1B, both donate protons to form hydrogen bonds with the same SO_4 ion. The OH groups in the two molecules form different kinds of hydrogen bonds. In molecule A, the OH group forms a hydrogen bond with the SO_4 ion directly behind the one shown in Fig. 3; in molecule B, the OH group is a donor to a hydrogen bond with a water molecule in site W-2 or W-3, which in turn is a donor to hydrogen bonds with two different SO_4 ions. Finally, a water molecule in site W-1 bridges the SO_4 ion with the quinoline N-13 atom in molecule A with hydrogen bonds. The quinoline N-13 in molecule B does not participate in any hydrogen bonding. Distances and angles involved in the hydrogen bonds are shown in Table 3. There is no intramolecular hydrogen bonding in the active antimalarial cinchona alkaloids.

Oleksyn and Lebioda (8) have calculated minimum energy conformers for cinchona alkaloids by use of the EENY program (7). The values τ_1(O-12–C-9–C-16–C-15) and τ_2(O-12–C-9–C-8–C-7) determined for quinidine in this investigation fall very near one of the four energy minima calculated for quinidine [see figure 2b of Oleksyn and Lebioda (8)].

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* Lists of structure factors, anisotropic thermal parameters for the nonhydrogen atoms, bond lengths, and bond angles and the approximate coordinates for the hydrogen atoms are available, on request, from the authors.