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Conformational Study of Cinchona Alkaloids. A Combined NMR, Molecular Mechanics, and X-ray Approach

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Abstract: A conformational study of some cinchona alkaloids is presented. The conformations of the alkaloids in solution have been determined by NOE difference and NOESY NMR techniques. The necessary chemical shift assignments of the cinchona alkaloids have been elucidated by combination of COSY and NOE NMR experiments. Minimum energy conformations of the alkaloids have been calculated by molecular mechanics. The first known X-ray structure of a cinchona alkaloid with a "closed" conformation is presented. Finally, the conformational effects of protonation and complexation on the tertiary nitrogen of the quinuclidine ring have been investigated.

Quinine and quinidine, two cinchona alkaloids so generously provided by Mother Nature, are not only important pharmacological drugs,[1] but their contribution to chemistry certainly deserves respect.[2] Examples where quinine is used as a chiral resolving agent are countless, and the activity in this area does not seem to slow down.[3] The chromatographic separation of enantiomers employing quinine- and quinidine-impregnated supports is a recent accomplishment.[4] But perhaps the most interesting application of cinchona alkaloids resides in their ability to induce asymmetry when employed as chiral adjuvants. The first attempt to achieve asymmetric induction with these alkaloids was made in 1912, when Bredig and Fiske[5] reported that reaction of benzaldehyde with hydrogen cyanide, catalyzed by quinine, yielded the corresponding cyanohydrin with an enantiomeric excess (ee) of 20%.

From the work of Wynberg it is known that catalytic amounts of quinine or quinidine are sufficient to provide moderate to excellent levels of asymmetric induction in a variety of synthetic transformations.[6] This group reported in 1975 the first case of a quinine-catalyzed asymmetric Michael addition.[7] Even more success was obtained in another asymmetric carbon–carbon bond forming reaction, the [2 + 2] cycloaddition between chloral and ketene yielded optically active lactones in up to 98% ee.[8] Another asymmetric bond formation, catalyzed by cinchona alkaloids, is the carbon–sulfur and carbon–selenium bond forming Michael reaction.[9] The use of these alkaloids as chiral phase-transfer catalysts is also well documented,[10] and from the work of Sharpless

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(1) Goodman, L. S.; Gilman, A. G. *The Pharmacological Basis of Therapeutics*, 7th ed.; McMillan Publishing Co.: New York, 1985; pp 756, 1041.
(2) For a general review on the chemistry of the cinchona alkaloids, see: Grethe, G.; Uskokovic, M. R. In *Heterocyclic Compounds, The Monoterpenoid Indole Alkaloids*; John Wiley and Sons Inc.: New York, 1983; Vol. 25, Part 4, Chapter XII, p 729, and references cited therein.
(3) Jacques, J.; Collet, A.; Wilen, S. H. In *Enantiomers, Racemates and Resolution*; John Wiley and Sons Inc.: New York, 1981; pp 254, 257.
(4) Salvador, P.; Rosini, C.; Pini, D.; Bertucci, C.; Altemura, P.; Uccello-Baretta, G.; Raffaelli, A. *Tetrahedron* **1987**, *43*, 4969.
(5) Bredig, G.; Fiske, P. S. *Biochem. Z.* **1912**, *46*, 7.
(6) (a) Wynberg, H. *Top. Stereochem.* **1986**, *16*, 87. For other reviews, see: (b) Pracejus, H. *Z. Fortschr. Chem. Forsch.* **1967**, *8*, 493. (c) Morrison, J. D.; Mosher, H. S. In *Asymmetric Organic Reactions*; Prentice-Hall, Englewood Cliffs, NJ, 1971.
(7) Wynberg, H.; Helder, R. *Tetrahedron Lett.* **1975**, 4057.
(8) (a) Wynberg, H.; Staring, A. G. J. *J. Am. Chem. Soc.* **1982**, *104*, 166. (b) Wynberg, H.; Staring, A. G. J. *J. Chem. Soc., Chem. Commun.* **1984**, 1181.
(9) (a) Hiemstra, H.; Wynberg, H. *J. Am. Chem. Soc.* **1981**, *103*, 417. (b) Pluim, H.; Wynberg, H. *Tetrahedron Lett.* **1979**, 1251.
(10) (a) Hummelen, J. C.; Wynberg, H. *Tetrahedron Lett.* **1978**, 1089. (b) Annunziato, R. *Synth. Commun.* **1979**, *9*, 171. (c) Marsman, B.; Wynberg, H. *J. Org. Chem.* **1979**, *44*, 2312. (d) Pluim, H.; Wynberg, H. *J. Org. Chem.* **1980**, *45*, 2498. (e) Harigaya, Y.; Yamaguchi, H.; Onda, M. *Heterocycles* **1981**, *15*, 183. (f) Dolling, U. H.; Davis, P.; Grabowski, E. J. J. *J. Am. Chem. Soc.* **1984**, *106*, 446.

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Table I. ^{1}H NMR Chemical Shifts (in ppm) from Internal TMS with Precision of ±0.01 ppm for Dihydroquinidine and Dihydroquinine Derivatives[a]

	DHQD					DHQ	
H	*p*-ClBz 1	$CONMe_2$ 2	Ac 3	H 4	Me 5	*p*-ClBz 6	H 7
1	7.40	7.34	7.43	7.26	7.43	7.40	7.48
2	8.75	8.74	8.83	8.50	8.76	8.72	8.54
3	8.02	8.00	8.13	7.90	8.04	8.02	7.89
4	7.39	7.35	7.47	7.26	7.37	7.38	7.24
5	7.45	7.46	7.54	7.17	7.30	7.49	7.20
8	6.72	6.43	6.42	5.52	5.01	6.72	5.60
9	3.38	3.29	3.38	3.04	2.7–3.1	3.47	3.06
10	1.85	1.81	2.00	1.96	1.97	1.63	1.30–1.45
11	1.55	1.55	1.50	1.06	1.13	1.88	1.62–1.80
12	1.75	1.73	1.89	1.66	1.68	1.85	1.62–1.80
13	1.46	1.45	1.55–1.75	1.3–1.55	1.35–1.55	1.76	1.62–1.80
14	1.56	1.5[b]	1.55–1.75	1.3–1.55	1.35–1.55	1.51	1.30–1.45
15	2.70	2.7[b]	2.8–3.0	2.65–3.05	2.7–3.1	2.67	2.61
16	2.79	2.7[b]	2.8–3.0	2.65–3.05	2.7–3.1	3.17	3.53
17	1.45	1.5[b]	1.55–1.75	1.3–1.55	1.35–1.55	1.50	1.30–1.45
18	2.68	2.7[b]	2.8–3.0	2.65–3.05	2.7–3.1	2.37	2.35
19	2.85	2.90	3.10	2.65–3.05	2.7–3.1	3.06	3.03
20	1.45	1.38	1.64	1.43	1.47	1.35	1.19
21	0.92	0.90	1.06	0.85	0.91	0.85	0.77
MeO	3.95	3.95	4.10	3.81	3.94	3.97	3.83
R-gr	7.46	2.89	2.30	5.15	3.31	7.44	4.96
	8.05	3.03				8.03	

[a] Spectra were obtained in chloroform-d_1 at room temperature, and alkaloid concentrations of 0.02 M were used. [b] Assignments may be reversed.

we know that cinchona alkaloid derivatives also act as excellent chiral ligands in the asymmetric dihydroxylation of olefins.[11]

Several reviews have discussed in detail the cinchona alkaloids and their use as chiral catalysts.[6] Although quinine and quinidine are diastereomeric pairs, as shown in Figure 1, their opposite stereochemistries at the crucial carbons 8 and 9 are more characteristic of enantiomers. It is therefore not surprising that in reactions where cinchona alkaloids are used as chiral catalysts, if quinine gives one enantiomer in excess, the other enantiomer will predominate when quinidine is employed. Although they act almost like enantiomeric catalysts, the diastereomeric nature of these alkaloids is revealed by a small, but constant difference in the ee of the products.[12] The epicinchona alkaloids have the opposite configuration at carbon 9 relative to the parent compounds (Figure 1). As far as we know, the results obtained with the epialkaloids as chiral catalysts are mostly disappointing.

In order to probe the intimate details of the mechanism of action of the cinchona alkaloids and their derivatives, a thorough understanding of their preferred conformation(s) in solution is needed. Several studies have been addressed to the conformation of quinine and quinidine,[6,13] the general result being that the C_8–C_9 and C_4–C_9 bonds are considered most important in determining the overall conformation. No studies, to the best of our knowledge, have dealt with the conformation of the quinuclidine ring of the cinchona alkaloids.[14]

In an exhaustive study of the cinchona alkaloid catalyzed Michael addition of thiols to enones, Hiemstra and Wynberg[9a]

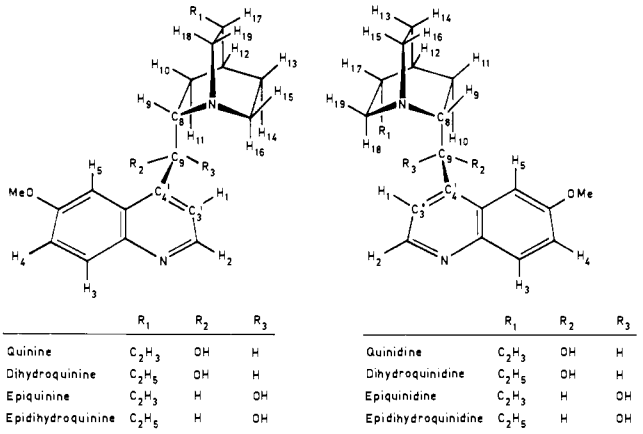


Figure 1. Structure, numbering, and absolute configuration of eight cinchona alkaloids.

proposed that the most stable conformation of quinine has the largest substituent—the quinuclidine ring—on one side of the quinoline ring, and hydrogen 8 and the hydroxyl at C_9 on the other side. This conformation was also considered the most favorable by Prelog and Meurling.[13a,b] In a conformational study of the cinchona alkaloid catalyzed Michael addition, Dijkstra has recently described a conformational analysis of quinine and quinidine by a combined NMR and molecular modeling approach.[15] The importance of the cinchona alkaloid catalyzed reactions, coupled with this eagerness to understand the mechanism of asymmetric inductions, induced us to extend this conformational study of the cinchona alkaloids.

In this paper we will describe in detail the salient features of the ground-state conformations of cinchona alkaloids, their N-protonated forms, as well as the conformation of the osmium tetraoxide–alkaloid complex, by a combined NMR, molecular modeling and X-ray analysis. The influence of different substituents at the benzylic carbon C_9 on the conformation will be described, and so a picture of the conformational behavior of

(11) (a) Hentges, S. G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 4264. (b) Jacobsen, E. J.; Marko, I.; Mungall, W. S.; Schroder, G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 1968. (c) Wai, J. S. M.; Marko, I.; Svendsen, J. S.; Finn, M. G.; Jacobsen, E. N.; Sharpless, K. B. *J. Am. Chem. Soc.* **1989**, *111*, 1123. In press. For cinchona alkaloids as chiral ligands, see also e.g.: Cabaret, D.; Welvart, J. *J. Organomet. Chem.* **1974**, *78*, 295. Boireau, G.; Abenhaim, D.; Henry-Basch, E. *Tetrahedron* **1979**, *35*, 1457. Bergstein, W.; Kleeman, A.; Martens, J. *Synthesis* **1981**, *76*, and references therein. Ohgo, Y.; Natori, Y.; Takeuchi, S.; Yoshimura, J. *Chem. Lett.* **1974**, *78*, 1327.

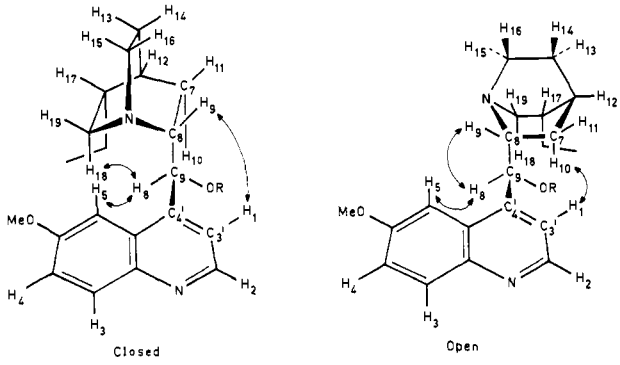
(12) For example, quinine derivatives employed as chiral catalysts in the catalytic asymmetric osmylation of olefins always gave a lower ee (~10%) than the corresponding quinidine derivatives. Similarly, cinchonine gave higher ee than cinchonidine when employed in the Michael addition of thiols to enones.

(13) (a) Prelog, V.; Wilhelm, H. *Helv. Chim. Acta* **1954**, *37*, 1634. (b) Meurling, L. *Chem. Scr.* **1975**, *7*, 90. (c) Hiemstra, H. Ph.D. Thesis, University of Groningen, The Netherlands, 1980.

(14) The quinuclidine ring should be and actually is twisted in order to relieve some hydrogen–hydrogen eclipsing interaction.

(15) Dijkstra, G. D. H.; Kellogg, R. M.; Wynberg, H. *Recl. Trav. Chim. Pays-Bas.* **1989**, *108*, 195.

(16) For the assignment of quinine and quinidine as hydrochlorides in DMSO-d_6, see: Chazin, W. J.; Colebrook, L. D. *J. Org. Chem.* **1986**, *51*, 1243.



1 R = *p*-ClBz
2 R = $CONMe_2$
3 R = Ac
4 R = H
5 R = Me

Figure 2. Schematic drawing showing (a) the closed and (b) the open conformation of the quinidine derivatives.

cinchona alkaloids and the relevance to asymmetric reactions will be presented.

Results

Assignment of ^1H NMR Spectra of Dihydroquinidines. The cinchona alkaloids all have complex ^1H NMR spectra. The spectra of the quinidines and quinines were very different, but, not unexpectedly, most of the quinidine spectra were mutually similar. The assignments for (*p*-chlorobenzoyl)dihydroquinidine (*p*-ClBzDHQD) (**1**) (Figure 2) will serve here as a model for all other quinidine derivatives.

The chemical shift assignments of dihydroquinidine **1** in chloroform-d_1 are presented in Table I.[16] The hydrogens in the quinoline ring could be assigned straightforwardly. The H_1 and H_2 hydrogens are located as doublets at δ 7.40 and 8.75, respectively, and are revealed by a ortho coupling of 4.6 Hz. The H_3 proton appears as a doublet at δ 8.02 with an ortho coupling of 9.2 Hz to H_4 at δ 7.39. In addition, H_4 is coupled to H_5 at δ 7.45 through a meta coupling of 2.6 Hz.

The assignment of the protons in the quinuclidine ring was a more challenging task. The H_8 hydrogen is apparent as a doublet at δ 6.72 with a vicinal coupling of 7.5 Hz to H_9 at δ 3.38. The COSY spectrum reveals that H_9 is coupled with the vicinal protons H_{10} and H_{11} at δ 1.85 and 1.55. These two signals showed similar NOE upon irradiation of H_9, rendering their relative assignment impossible by this strategy. Irradiation of H_9 gave rise to an additional NOE at δ 2.79, which was assigned to the closest methylene proton H_{16}. This proton yields a strong NOE at δ 1.56 and a weaker enhancement at δ 1.46. These two signals were assigned to H_{14} and H_{13}, respectively, the former giving the strongest NOE due to its cis relationship with H_{16}. The H_{16} proton is coupled with the geminal proton H_{15} at δ 2.70 and displays a strong vicinal coupling to H_{14} and a weak coupling to H_{13}. On the other hand, H_{15} has a strong coupling to both H_{14} and H_{13}.

The two remaining unassigned protons α to the quinuclidine nitrogen, H_{18} and H_{19}, observed as multiplets at δ 2.68 and 2.85, are both coupled with H_{17} at δ 1.45. The signal at δ 2.85 showed a strong NOE to H_{17} and was assigned to the cis proton H_{19}, thereby locating H_{18} at δ 2.68.

The two methylene hydrogens in the ethyl group, H_{20}, could easily be assigned to the δ 1.45 absorption due to their coupling with the three hydrogens, H_{21}, of the methyl group. The H_{20} protons showed a small NOE with the signal at δ 1.85, which then was assigned to H_{10}, located at the same side of the quinuclidine ring. This resolves the ambiguity of the H_{10}, H_{11} assignment (vide supra).

The remaining unassigned proton in the quinuclidine ring, H_{12}, is observed as a narrow multiplet at δ 1.75, with only minute couplings to other protons.

Conformational Assignment of (*p*-Chlorobenzoyl)dihydroquinidine. With the complete assignment of the hydrogens of **1** in hand, we turned to investigate the conformation of the alkaloid. The gross conformation of the cinchona alkaloids is determined by the torsions of the C_8–C_9 and C_9–$C_{4'}$ bonds. These bonds link the two rigid quinuclidine and quinoline ring systems, thus defining the spatial relationship between these rings. Our strategy was to use inter-ring NOE's in order to establish the overall conformation of dihydroquinidine **1**. The presence of NOE between H_5, H_8, and H_{18} suggests that these three nuclei are in close spatial proximity. An additional, but weaker inter-ring NOE was observed between H_9 and H_1. These interactions are only possible with an alkaloid conformation in which the quinuclidine nitrogen lone pair points over the quinoline ring. Such a "closed" conformation is depicted in Figure 2a. This alkaloid structure suggests that the $C_{3'}$–$C_{4'}$–C_9–C_8 dihedral angle is close to −90°, and that the H_8–C_9–C_8–H_9 dihedral angle approaches an anti conformation. The most apparent indication of the H_8–C_9–C_8–H_9 dihedral angle was obtained from the $^3J_{H_8H_9}$ coupling constant of 7.5 Hz. By application of the Altona equation[17] this angle is estimated to be ~155°. This is in good agreement with the conformation suggested by the NOE interactions.

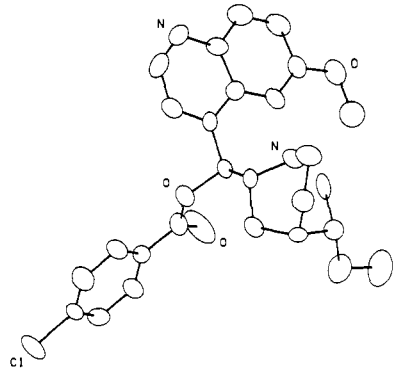


Figure 3. ORTEP view of the crystal structure of (*p*-chlorobenzoyl)dihydroquinidine (**1**).

In an attempt to substantiate further the conformation of dihydroquinone **1**, a single-crystal X-ray diffraction analysis was undertaken.[22] This X-ray analysis showed that the alkaloid in the solid state exists in a closed conformation (Figure 3). All essential features of the conformation in solution, as discussed above, are present in the solid state. This is particulary evident for the important dihedral angles, $C_{3'}$–$C_{4'}$–C_9–C_8 and H_8–C_9–C_8–H_9, which are approximately −86 and 170° in the crystal structure, closely resembling the corresponding angles of −90 and 155° suggested by the NMR experiments of the alkaloid in solution. Furthermore, the X-ray analysis revealed that H_5, H_8, and H_{18} are positioned in a close spatial arrangement, with internuclear distances of 2.23 (H_5, H_8), 2.41 (H_5, H_{18}), and 2.24 Å (H_8, H_{18}). This is in agreement with the strong NOE observed between these nuclei. The weaker NOE observed between H_1 and H_9 was reflected in the solid-state structure by a longer internuclear distance of 2.89 Å.

Conformational Assignment of Other Dihydroquinidines. The ^1H NMR spectra of (dimethylcarbamoyl)dihydroquinidine (**2**) and acetyldihydroquinidine (AcDHQD) (**3**) are very similar to that of **1** (cf. Table I). The ^1H NMR spectra of dihydroquinidine

(17) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2387.
(18) CHEMX, developed and distributed by Chemical Design Ltd., Oxford, England.
(19) QCPE Program 395/400. Allinger Force Field Molecular Mechanics Calculations. Allinger, N. L., Ed., Dept. of Chemistry, University of Georgia, Athens, GA 30602.
(20) MMX, derived from MM2 (QCPE 395) with MMP1 π subroutines (QCPE 318) incorporated for delocalized π-electron systems, N. L. Allinger, Dept. of Chemistry, University of Georgia, written by Y. H. Yuh.
(21) Kolthoff, J. *Biochem. Z.* **1925**, 162, 289.
(22) These results are summarized in the supplementary material.
(23) Schipper, P. E. *J. Phys. Chem.* **1986**, *90*, 4259.
(24) Smaardijk, A. A.; Wynberg, H. *J. Org. Chem.* **1987**, *52*, 135.
(25) Williams, T.; Pitcher, R. G.; Bommer, P.; Gutzwiller, J.; Uskokovic, M. R. *J. Am. Chem. Soc.* **1969**, *91*, 1871.

(DHQD) (**4**) and methoxydihydroquinidine (MeDHQD) (**5**) showed several differences relative to those of compounds **1–3**. In the dihydroquinidine esters (**1–3**) H_{11} appeared between δ 1.50 and 1.55, whereas in **4** and **5**, H_{11} appeared at δ 1.06 and 1.13, respectively. Furthermore, the $^3J_{H_8H_9}$ coupling constants for **1–3** are between 7.5 and 8.3 Hz, whereas for **4** and **5**, this coupling constant decreases to 3.5 and 3.9 Hz, respectively. Due to the similarities in chemical shifts and the $^3J_{H_8H_9}$ coupling constants between **1** and the two other dihydroquinidine esters (**2** and **3**), we conclude that these too have closed conformations.

The $^3J_{H_8H_9}$ coupling constants of **4** and **5**, suggest that the torsional angle H_8–C_9–C_8–H_9 is very different from those of the dihydroquinidine esters. Application of the Altona equation gives a H_8–C_9–C_8–H_9 dihedral angle of either close to 120°, indicating an eclipsed conformation, or approximately 60°, indicative of a staggered conformation. In order to resolve this ambiguity, detailed NOE difference studies of **5** were undertaken. Irradiation of H_8 yielded strong NOEs both in H_5 and H_9; this requires a staggered conformation of the C_8–C_9 bond. This conformation brings H_1 close to H_{10} and thus accounts for the strong inter-ring NOE found between these nuclei. H_{11} did not show a NOE with H_1, which means that H_1 is placed below H_{10} at a distance too far from H_{11} to yield any significant NOE. This places H_{11} in the shielding cone of the quinoline ring and explains the pronounced upfield shift (0.4 ppm) relative to the corresponding hydrogen in the closed conformation. The conformation defines the "open" conformation, where the lone pair of the quinuclidine nitrogen points away from the quinoline ring as depicted in Figure 2b. Based on the similarities in chemical shifts and $^3J_{H_8H_9}$ coupling constants, it is concluded that compound **4** too has the open conformation. This is in good agreement with the work of Dijkstra,[15] who found an open conformation for the parent quinidine.

Solvent Effect on the Conformation of Dihydroquinidines. The surprising result that the dihydroquinidines have different conformations in chloroform-d_1 depending on the nature of the substituent at C_9, encouraged us to investigate possible solvent effects on the alkaloid conformation. The *p*-chlorobenzoate **1** with a closed conformation, and the methyl ether **5** with an open conformation, were chosen as model compounds.

The ^{1}H NMR spectra of **1** were measured in acetone-d_6, acetonitrile-d_3, dichloromethane-d_2, and toluene-d_8. The chemical shifts have been compiled[22] and the results reveal that, with the exception of toluene-d_8, there are only small differences in the chemical shifts (including H_{11}). Furthermore, the coupling constants $^3J_{H_8H_9}$ were in the range of 7.5–8.6 Hz, with one exception, in toluene-d_8, in which a coupling constant of 6.8 Hz was measured.

These results suggest that there are only small variations in the conformation of **1** in the different solvents. The $^3J_{H_8H_9}$ coupling in toluene-d_8 implies that the conformation of **1** opens up slightly, yielding a H_8–C_9–C_8–H_9 dihedral angle of 140°, but still retaining the closed overall conformation. This conclusion is also substantiated by the fact that the chemical shift of H_{11} was virtually unchanged in all solvents.

The effect on the ^{1}H NMR spectrum of **5** on changing the solvent from chloroform-d_1 to dichloromethane-d_2 is, however, striking. The H_{11} hydrogen moved from δ 1.13 to approximately δ 1.5, and the change was accompanied by a substantial increase in $^3J_{H_8H_9}$ from 3.9 to 6.6 Hz. These observations indicate that the methyl ether **5** attains a closed conformation in dichloromethane-d_2. Differential NOE confirmed this conclusion; irradiation of H_8 yielded NOEs at H_5 and H_{18}, and also a substantial NOE was observed between H_9 and H_1.

Molecular Mechanics Calculations of Dihydroquinidine Derivatives. To gain understanding of the principles that determine the different conformations of the alkaloids, we turned to molecular mechanics calculations. With the molecular modeling program CHEMX,[18] the conformational freedom with respect to the C_8–C_9 and C_9–$C_{4'}$ bonds was investigated. Contour plots were calculated in which the molecular mechanics energy is plotted as a function of the two dihedral angles $C_{3'}$–$C_{4'}$–C_9–C_8 and $C_{4'}$–C_9–C_8–C_7 on the *x* and *y* axis, respectively. Figure 4 shows a typical example of such a plot for DHQD. The geometries of the energy minima were optimized with MM2P[19] and MMX[20] force field calculations. The preferred conformations of the three side groups (methoxy, ethyl, and benzylic substituents) were calculated in a similar manner. Three different dihydroquinidine derivatives were chosen as model substances: AcDHQD (**3**), which exhibits the closed conformation, DHQD (**4**), with the open conformation, and MeDHQD (**5**), with the open or closed conformation depending on the solvent.

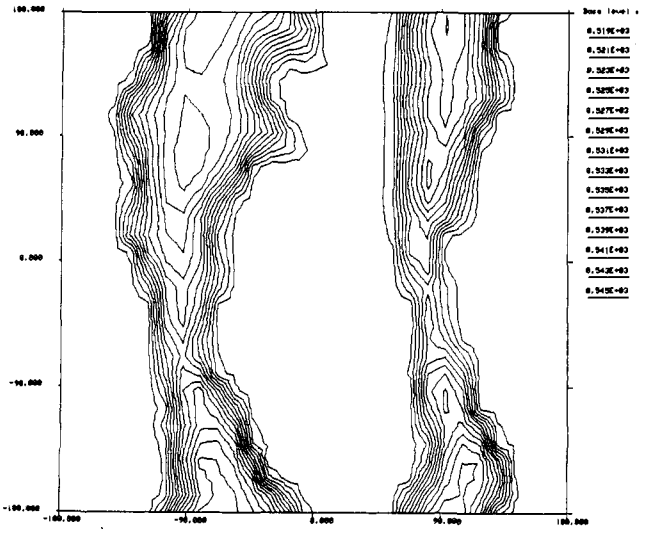


Figure 4. Contour plot of the molecular mechanics energy as a function of the torsional angles $C_{3'}$–$C_{4'}$–C_9–C_8 and $C_{4'}$–C_9–C_8–C_7 on the *x* and *y* axis, respectively. The energy spacing between the contours is 2 kcal/mol.

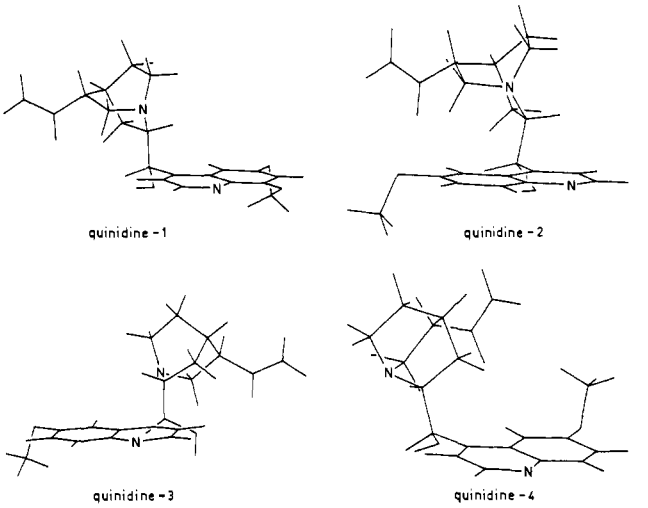


Figure 5. The four minimum energy conformations of dihydroquinidine (**4**). Upper left (a), upper right (b), lower left (c), lower right (d).

The results of the calculations, compiled in Table II show that for all model substances four different minimum energy conformations have been found.[26] In two of these, conformations 1 and 2, H_8 and H_9 are in an almost anti orientation forming closed conformations. The other two conformations, 3 and 4, place H_8 and H_9 in a staggered orientation forming open conformations. The difference between the two closed conformations is the orientation of the quinoline ring, defined by the $C_{3'}$–$C_{4'}$–C_9–C_8 torsional angle. In conformation 1, the quinoline ring is turned away from the quinuclidine ring (Figure 5a), whereas the other closed conformer (2) places the quinoline ring toward the quinuclidine ring (Figure 5b), resembling the closed conformation

(26) This is in good agreement with calculational data obtained by: Oleksyn, B. J.; Lebioda, L. J. *Pol. J. Chem.* **1980**, *54*, 755.

Table II. Minimum Energy Conformations of Some Chinchona Alkaloid Derivatives

	compound conformation														
	AcDHQD (3)				MeDHQD (5)				DHQD (4)				DHQ (7)		
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
MM2P															
Δ energy[a]		0.33	0.57	2.43		0.24	1.98	4.33		0.11	1.18	5.30		0.35	1.41
dipole moment[b]	3.04	2.60	5.95	2.58	3.22	3.33	3.32	3.78	4.38	4.46	3.21	4.40	3.06	3.28	3.25
T_1[c]	106.2	−64.6	−91.9	83.7	104.6	−69.8	−92.8	85.1	104.4	−71.3	−93.7	87.4	−108.6	72.9	99.5
T_2[d]	−42.1	−41.1	−155.5	−172.8	−51.5	−54.8	−155.6	−176.9	−51.6	−54.6	−155.3	−150.7	47.7	59.4	160.4
MMX															
Δ energy[a]	1.24		0.72	3.28	0.18		1.66	4.11		0.02	0.55	2.76		0.19	0.96
dipole moment[b]	2.65	2.88	3.74	4.17	3.15	2.51	2.02	2.14	4.62	3.97	1.91	4.17	3.12	2.44	1.84
T_1[c]	107.3	−62.7	−90.2	87.8	105.4	−69.2	−91.1	87.6	104.5	−71.2	−92.4	86.0	−109.2	71.9	98.2
T_2[d]	−47.9	−50.5	−157.8	−176.4	−51.1	−54.1	−152.7	−176.3	−51.7	−55.5	−153.8	−177.5	48.7	59.7	160.4

[a] Energy difference given in kcal/mol. [b] Dipole moment in Debye. [c] T_1 denotes the C_3–C_4–C_8–C_9 torsional angle. [d] T_2 denotes the C_4–C_8–C_9–N_1 torsional angle.

discussed previously. A similar difference in the orientation of the quinoline ring is observed in the two open conformations. Conformation 3, depicted in Figure 5c, resembles the open conformation discussed before, with a C_3–C_4–C_9–C_8 torsional angle of approximately −90°, whereas conformation 4 exhibits a C_3–C_4–C_9–C_8 torsional angle of approximately +90° (cf. Figure 5d).

Examination of the difference in molecular mechanics energy by MM2P and MMX force field calculations between the different conformers reveals that for all model compounds, conformations 1–3 are closely spaced in energy, whereas conformation 4 is substantially less stable.

In AcDHQD (**3**), the difference in molecular mechanics energy between conformations 2 and 3 is very small, ~0.5 kcal/mol, in favor of the closed conformer 2. The difference in dipole moments between the closed and open conformation is more striking, with the closed conformation 2 ~2.1 D lower than the open conformation 3. A similar trend is observed in MeDHQD (**5**), where the two conformations 2 and 3 are separated by ~1.7 kcal/mol, favoring the closed conformation. The difference in dipole moments between conformations 2 and 3 is not as pronounced as for compound **3**, with the closed conformation less than ~0.5 D lower than the open. For DHQD (**4**) the difference in molecular mechanics energy is ~1.1 kcal/mol, favoring the closed conformation 2. The difference in dipole moments is, however, larger, with the closed conformation 2 ~1.2 D *higher* than the open conformation 3. It is important to note that the difference in dipole moments for **4** is opposite to that of compounds **3** and **5**.

Spectral and Conformational Assignment of Dihydroquinines. The ^{1}H NMR spectra of the dihydroquinines are different from those of the dihydroquinidines. The aromatic hydrogens, however, show close resemblance between the two groups of alkaloids, and these protons were assigned as described for the corresponding quinidine derivative **1** ((vide supra).

The chemical shift assignment of the aliphatic protons in the dihydroquinines is exemplified for (*p*-chlorobenzoyl)dihydroquinine (*p*-ClBzDHQ) (**6**). The benzylic hydrogen H_8 is coupled through a vicinal coupling of 6.1 Hz to the quinuclidine ring hydrogen H_9 at δ 3.47. The H_9 proton is coupled to both H_{10} at δ 1.90 and H_{11} at δ 1.41 with coupling constants of approximately 7.7 Hz. The H_{10} hydrogen is assigned by a large NOE interaction with its cis hydrogen H_9. In addition, H_9 is coupled by a *W* coupling to H_{15} at δ 2.67. Furthermore, H_{15} is coupled with H_{16} at δ 3.17, H_{13} at δ 1.76, and H_{14} at δ 1.51. The assignment of H_{13} and H_{14} was based on strong NOE interactions with their corresponding cis hydrogens H_{15} and H_{16}. Another *W* coupling connects H_{16} to H_{18} at δ 2.37, which in turn was coupled to H_{19} at δ 3.06 and H_{17} at δ 1.50. The ethyl group with H_{20} at δ 1.17 and H_{21} at δ 0.78, and H_{12} at δ 1.85, complete the assignment of **6**. The proton assignment for dihydroquinine (DHQ) (**7**) was performed in a similar manner and the results are presented in Table I.

The conformations of the dihydroquinines were determined from the inter-ring NOEs and $^3J_{H_8H_9}$ coupling constants in a manner analogous to the dihydroquinidines (vide supra). The presence of substantial NOE between H_8, H_5, and H_{16}, and NOE between H_9 and H_1, shows that dihydroquinine **6** has a closed conformation similar to that depicted in Figure 2a. The $^3J_{H_8H_9}$ coupling constant of 6.1 Hz, which indicates a H_8–C_9–C_8–H_9 dihedral angle of 140°, is also indicative of the closed conformation. The parent compound **7** showed NOEs between H_5, H_8, an H_9, and between H_1 an H_{11}. The $^3J_{H_8H_9}$ coupling constant of 3.4 Hz corresponds to a H_8–C_9–C_8–H_9 dihedral angle of −55°, all in accordance with the open conformation. This is again in agreement with the open conformation found for the parent quinine in solution.[15]

Table III. ^{1}H Chemical Shift Values (in ppm) from Internal TMS with Precision of ±0.01 ppm for the Epicinchona Alkaloids at Room Temperature

H	epi-DHQD	Epi-QD	epi-Q
1	7.40	7.42	7.27
2	8.69	8.70	8.60
3	7.98	7.98	7.92
4	7.32	7.31	7.25
5	7.59	7.53	7.56
8	5.02	5.08	4.92
9	2.8–3.0	2.9	2.95–3.05
10	1.20	1.27	0.79
11	0.95	0.95	1.29
12	1.54	1.63	1.54
13	1.28–1.50	1.5	1.4
14	1.28–1.50	1.5	1.4
15	2.8–3.0	2.9	2.61–2.67
16	2.8–3.0	2.9	2.95–3.15
17	1.28–1.50	2.28	2.15
18	2.57	2.9	2.61–2.67
19	2.8–3.0	2.9	2.95–3.15
20	1.28–1.50	5.86	5.58
21	0.84	5.05	4.83
MeO	3.88	3.87	3.77
HO	4.78	4.77	4.8

Molecular Mechanics Calculations of Dihydroquinines. The minimum energy conformations of the DHQ derivatives were determined in the same way as described for the DHQD compounds. The similarities in conformation found by NMR between dihydroquinidines and dihydroquinines are also reflected in the molecular mechanics calculations. However, the molecular modeling study suggests that only three conformational minima exist for dihydroquinines, corresponding to conformations 1–3 of the dihydroquinidines (cf. Table II). Nevertheless, only two of these conformations, 2 and 3, were observed in solution for *p*-ClBzDHQ (**6**) and DHQ (**7**), respectively (vide supra).

Spectral and Conformational Assignment of Epicinchona Alkaloid Derivatives. The ^{1}H NMR chemical shift assignments for epidihydroquinidine (epiDHQD) (**8**), epiquinidine (epiQD) (**9**), and epiquinine (epiQ) (**10**) are presented in Table III.

The conformations of the epialkaloids were determined by inter-ring NOEs and $^3J_{H_8H_9}$ coupling constants. The presence of NOEs between H_8, H_{18}, H_{10}, and H_5 in compounds **8** and **9** shows that the epiquinidines have an open conformation, depicted in

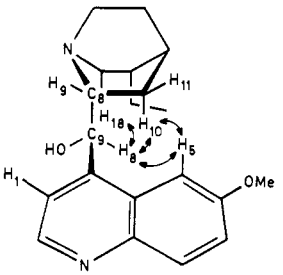


Figure 6. Schematic drawing showing the open conformation of epidihydroquinidine (8).

Figure 6. This conformation differs, however, from the open conformation 3, which was observed in compounds **4**, **5**, and **7**. The open conformation of the epi derivatives resembles the open conformation **4**, which was found by molecular mechanics calculations of the quinidines. The $^3J_{H_8H_9}$ coupling constant of 10.1 Hz in compound **8** corresponds to the anti arrangement of H_8 and H_9, as expected in this conformation.

The open conformation 4 is also preferred by compound **10**, evident by NOE interactions between H_5, H_8, and H_{11}, and a $^3J_{H_8H_9}$ coupling constant of 9.9 Hz, once again corresponding to an anti relationship between H_8 and H_9.

Conformation of the Quinuclidine Ring. Quinidines and quinines are diastereomers, with opposite configurations at C_8 and C_9. There is still another conformational feature of these alkaloids, which has not yet been discussed, the conformation of the quinuclidine ring. It is highly unlikely that the quinuclidine ring will have its methylene groups opposed to form an unfavored all-eclipsed conformation. Rather, the excess steric strain is reduced by a twist in the quinuclidine ring, allowing the methylene groups to approach staggered conformations. This twist can take place in two different directions, either forming a right-handed or left-handed screw (viewed from the quinuclidine nitrogen atom along the pseudo C_3 symmetry axis). We were interested to see if the pseudoenantiomeric relationship between the quinidines and quinines is also reflected in the direction of the twist in the quinuclidine ring.

The twist in the quinuclidine ring gives rise to differences in dihedral angles of the vicinal hydrogens and should, hence, be reflected in differences in the vicinal coupling constants. Because most of the signals of the quinuclidine hydrogens in the ^{1}H NMR spectrum of dihydroquinine **6** are well resolved, all vicinal coupling constants could be obtained, either directly from the spectrum or estimated by computer simulations of the spin systems involved. The magnitude of the twist is not necessarily the same in all bonds, and the C_5–C_6 bond was addressed first. The vicinal couplings between H_{16} and H_{13} and between H_{16} and H_{14} are 6.1 and 10.3 Hz, respectively, corresponding to dihedral angles of −135 and −15°, suggesting a left-handed twist of approximately 15° in the C_5–C_6 bond. The direction and size of this twist is supported by the $^3J_{H_{15}H_{13}}$ coupling constant of 10.3 Hz corresponding to a dihedral angle of 15°. The $^3J_{H_{15}H_{14}}$ coupling constant was harder to assess directly from the spectrum, but computer simulation showed that this coupling is between 3.5 and 4 Hz, which also is in accordance with the left-handed twist (Figure 7a).

Similarly, the torsional angle of the C_7–C_8 bond was obtained from the couplings of H_9 with both H_{10} and H_{11}. These couplings were both 7.7 Hz, which corresponds to dihedral angles of 20° for the H_9H_{10} and 140° for the H_9H_{11} dihedrals. These angles show that the C_7–C_8 bond is twisted approximately 20° in a left-handed screw.

Finally, the torsional angle of the C_2–C_3 bond was obtained from the couplings between H_{17} and the vicinal protons H_{18} and H_{19}, which were 3.3 (−115°) and 9.8 Hz (−10°), respectively. These couplings indicate a C_2–C_3 torsion of approximately 10° to form a left-handed screw.

These results clearly show that the quinuclidine ring in the dihydroquinine **6** is twisted as a left-handed screw (Figure 7a), and that the twist is largest in the C_7–C_8 bond, less in the C_5–C_6 bond, and least in the C_2–C_3 bond. A similar twist of the quinuclidine ring is observed with the molecular mechanics calculations of the dihydroquinines.

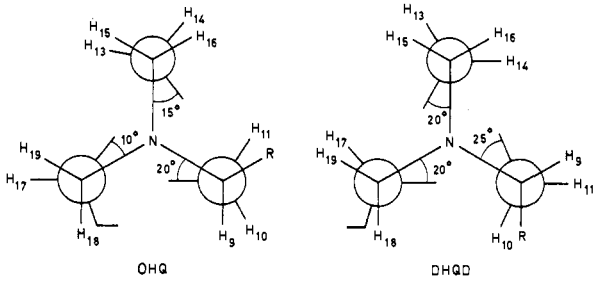


Figure 7. Schematic drawing of the quinuclidine ring of (a) (*p*-chlorobenzoyl)dihydroquinine (**6**) and (b) (*p*-chlorobenzoyl)dihydroquinidine (**1**).

The twist was also investigated for dihydroquinidine **1**, but due to severe overlap of several signals in the spectrum, not all vicinal coupling constants could be obtained. The couplings available proved, however, to be sufficient to determine the twist of the quinuclidine ring. The spectrum in toluene-d_8 revealed that $^3J_{H_{15}H_{13}}$ is 9.7 Hz, corresponding to a dihedral angle of 20°, and that $^3J_{H_{15}H_{14}}$ was 7.8 Hz, corresponding to a dihedral of 140°. The analogous vicinal couplings with H_{16} were derived by computer simulations, yielding a coupling constant of 9–10 Hz for $^3J_{H_{16}H_{14}}$, and a coupling constant of 1–2 Hz for $^3J_{H_{16}H_{13}}$. The corresponding dihedral angles obtained by the Altona equation were 20 and −110°, respectively, establishing a right-handed screw of the quinuclidine ring with a torsional angle of 20° in the C_5–C_6 bond (Figure 7b).

The H_9 couplings to both H_{10} and H_{11} are 8.9 Hz, in accordance with dihedral angles of 145° for the H_9H_{10} dihedral and 25° for the H_9H_{11} dihedral, suggesting a right-handed twist of ~25° of the C_7–C_8 bond. Similarly, the $^3J_{H_{17}H_{19}}$ coupling of 7.7 Hz suggests a torsional angle of 20° in the C_2–C_3 bond, also in accordance with the right-handed twist.

These results show that the quinuclidine ring in dihydroquinidine **1** has a righ-handed twist, the twist being largest in the C_7–C_8 bond and smaller in the C_2–C_3 and C_5–C_6 bonds (Figure 7b). The right-handed twist in **1** was also observed with the molecular mechanics calculations, as well as in the X-ray structure (Figure 3), where the N_1–C_8–C_7–C_4 dihedral angle was 22.2°, the N_1–C_6–C_5–C_4 angle was 17.6°, and the N_1–C_2–C_3–C_4 angle was 19.6°, all in excellent agreement with the angles obtained from the NMR study.

Conformational Effects of Cinchona Alkaloid–Substrate Interactions. The use of cinchona alkaloids as chiral bases and chiral ligands in catalytic asymmetric synthesis is well established. With detailed conformational information in hand, forthcoming from direct measurements in solution and in the solid state, as well as from calculations, we began a study of the conformational effects of alkaloid–substrate interactions. When cinchona alkaloids are used as chiral bases, the main interaction with the substrate is a protonation of the tertiary nitrogen in the quinuclidine ring and subsequent formation of an ion pair between the protonated alkaloid and the deprotonated substrate molecule. When the alkaloids are used as chiral ligands, the main interaction is the formation of a dative bond between the tertiary nitrogen of the quinuclidine ring and the metal atom of the substrate molecule.

In order to gain more insight into the mechanistic details of cinchona alkaloid catalyzed reactions, we have investigated the effects of protonation and complexation on the conformation of the alkaloids. When osmium tetraoxide was added to a solution of **1** in chloroform-d_1, a yellow-orange complex of **1** and osmium tetraoxide was formed and only intermediate signals are observed in the ^{1}H NMR spectrum. Separate signals for complexed and uncomplexed **1** are not observed even by cooling down to −80 °C in dichloromethane-d_2. The formation of an osmium tetraoxide complex caused several changes in the ^{1}H NMR spectrum.[22] These changes are partly due to direct shielding contributions of the heavy-metal oxo species; more important, however, is the notable reduction of the $^3J_{H_8H_9}$ coupling constant, accompanied

by a large upfield shift of H_{11}, which suggests that the binding of osmium tetraoxide imposes conformational changes in the alkaloid.

The conformation of the (*p*-chlorobenzoyl)dihydroquinidine osmium tetraoxide complex was investigated further by using NOE difference and NOESY techniques (vide supra). The presence of strong NOEs between H_8 and both H_5 and H_9 together with a strong NOE between H_{10} and H_1 confirms that the osmium tetraoxide complex of **1** has the open conformation 3. Similar investigation of the NMR spectra of all closed alkaloids (dihydroquinidines **2** and **3**, together with dihydroquinine **6**) reveals that all ester alkaloids attain the open conformation 3 upon complexation with osmium tetraoxide.

The methoxy derivative **4**, which has an open conformation in chloroform-d_1 and a closed conformation in dichloromethane-d_2, attained an open conformation upon complex formation, irrespective of the solvent.

In order to assess if similar conformational changes occurred when the alkaloids are protonated, **1** was treated with trifluoroacetic acid-d_1. The alkaloid contains two different basic sites, the quinoline and quinuclidine nitrogens, of which the latter is most basic.[21] The ^1H NMR spectrum[22] reveals that protonation takes place on the quinuclidine nitrogen as revealed by extensive line broadening of the α-hydrogens, H_9, H_{15}, H_{16}, H_{18}, and H_{19}. Furthermore, the ^1H NMR spectrum underwent several by now familiar changes, the $^3J_{H_8H_9}$ coupling constant almost disappeared, and the chemical shift of H_{11} moved 0.47 ppm upfield, all in accordance with a transition from a closed to an open conformation.

Discussion

The unique potential of cinchona alkaloids for asymmetric synthesis has been widely recognized. In most of these reactions, the alkaloids act either as chiral bases or as chiral ligands to metals, and our attention has been focused on these aspects.

In the chiral Michael additions the cinchona alkaloids are used as chiral bases. The alkaloid-catalyzed addition of aromatic thiols to α,β-unsaturated ketones has been studied in detail.[9a,15] In a fast preequilibrium, the aromatic thiol is deprotonated by the alkaloid and the so formed tight ion pair reacts in the rate-determining step with the unsaturated ketone to form the chiral addition product. In the catalytic asymmetric dihydroxylation reactions of alkenes, derivatives of dihydroquinidine and dihydroquinine play a complex role as chiral ligands to osmium. In addition to imparting fair to high asymmetry into the diol products, these ligands accelerate addition of alkenes to osmium tetroxide by 1–2 orders of magnitude.

The results of the conformational study on quinine and quinidine have already led to a new proposal for the transition state of the alkaloid-catalyzed Michael addition.[15] In the Hiemstra model,[9a] advanced some years ago on the basis of kinetic data and product studies, a transition from an open to a closed alkaloid conformation upon the formation of the ion pair between aromatic thiol and the quinuclidine nitrogen was postulated. Evidence from NMR revealed that the open conformation 3 did not close upon protonation on the quinuclidine nitrogen, and a mechanism consistent with the newly available conformational information has been advanced. The results of the conformational study will also serve as a basis for the study of the chiral discrimination in the asymmetric dihydroxylation reactions, in the diethylzinc additions to aldehydes,[24] and in the [2 + 2] cycloadditions between chloral and ketene.[8a,8b]

The results of the present study have revealed the valuable and unexpected information that, although the dihydroquinidines and the dihydroquinines display very similar conformational behavior, their conformations can be influenced by varying the substituent at the benzylic position, by protonation on the quinuclidine nitrogen, or by complexation with osmium tetraoxide. The ester derivatives always have the closed conformation 2, the ether derivative exhibits intermediate behavior between the closed conformation 2 and the open conformation 3, depending on the solvent, and finally, if a hydroxyl group is present at the benzylic position the open conformation 3 is preferred. However, when the alkaloids are protonated or complexed to osmium tetraoxide, they all form the open conformation 3, irrespective of both their starting conformation and the solvent. The quinine and quinidine derivatives have an erythro configuration between the C_8 and C_9 atoms. Epiquinine and epiquinidine, on the other hand, have a threo relationship between these two chiral atoms. The threo alkaloids prefer to stay in an open conformation; however, it should be noted that this open conformation is different from the open conformation observed in the erythro alkaloids. The open conformation in the threo alkaloids resembles conformation 4, suggested by the molecular mechanics calculations on the dihydroquinidines.

The molecular modeling study of the dihydroquinidines revealed that four different minimum energy conformations could be identified. One of these conformations (open conformation 4) was substantially higher in energy than the other three, and for the dihydroquinine derivatives, the equivalent of this conformation was not even identified as a minimum energy conformation. The three minimum energy conformations of the dihydroquinines are related pseudoenantiomerically with conformations 1–3 of the dihydroquinidines. Molecular mechanics calculations performed on quinine and quinidine derivatives themselves, gave results very similar to those of the dihydro analogues; however, the energy difference between conformations 1 and 2 became more pronounced.[22] The similarities between the two classes of compounds extend to their conformations in solution, where quinine and quinidine both adopt the open conformation.[15,22]

Of the closed conformations 1 and 2 predicted by molecular mechanics, only 2 has been found in solution. No evidence from the NMR data for the existence of conformation 1 has been obtained for any of the studied derivatives of the cinchona alkaloids. Judging from the results of the molecular mechanics calculations, this is unexpected, because the energy difference between conformations 1 and 2 is predicted to be minimal. Furthermore, the molecular mechanics calculations did not reveal the gradual change in energy difference between conformations 2 and 3 as a function of the benzylic substituent. However, from Table III and results from other calculations[22] it can be seen that the conformations found in solution are the ones with lowest dipole moments. Because the observed conformational phenomena are not consistent with the gas-phase molecular mechanics calculations, an explanation must be sought in terms that are not taken in account by molecular mechanics. One possibility is a stabilizing interaction between specific conformations of the alkaloids in solution. Another possibility is the existence of other contributions to the total energy of the system, which are not described by molecular mechanics. In the closed conformation 2, the lone pair of the quinuclidine nitrogen points toward the electrons of the π system of the quinoline ring. This is not the case for the other closed conformation 1, nor for any of the open conformations. The possibility of a stabilizing interaction between the quinuclidine nitrogen lone pair and the aromatic π system[23] is currently under investigation. When one compares the three different benzylic substituents, it is seen that the ester groups are most electron withdrawing, followed by the ether group, and finally the hydroxyl group, which is the least electron-withdrawing substituent of the three.

We note seminal observations of Uskokovic et al.,[25] who published in 1969 evidence for interaction between cinchona alkaloids in solution, based on the difference between the NMR spectra of optically pure and racemic dihydroquinidines in chloroform-d_1 obtained under the same conditions. The spectral differences were greatly reduced when methanol-d_4 was used as solvent. Furthermore, Uskokovic reported that the acetates of optically active and racemic dihydroquinine showed significantly smaller differences than those observed with dihydroquinine. The authors concluded that the observations could be rationalized by solute–solute interactions of the enantiomers. Further work will be done to resolve the ambiguity of the driving force(s) behind the conformational behavior of these cinchona alkaloids.

A fairly detailed picture of the conformation of the studied cinchona alkaloids as a function of the benzylic substituent, the

solvent, and the type of complex is now available from this combination of X-ray, NMR, and calculational data. These data can serve as a basis for understanding the chiral recognition observed when the alkaloids are used as resolving agents, help to elucidate details of asymmetric synthesis, and make predictions subject to experimental verification.

Experimental Section

Energy calculations and total energy minimizations were performed with the CHEMX[18] and MODEL molecular modeling programs on VAX 11/750 and 11/785 computers. MODEL is a modified and updated version of C. Still's program, written by K. Steliou, University of Montreal (1988). To obtain the energetically preferable conformations we used Allinger's MM2P[19] and MMX[20] minimization algorithms until the root-mean-square of the gradient of the energy was less than 0.01 kcal/Å. All calculations were performed by using a dielectric constant of 1.5. The NMR experiments were performed on Varian VXR-300, XL-300, and Gemini-300 spectrometers. The NMR spectra were obtained at ambient temperatures and the alkaloid concentrations were 0.02 M. All the alkaloid derivatives were prepared by literature procedures. Because of the low equilibrium constant between free and osmium-bound alkaloid, it had to be ensured that complete complexation had taken place before analyzing the NMR spectra of the osmium–alkaloid complexes. For that purpose, small amounts of osmium tetraoxide were gradually added to the alkaloid and the $\Delta\delta$ values were plotted against the total osmium tetroxide concentration. An asymptotic curve was obtained, which leveled off when maximum complexation had been reached. The NMR experiments were then performed at this point. A similar approach was applied in the study of protonation on the quinuclidine nitrogen. X-ray structure determination of (*p*-chlorobenzoyl)dihydroquinidine (**1**): Suitable crystals for X-ray structure determination were obtained from solution of **1** in a minimum amount of acetonitrile. Data were collected on a Enraf-Nonius CAD 4 defractometer. The structure was solved and refined by standard procedures. Crystallographic data are summarized in Table II.

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Supplementary Material Available: Tables of crystallographic data (for **1**), ^{1}H NMR data (for **1** and **5**), energy calculations for the substituted quinine and quinidine compounds, and an ORTEP drawing and tables of bond distances and bond angles (for **1**) (36 pages). Ordering information is given on any current masthead page.

An ENDOR Study of the Tyrosyl Free Radical in Ribonucleotide Reductase from *Escherichia coli*

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Abstract: Tyrosyl radicals have been identified as components of several proteins whose function is redox chemistry. Ribonucleotide reductase is representative of this class of enzymes as its B2 subunit contains a tyrosine residue that is necessarily a radical for activity. The EPR spectrum of the immobilized enzyme is broadened, however, and only limited information can be extracted from an analysis of its line shape. In this situation, ENDOR spectroscopy is a higher resolution technique, and we used it to characterize the enzyme-bound radical in detail. ENDOR enhancement in ribonucleotide reductase is observed only at temperatures below 110 K due to a temperature-dependent relaxation enhancement of the radical by a μ-oxo-bridged pair of high-spin ferric ions. Below this temperature, excellent spectra are obtained and specific deuteration of the tyrosine residues in the protein was used to assign measured hyperfine tensors to the various protons in the radical. An analysis of the principal tensor components of the ortho protons ($A_x = -26.9$ MHz, $A_y = -7.8$ MHz, $A_z = -19.7$ MHz) and the strongly coupled β-methylene proton establishes that the radical has characteristics of a seven-member odd-alternate species with a spin density distribution of 0.16 (phenol oxygen), 0.26 (ortho), −0.07 (meta), −0.03 (ring carbon carrying phenol oxygen), and 0.49 (para). These calculations also provide a determination of the McConnell Q value for ring protons in this class of radical. The β protons are situated with dihedral angles of 30° and 90° with respect to the p_z orbital on carbon-1 of the aromatic ring. The results indicate that the tyrosyl radical of ribonucleotide reductase is uncharged and not hydrogen bonded to donors in its environment within the protein.

Ribonucleotide reductase is an essential enzyme that catalyzes the direct reduction of ribonucleotides to their corresponding deoxyribonucleotides. In a strictly regulated reaction all four ribonucleotides compete for the same enzyme active site, and accordingly the protein provides a balanced supply of precursors for DNA synthesis in living cells.[1] One fascinating feature of several ribonucleotide reductases is the presence of a stable tyrosyl free radical.[2] This type of enzyme is found in some bacteria, eukaryotic cells, and it is also coded for by certain bacteriophages and eukaryotic viruses.[3]

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(1) Lammers, M.; Follman, H. *Struct. Bonding (Berlin)* **1983**, *54*, 29. Reichard, P. *Annu. Rev. Biochem.* **1988**, *57*, 349.
(2) Gräslund, A.; Sahlin, M.; Sjöberg, B.-M. *Environ. Health Persp.* **1985**, *64*, 139. Stubbe, J. *Biochemistry* **1988**, *27*, 3893.

0002-7863/89/1511-8076$01.50/0