Conformational Studies of a Family of Related Antimalarial Phenanthrene Amino Alcohols

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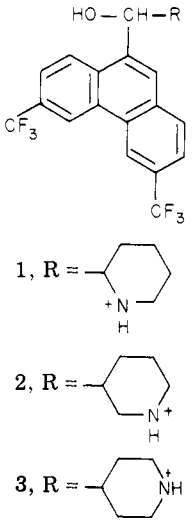
A conformational study utilizing quantum chemical methods was performed on a family of antimalarial α-(piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanols whose structures differ by the placement of the substituent on either the 2, 3, or 4 position of the piperidyl ring. The antimalarial activity of these 3-substituted compounds has been shown experimentally to be highly stereospecific while the 2-substituted compounds are not and the 4-substituted compounds are inactive. In this study, such observed differences in behavior are correlated with conformational results and a pharmacophore is postulated. The identity of the active racemate of the 3-piperidyl compound is predicted.

Interesting antimalarial activity possibly related to conformational behavior has been observed for a family of related α-(piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanols.[1,2]

Compounds **1** and **2** exist as diastereomers due to the presence of two chiral centers. All four of the 2-piperidyl optical isomers (**1**) are highly active against *Plasmodium*



berghei,[1] while only one racemate of the 3-piperidyl isomers (**2**) displays activity, an unusual stereospecificity for this class of antimaterials. It is not known which is the active racemate of **2**. The racemate of the 4-piperidyl compound (**3**), which has only one chiral center, is inactive.[2]

NMR studies[3] of compound **1** suggest a preferred conformation in solution where the rotation of the piperidyl ring leads to a staggered configuration with the quaternary nitrogen atom directed away from the phenanthrene ring. Evidence[4] involving the correlation of activity with the basicity of the nitrogen atom suggests[5] that the protonated form of the nitrogen is active. Structure–activity studies demonstrate that the aryl and hydroxyl functions are required for activity.[4]

While little is known of the mode of action of these compounds, it has been suggested that they act in a fashion similar to quinine due to similarities in structure,[6] in their ultrastructural effects on *P. berghei*,[6] and from results of binding and cross resistance studies.[7,8] The basic amino center on quinine is regarded to be important for activity.[9]

With these data, a pharmacophore of definite dimensions and character involving a hydrogen-bonded ring between the hydroxyl group and the amino group as well as the presence of an aromatic ring has been suggested.[10,15]

We have used a semiempirical molecular orbital method to study the behavior of this family of compounds. The goal of this work was to identify the preferred conformations of each molecule, relate these conformational aspects to their potencies, and from this deduce a geometric description of the pharmacophore which would explain the different stereospecificities of compounds **1–3**. We have assumed that all three structural isomers (**1–3**) of this family act similarly on *P. berghei*.

Experimental Section

The method utilized in this work is a refined all-valence-electron procedure, designated the perturbative configuration interaction using localized orbitals (PCILO).[11] Molecular orbital calculations were made on isolated molecules in their ground electronic state.

In addition, use was made of an interactive program which could monitor interatomic distances as single bonds in the molecule were rotated. Molecular models were used to determine gross aspects of rotational behavior and to select promising conformers for detailed study.

The structural geometries were derived from standard bond lengths and angles[12] with the exception of those immediately adjacent to the carbinol carbon (C_{17}). Here it was assumed that the crystal structure of ephedrine hydrochloride[13] would more closely approximate the real geometry of this portion of the molecule.

Measured irregularities in the structure of the protonated piperidine ring are within the experimental accuracy in the x-ray analysis[14] and these need not necessarily be retained in solution or gas phase. Thus, we have used an entirely symmetric piperidine ring structure with r_{CH} = 1.09 Å, r_{NH} = 1.03 Å, $r_{CC} = r_{NC}$ = 1.52 Å and tetrahedral bond angles (109.47°). This compares with the crystal structure values, determined for heavy atoms only, of $r_{CC} \simeq r_{NC}$ = 1.50 ± 0.05 Å and bond angles varying from 110 to 113°. The protonated form of the nitrogen was used for the study since it is believed to be the active form.

The main conformational characteristics are determined by the three axes of rotation which are listed in Figure 1 and the orientation of the phenanthrene ring in either an axial or equatorial position relative to the piperidyl ring. The convention used to define the torsion angles τ(ABCD) was clockwise rotation of atom D into atom A while looking along the C–B axis from atom C to atom B.

Detailed study was limited to compounds 1-*RS*, 1-*SS*, 2-*RS*, and 2-*SS* since their enantiomers would simply display complementary behavior.

The number of conformations that had to be considered for each isomer was limited by the observation that rotations about τ_3 were found to be highly sterically hindered for both the axial and equatorial forms of all the isomers regardless of τ_1 and τ_2 values. The piperidyl ring must point away from the plane of the phenanthrene ring corresponding to values of τ_3 very close to 90 and 270°.

For equatorial conformers full rotations with 60° increments about τ_1 were made for a specific value of τ_2 determined from steric considerations to be the least interfering to rotational behavior. The value of τ_2 chosen for the 1- and 2-*RS* isomers was 240° and for the *SS* isomers was 120°. These values produced equivalent conformers with minimal steric interaction between the hydroxyl group and both the piperidyl and phenanthrene rings.

To further verify the value of τ_2 chosen, variations of τ_2 were performed for conformers that exhibited relatively low-energy

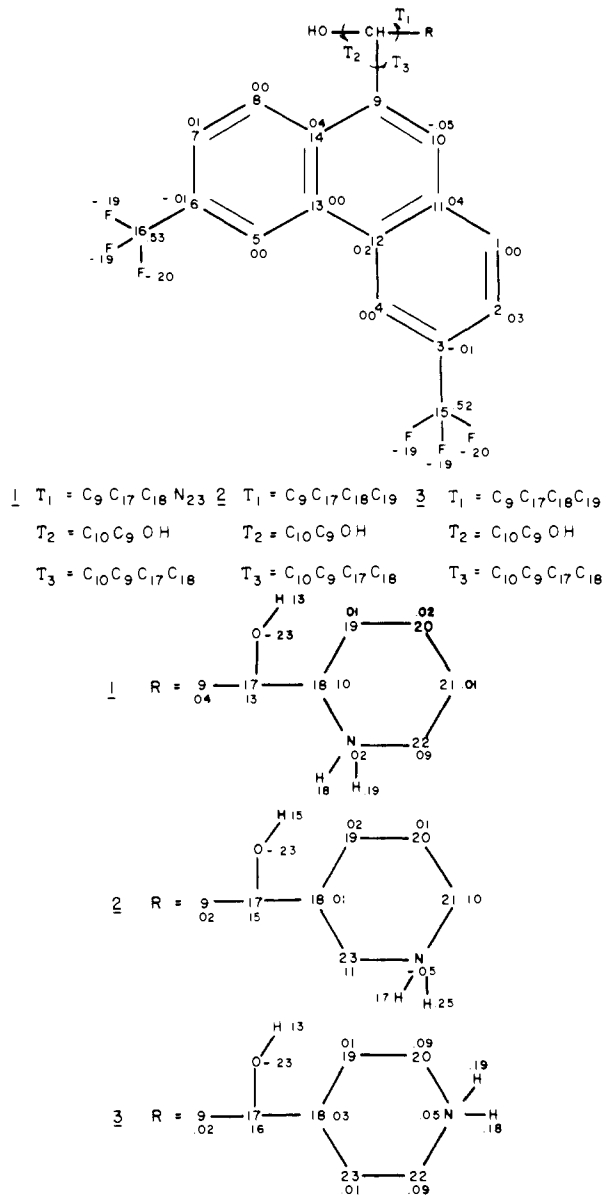


Figure 1. Net atomic charges and torsion angle definitions for three compounds studied.

behavior with respect to rotation about τ_1. It was determined that while rotation about τ_2 could uniformly depress the energy curves by as much as 2 kcal/mol, they did not affect the relative energies or positions of the local minima. The 60° increments of τ_1 are satisfactory for the purpose of this study for several reasons. First, detailed study of models showed that from steric considerations no local minima far from these intervals were possible. Secondly, the relative minima centered at these points were explored and found to be fairly broad in that variations of $\sim\pm5$ Å did not change the energy appreciably.

From model building and monitoring of nonbonded distances the axial conformers of both 1 and 2 were found to have an obvious region of values of τ_1 with large steric hindrance between the piperidyl and phenanthrene rings. Energy calculations to be performed were therefore selected from the remaining plausible region of τ_1 = 60–180°. τ_2 was again kept constant at 240 and 120° for the *RS* and *SS* isomers, respectively.

Study of the inactive 3 compound was limited to a search for any conformation which could mimic low-energy conformers of the other two sets of isomers (compounds 1 and 2). No such conformation could be found and, hence, no energy calculations were made.

Results

Figure 2 presents the energy variations as a function of rotation of τ_1 for the equatorial forms of the 1-*RS* and 1-*SS* isomers for two given values of τ_3. For each isomer several local mimima are found within 2 kcal/mol of the absolute energy minima.

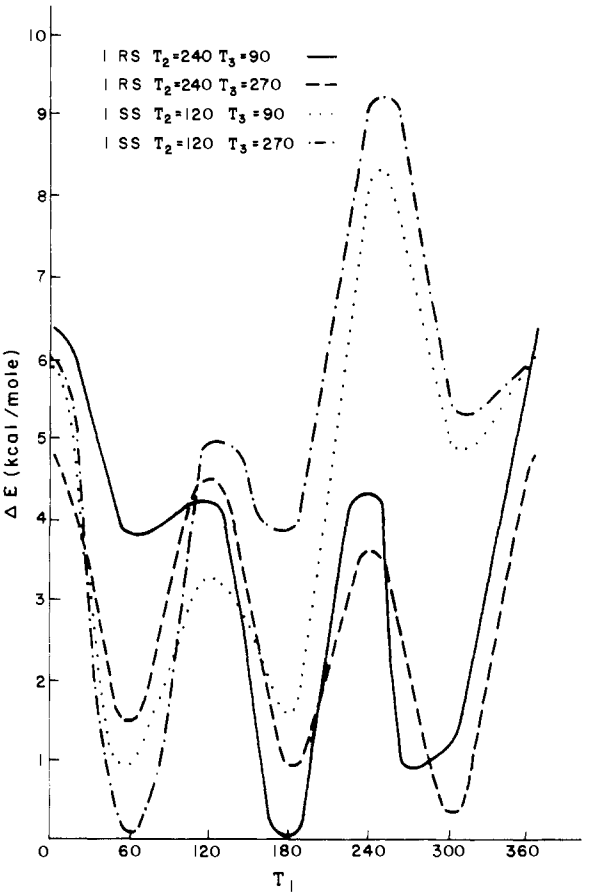


Figure 2. Energy conformation behavior of the equatorial forms of compound 1. Relative energy (ΔE, kcal/mol) as a function of τ_1 for given values of τ_2 and τ_3.

Figure 3 gives the same information for the 2-*RS* and 2-*SS* isomers. For these isomers also a number of energy minima are found.

All of the axial conformers of the 1-*RS* and 1-*SS* isomers considered were found to be at least 5 kcal/mol higher in energy than the best equatorial conformers. The axial conformers of the 2-*RS* and 2-*SS* isomers behaved similarly with the exception of a single 2-*RS* isomer (τ_1 = 150°, τ_2 = 240°, τ_3 = 90°) for which the energy was only 2.5 kcal/mol above the absolute minimum for the equatorial form.

The net atomic charges for compounds 1 in an equatorial, 2 in an axial, and 3 in an equatorial position are given in Figure 1.

Discussion

The rotational behavior of both the 1-*RS* and 1-*SS* isomers with equatorially attached piperidyl rings suggests that a hydrogen bond may occur between the oxygen and protonated nitrogen atoms. This behavior is manifest in Figure 2 where the four lowest energy local minima for *RS* and for *SS* isomers correspond to relative positions of the N and O atoms which allow at least weak hydrogen bond formation. A perfectly linear intramolecular hydrogen bond is not possible for these compounds. For the 2 isomers with an equatorial phenanthrene ring, no intramolecular hydrogen bonding is possible because the OH containing substituent in a 3 position is too far away from the nitrogen atom.

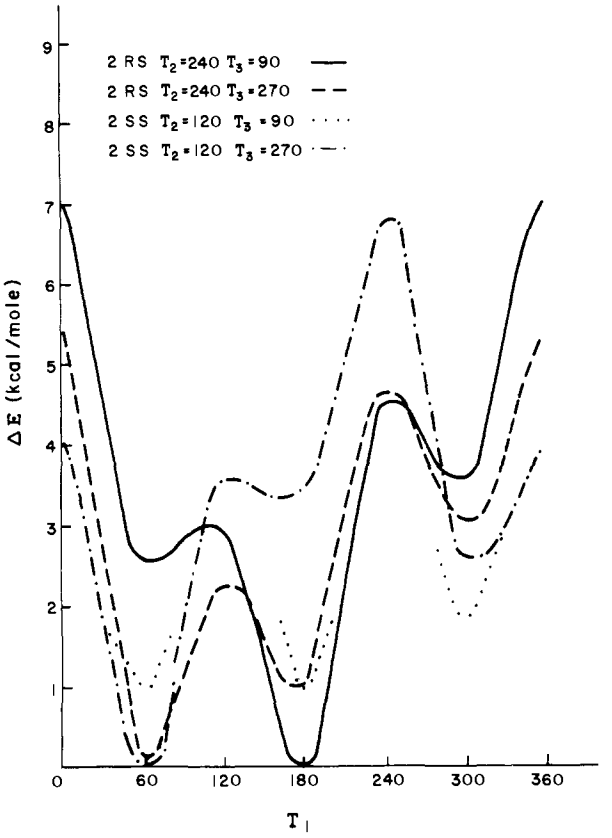


Figure 3. Energy conformation behavior of the equatorial forms of compound 2. Relative energy (ΔE, kcal/mol) as a function of τ_1 for given values of τ_2 and τ_3.

The local minima of the 1 isomers are 3–4 kcal/mol lower in energy than the corresponding minima of the 2 isomers, which is consistent with the presence of weak hydrogen bond formation in the former compounds.

There are a number of relatively low-energy equatorial conformers of compound 1 that could conceivably act at a receptor site. Similarly, there are a number of relatively low-energy equatorial conformers of compound 2. There are no low-energy axial conformers of compound 1 and only one low-energy axial conformer of compound 2 which are plausible candidates for receptor interaction ($\Delta E \leq 5$ kcal). This single axial conformer allows weak hydrogen bond formation.

In connection with the net charges obtained (Figure 1), it may be noted that the positive charge is not localized on the quaternary nitrogen, a feature shared by other classes of amine-containing drugs.[15] The protons on the nitrogen atom are the most positive. Other very polar regions are the CF_3 group and OH group, as would be expected. The net charge on the phenanthrene ring and the CF_3 groups does not vary significantly from compound to compound. The greatest variations among isomers 1, 2, and 3 are on the nitrogen and its hydrogen atoms. However, differences in net atomic charges alone do not correlate with the observed differences in potency and biological specificity among these isomers, leaving conformation characteristics the main distinction among them.

Assuming that all the active isomers act at the same receptor site, and knowing the dependence of biological activity on isomeric structure, the question is, can a pharmacophore be designed that all four 1 isomers can emulate while only one racemic pair of the 2 isomers and neither of the 3 isomers could emulate? Since structure–activity studies indicate the importance of the OH, amino, and aryl ring functions, they should form the major features of the pharmacophore as suggested by Cheng.

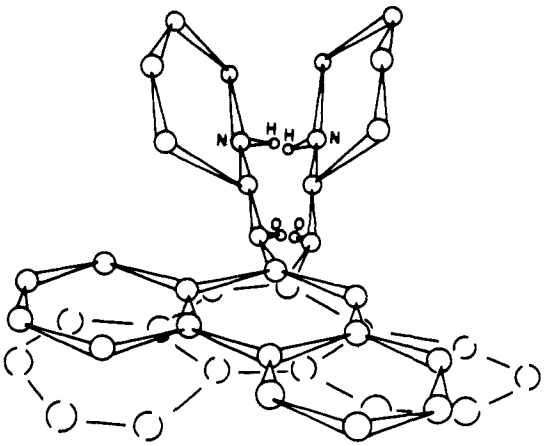


Figure 4. Superposition of low-energy 1-*RR* and 1-*SS* equatorial conformers to show overlapping regions that can determine a common pharmacophore: 1-*RR* (τ_1 = 180°; τ_2 = 240°; τ_3 = 90°), 1-*SS* (τ_1 = 180°; τ_2 = 120°; τ_3 = 270°).

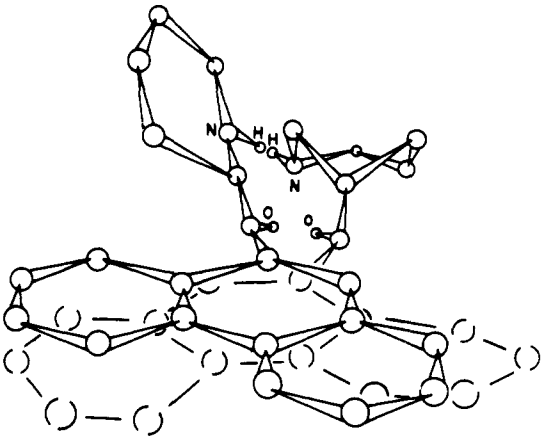


Figure 5. Superposition of the unique low-energy 2-*RS* axial conformer on the 1-*SS* conformer shown in Figure 4 indicating similar overlapping regions: 1-*SS* (τ_1 = 180°; τ_2 = 120°; τ_3 = 270°), 2-*RS* (τ_1 = 150°; τ_2 = 240°; τ_3 = 90°).

Table I. Distances[a] between Key Features of Proposed Pharmacophore

	N-O	Ctr[b]-N	Ctr[b]-O	H_N-O	Ctr[b]-H_N
1-*RS* (*SR*)[c]	2.723	5.198	3.770	3.024	5.497
1-*SS* (*RR*)[c]	2.723	5.198	3.770	3.024	5.497
2-*RS*[d]	2.272	5.215	3.770	3.120	5.853

[a] Separations given in ångströms. [b] Center of central aromatic ring. [c] N, H, and O defined in Figure 4. [d] N, H, and O defined in Figure 5.

It is possible to find several low-energy equatorial conformers of all four isomers of 1 with nearly superimposable functional groups and aryl rings as, for example, shown in Figure 4. This similarity then could account for their observed lack of biological stereospecificity.

In comparing the 1 and 2 isomers it is not possible to make any of the low-energy equatorial conformers of 2 superimpose on those of 1. Interestingly, however, the single low-energy 2-*RS* axial conformer can match reasonably well to the pharmacophore determined by the 1 isomers (Figure 4) as shown in Figure 5. This pharmacophore allows the oxygen atoms, the protons on the nitrogen atoms, as well as the N atoms, and the central aromatic ring of the phenanthrene ring to be superimposed

over each other for all the active isomers. This superimposibility is also shown by comparison of distances between key features of the proposed pharmacophore as shown in Table I. Since there are no low-energy 2-*SS* axial isomers, no 2-*SS* (or *RR*) isomer can superimpose on this pattern. No conformer of the **3** compounds can be matched to the pattern shown in Figures 4 and 5, thus possibly accounting for their activity.

The active conformation of each molecule is such that it allows a hydrogen bond to form between the OH and amine functions. The existence of a hydrogen bond is not necessarily required for activity but helps to stabilize each active molecule in the conformation that contains the proposed pharmacophore. Cheng[16] has also suggested that the axial conformation of compound **2** must be invoked to explain its stereospecific activity and uses mass spectral data to lend support to this conclusion. Our results, based on direct conformational analysis, independently describe the same pharmacophore to account for the stereospecificity of the **2** isomers, the lack of it in the **1** isomers, and the inactivity of the **3** isomers. They further point to the 2-*SS* and 2-*RR* as the inactive forms, a prediction that can be tested by experiment.

Subject to further verification then, our results have led to a description of a pharmacophore for this subclass of antimalarial drugs which can account for their observed behavior and is similar in dimension and character to that proposed by Cheng.

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Synthetic Models of DNA Complexes with Antimalarial Compounds. 2. The Problem of Guanine Specificity in Chloroquine Binding

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Stacking interactions between the aminoquinoline ring of the antimalarial chloroquine and the purine bases have been studied by preparing and examining models in which the quinoline is linked to the base by a trimethylene chain. The degree of stacking of the models which reflects the strength of the interaction was quantitatively determined in water at different temperatures by hypochromism measurement in the uv. Adenine and guanine exhibit equal affinity for the quinoline nucleus as reflected by very close hypochromism values observed for the two models at all temperatures studied.

Chloroquine (**1**),[1] a widely used antimalarial, inhibits several biological functions of plasmodia, the microorganism responsible for the disease, acting at the level of the DNA and RNA polymerases and interfering with protein synthesis.[2] Its mode of action involves binding to the nucleic acids; the influence of this on nucleic acid biosynthesis has been demonstrated in vitro.[3]

The complexation of chloroquine with DNA[4] and with synthetic polynucleotides[5] has been studied. Two types of interaction are involved in complex formation, an electrostatic attraction between the amine group (protonated at physiological pH[6]) of the chloroquine side chain and the phosphate groups of the DNA, and a more specific interaction between the aromatic system of chloroquine and the nucleotide bases.[7] It has been suggested that the protonated aminoquinoline ring is actually intercalated between base pairs in the DNA helix.[8]

As an approach to the problem of the ring–ring interactions which may take place in the intercalation process, we studied the stacking interactions between the aminoquinoline ring of chloroquine and the nucleotide bases. Models of the form B-C_3-Q (**2**, **3**, and **4**) were prepared, in which the aminoquinoline Q and the base B are joined by a trimethylene chain;[9] such molecules are capable of adopting a folded conformation in which the two aromatic systems interact. The proportion of molecules in the folded conformation will be an indication of the affinity of quinoline for the base. In a previous publication[10] we have thus reported a study of the interaction of aminoquinoline with a purine (adenine) and a pyrimidine (thymine); the extent of interaction with adenine was much greater than that with thymine. This result is in agreement with studies of chloroquine–polynucleotide complexation[4,5] which demonstrated only a weak affinity of the quinoline nucleus for pyrimidine bases. However, the studies of the complexation of chloroquine with polynucleotides did not clearly establish the role of the purine bases, adenine and guanine.

An ambiguity arises from the fact that most of these studies were based on measurements of the perturbation "hypochromicity"[11] in the uv absorption of chloroquine in the complex. The degree of perturbation depends on the nature of the polynucleotide, being stronger for polyG than for polyA, for polydGdC than for polydAdT, and increasing with the (G–C) content in a series of natural DNA's from different sources, while the equilibrium