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Determining the Absolute Configuration of (+)-Mefloquine HCl, the Side-Effect-Reducing Enantiomer of the Antimalaria Drug Lariam

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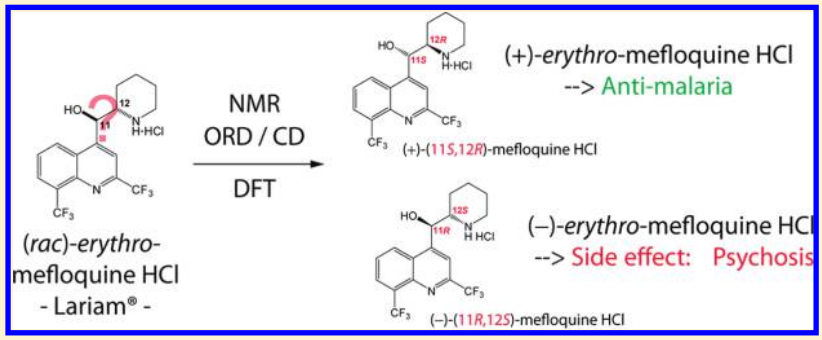
Supporting Information



ABSTRACT: Even though the important antimalaria drug *rac-erythro*-mefloquine HCl has been on the market as Lariam for decades, the absolute configurations of its enantiomers have not been determined conclusively. This is needed, since the (−) enantiomer is believed to cause adverse side effects in malaria treatment resulting from binding to the adenosine receptor in the human brain. Since there are conflicting assignments based on enantioselective synthesis and anomalous X-ray diffraction, we determined the absolute configuration using a combination of NMR, optical rotatory dispersion (ORD), and circular dichroism (CD) spectroscopy together with density functional theory calculations. First, structural models of *erythro*-mefloquine HCl compatible with NMR-derived $^{3}J_{HH}$ scalar couplings, ^{15}N chemical shifts, rotational Overhauser effects, and residual dipolar couplings were constructed. Second, we calculated ORD and CD spectra of the structural models and compared the calculated data with the experimental values. The experimental results for (−)-*erythro*-mefloquine HCl matched our calculated chiroptical data for the 11*R*,12*S* model. Accordingly, we conclude that the assignment of 11*R*,12*S* to (−)-*erythro*-mefloquine HCl is correct.

Notwithstanding its importance as an antimalaria agent, the absolute configuration of neither (+)- nor (−)-*erythro*-mefloquine HCl has been solved conclusively to date. Although it is currently applied as a racemate (Lariam), the pharmacological data differ for the two enantiomers,[1] and consequently, a patent on the (+) enantiomer was filed in 2003.[2] This enantiomer binds less tightly to the adenosine receptor in the brain; such binding is thought to infer adverse malaria treatment effects such as depression and psychosis.[3,4] Historically, the first attempt to determine the absolute configuration was made in 1974, based on circular dichroism (CD) data and empirical rules.[5] This first assignment was rejected and inverted by anomalous X-ray diffraction in 2002.[6] In 2008, however, a total synthesis using a proline-catalyzed asymmetric direct aldol reaction (recently reviewed for its broad applicability[7]) and Beckmann rearrangement followed by a Mosher analysis[8] reconfirmed the original assignment.[9] Because these established methods for the determination of the absolute configuration, namely, anomalous diffraction and stereoselective synthesis, gave contrary results, we determined the absolute configuration using residual dipolar coupling (RDC)-enhanced NMR spectroscopy in combination with optical rotatory dispersion (ORD) and CD spectroscopy.

The key to successfully combining RDC-enhanced NMR and chiroptical methods is that RDC-enhanced NMR spectroscopy provides accurate ensembles for flexible molecules that are required for accurate density functional theory (DFT) calculations of chiroptical properties to determine absolute configurations. Through the use of RDCs in conjunction with conventional NMR restraints such as nuclear Overhauser effects (NOEs) and *J* couplings, ensembles of flexible molecules

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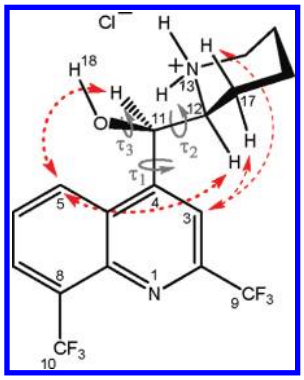
in solution can be determined with the highest precision possible today.[10−15] The experimental chiroptical properties can then be compared with calculated ones. In the case of a Michael reaction product with a known absolute configuration, the correct assignment of the enantiomers with chiroptical methods could only be done after the conformational ensemble of this flexible molecule had been determined by NMR analysis.[16] Here, we used this approach for a (−)-*erythro*-mefloquine HCl adduct of unknown absolute configuration that is flexible and in addition is a salt, thereby requiring that the conformational ensemble accurately describe the locations of the ions. We used the (−)-enantiomer, since it had been investigated by Karle. Of course, the (+)-enantiomer mentioned in the title has the inverted configuration.

(−)-*erythro*-Mefloquine HCl is a semirigid compound (Scheme 1). The planar quinoline fragment and the piperidine

Scheme 1. Molecular Structure of (−)-*erythro*-Mefloquine HCl[a]



[a]The 11*R*,12*S* enantiomer is depicted, since this was found in this study for (−)-*erythro*-mefloquine HCl. ROESY contacts: arrows in bold indicate a strong contact, normal arrows a weak to medium contact. The relative configuration was confirmed by comparison with literature data.[17,18]

ring, in a chair conformation with an equatorial alkyl substituent at C12, are rigid,[6] the latter being verified by ^3J couplings of the ring protons (see the Supporting Information). The conformational freedom of mefloquine HCl relies on only three rotatable bonds [C3−C4−C11−C12 (τ_1), C4−C11−C12−N13 (τ_2), and H18−O−C11−H11 (τ_3)] and the position of the chloride ion (see below). We therefore derived conformations that fit with the short-range NMR data [J couplings, chemical shifts, rotational Overhauser effects (ROEs)] and the long-range residual dipolar couplings (RDCs) and then optimized their atomic coordinates further using DFT. The populations of the conformations in the ensemble were subsequently determined by ^3J couplings and RDCs independently.

For the three torsion angles τ_1, τ_2, and τ_3, the ROE spectroscopy (ROESY) cross-peaks (Scheme 1) indicated that there are preferred conformations that clearly distinguish between the two sides of the quinoline fragment relative to the substituent at C4: on the one hand, the strong ROEs between 5 and 11 and between 5 and 12ax, and on the other hand, the missing ROEs between 5 and 17eq and between 5 and 17ax (eq = equatorial, ax = axial). Additionally, the $^3J_{H11H12}$ = 2.7 Hz coupling and two strong $^3J_{HC}$ heteronuclear multiple-bond correlation (HMBC) signals ($^3J_{H11C17}$ and $^3J_{H11C3}$) indicated preferred values of τ_1 and τ_2.

On the basis of this information, we constructed a model of N_{13}-protonated (11*R*,12*S*)-mefloquine with τ_1 = 90° and τ_2 = 180° that approximately fulfilled the above-mentioned *J*-coupling and ROE data. Optimizations and spectroscopic data were calculated at the B3LYP/6-311+g(2d,p) level of theory with the IEFPCM solvent model.[19,20] For the protonated model, we obtained τ_1 = +99.6°, τ_2 = +176.6°, and τ_3 = +42.1° (Table 1).

However, the calculated ^{15}N chemical shift difference between N_1 and N_{13} clearly violated the experimental restraint [experimental $\Delta\delta(^{15}N)$, 254 ppm; $\Delta\delta(^{15}N)$ of the protonated model, 285 ppm; Table 1). The $\Delta\delta(^{15}N)$ of the nonprotonated model (254 ppm) indicated that the positive charge must be compensated.

After positioning of a chloride anion close to either the equatorial or axial proton at N_{13} and subsequent optimization, we obtained two new conformations: BRIDGE_ax and BRIDGE_eq (Figure 1 and Table 1). In both ion pairs, the

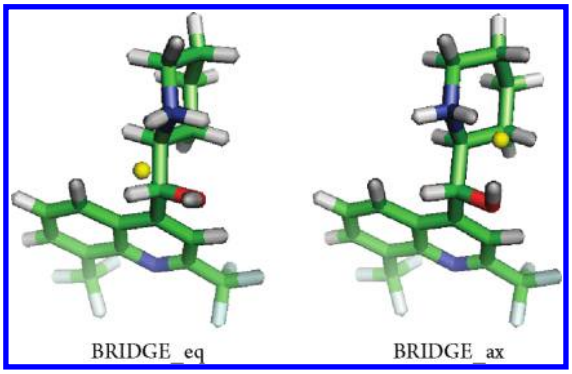


Figure 1. Optimized models of mefloquine HCl (BRIDGE_eq and BRIDGE_ax). Chloride ions appear as yellow spheres.

chloride anion links two hydrogens: the OH hydrogen and one of the NH hydrogens (equatorial in BRIDGE_eq and axial in BRIDGE_ax). The weights of the populations were then found by matching the ^3J couplings (see the Supporting Information), which gave weights of 60% for BRIDGE_ax and 40% for BRIDGE_eq (Table 1).

Table 1. Calculated and Experimental NMR Data: Δ^{15}N Chemical Shifts, Dihedral Angles, and ^3J Couplings

	$\Delta\delta(^{15}N_{13-1})$ (ppm)	τ_1 (deg)	τ_2 (deg)	τ_3 (deg)	$^3J_{H11H12}$ (Hz)	$^3J_{H11H18}$ (Hz)
protonated model	285	99.6	176.6	42.1	2.7	3.6
BRIDGE_ax	249	99.7	163.5	−55.1	1.0	2.2
BRIDGE_eq	248	103.7	−170.5	−18.3	4.9	8.0
0.6(BRIDGE_ax) + 0.4(BRIDGE_eq)	249	n.a.[a]	n.a.[a]	n.a.[a]	2.6	4.5
exptl	254	n.a.[a]	n.a.[a]	n.a.[a]	2.7[b]	4.2[b]

[a]n.a. = not applicable. [b]258 K.

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At this point we want to emphasize that the two ion pairs are only representatives of the "true" structures. Obviously, many slightly different positions of the chloride ion are possible, as indicated by the four conformers found in the X-ray analysis of mefloquine hydrochloride crystals from ethanol/water solutions.[6]

The result of our RDC-based population analysis using MSpin[21] was a 57:43 BRIDGE_ax:BRIDGE_eq mixture (Q = 0.035; Figure 2). This matches remarkably well with the 60:40

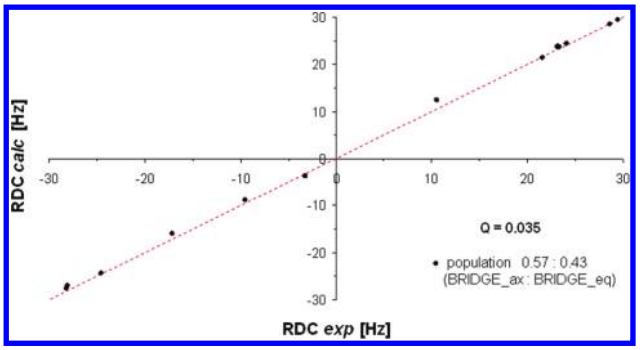

Figure 2. Single-tensor fitting performed on BRIDGE_eq and BRIDGE_ax as structural models using an average structure (NMRDev[21]). Data for individual structural models as well as synthetic details about the polyacrylamide gel [(*S*)-APhES/DMAA gel] used as an alignment medium are presented in the Supporting Information.

BRIDGE_ax:BRIDGE_eq mixture independently obtained by *J*-based population analysis as summarized in Table 1. Thus, the two ion pairs of mefloquine HCl in methanol with two sets of populations determined independently from two sets of experiments were used as a valid reflection of the solution ensemble of mefloquine HCl. The chemical shifts based on which the positions of the ions were determined were different in the anisotropic solution. However, this was also observed previously for the conformationally rigid molecule estrone.[22] Thus, we have no indication that the ion pairs would be different in isotropic or anisotropic solution.

To establish the absolute configuration, at least two chiroptical methods should be used simultaneously.[23] Consequently, we first calculated ORD spectra for the two ion pairs. Comparison with the experimental ORD spectrum (Figure 3)

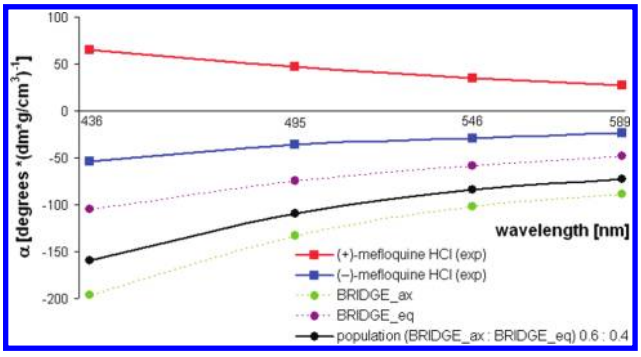

Figure 3. Experimental ORD spectra of (+)-mefloquine HCl (red) and (−)-mefloquine HCl (blue) in methanol and ORD data calculated for (11*R*,12*S*)-mefloquine HCl at four wavelengths (purple, BRIDGE_eq; green, BRIDGE_ax; black, 0.6:0.4 BRIDGE_ax:RIDGE_eq).

suggested that the 11*R*,12*S* configuration is the (−)-antipode of mefloquine HCl. However, the individually calculated values differed by more than a factor of 2, emphasizing the importance of the structural analysis. As can easily be seen, the experimental values for the two enantiomers are not perfect mirror images. This is most probably due to a higher optical purity of the (+)-enantiomer compared with the (−)-antipode, which was produced by the formation of a diastereomeric salt (see the Supporting Information).

As a second chiroptical method, we compared the CD spectra[24,25] for the two ion pairs with experimental values (Figure 4). Again, our structural models were consistent with

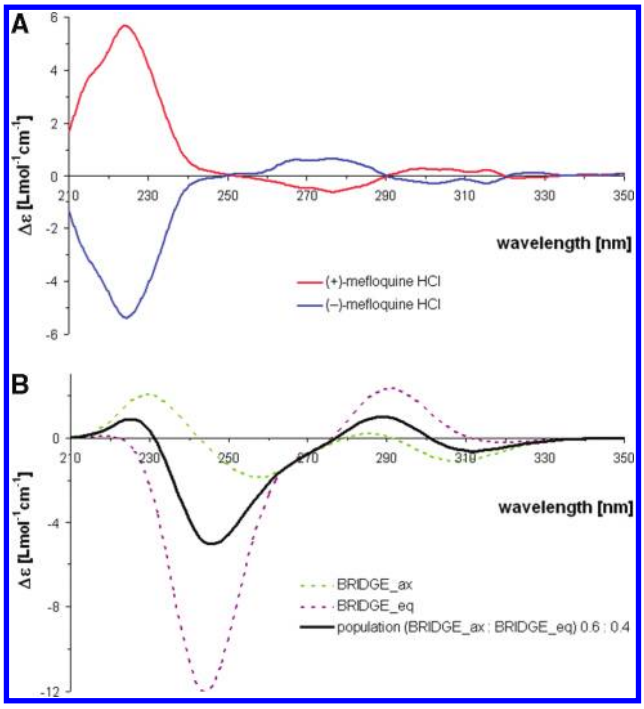

Figure 4. (A) Experimental CD spectra of (+)-mefloquine HCl (red) and (−)-mefloquine HCl (blue) in methanol. (B) Unshifted calculated CD spectra of (11*R*,12*S*)-mefloquine HCl (green, BRIDGE_ax; purple, BRIDGE_eq; black, 0.6:0.4 BRIDGE_ax:BRIDGE_eq).

the (−)-antipode with respect to the sign and position of the three experimentally observed signals, with two negative bands around 310 and 230 nm and one positive band at 280 nm.

In summary, we conclude that (−)-*erythro*-mefloquine HCl can be assigned as 11*R*,12*S*, indicating that the configurational analysis in the recently published enantioselective synthesis as well as the old proposition based on CD and empirical rules are incorrect and that the anomalous X-ray diffraction from the crystal delivered the correct assignment. Obviously, its occurrence as a chloride ion pair restricts the conformational space of mefloquine compared with studies of the related cinchonidine as the base.[26,27] With the correct assignment presented here, syntheses for (+)-(11*S*,12*R*)-mefloquine HCl, a promising malaria treatment with less adverse side effects, can now be designed. Similarly important is the correct usage of the configurational descriptors in the growing literature of medicinal applications of mefloquine HCl.[28,29]

■ ASSOCIATED CONTENT

Supporting Information

Assignment and J couplings of *erythro*-mefloquine HCl in methanol; synthesis of the alignment medium; computational details; Cartesian coordinates of the calculated structures; experimental and back-calculated RDC values; and complete ref 19. This material is available free of charge via the Internet at http://pubs.acs.org.

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