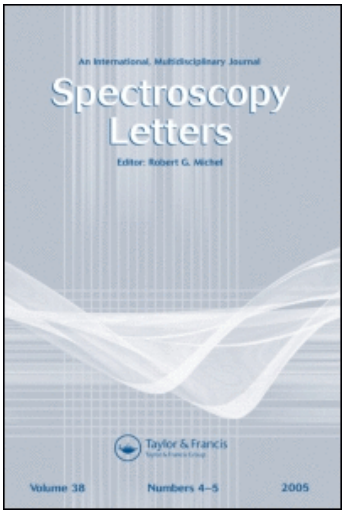

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Nuclear Overhauser Effect Spectroscopy (NOESY) and $^3J_{HH}$ Coupling Measurements in the Determination of the Conformation of the Sesquiterpene Antimalarial Arteether in Solution

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NUCLEAR OVERHAUSER EFFECT SPECTROSCOPY (NOESY) AND $^3J_{HH}$ COUPLING MEASUREMENTS IN THE DETERMINATION OF THE CONFORMATION OF THE SESQUITERPENE ANTIMALARIAL ARTEETHER IN SOLUTION

Keywords: modified Karplus equation, X-ray structure, molecular modeling

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ABSTRACT

The twisting of the A-, B-, and D- rings of β-arteether were determined from dihedral angles derived from $^3J_{HH}$ coupling constants and a Karplus-type equation that takes into account heteroatom substitution. The angles obtained by NMR were also compared to the angles obtained by molecular modeling and to those obtained from the methyoxyl analog (artemether) using x-ray crystallography. The NMR results showed that the A-ring was a relatively undistorted chair conformation with the 14-methyl group in an equatorial position. The NMR data indicated that the B-ring was very distorted and the D-ring was twisted outward because of the steric interactions with the ethoxy group. Two-dimensional NOESY data was used to measure selected proton-proton distances for arteether in solution and these distances were also compared to those obtained by molecular modeling and from the x-ray data for artemether. The NOESY data also indicated that the conformation of the A-ring was fairly normal, the B-ring was twisted, and the ethoxy group was in an extended conformation (C_{11}-C_{12}-O-C_{16} = +170°, and C_{12}-O-C_{16}-C_{17} = +169°).

INTRODUCTION

Arteether is a semi-synthetic antimalarial derived from the naturally occurring sesquiterpene product artemisinin (also known as Qinghaosu) which has a slightly higher antimalarial activity compared to the parent compound (1). Arteether has shown sufficient promise as a new drug that the steering committee of the Scientific Working Group on Malaria Chemotherapy of the World Health Organization has chosen this new agent for clinical evaluation in high-risk malaria patients (1). Even though arteether might be expected to be fairly ridged because of the interlocking ring system (Fig. 1), there is still a moderate degree of flexibility in both the A-ring, in the D-ring, and in the ethoxy group. The major objective of the present study was to determine the preferred conformation of arteether in solution which may prove useful in the design of a simpler, totally synthetic antimalarial having the same spatial relationships of the key functional groups needed for biological activity.

RESULTS AND DISCUSSION

^{1}H-NMR Assignments.

The proton NMR assignments of artemisinin, an antimalarial structurally related to artemether, have been made by Zhongshan *et. al.*[2] but the assignments where later corrected by Blasko *et. al.*[3]. The proton NMR spectrum observed for arteether in the present study was very similar to the assignments reported for artemisinin, except for protons 11, 5, and 8β which are strongly affected by the anisotropic shielding of the carbonyl group in artemisinin.

The proton NMR assignments made for arteether in the present study (Table 1) are the same as previously reported by this laboratory,[4] except that proton 8β has been reassigned and the individual assignments for 9α and 9β have been made. For arteether in deuterochloroform, the signal for 8β had been originally placed at approximately 1.24 ppm based largely by comparison with the artemisinin spectrum and on the presence of a cross peak at this chemical shift in the 2-D heteronuclear correlation (HETCOR) spectra. This small cross peak has proven to be an artifact and the true chemical shift of 8β was found to be at 1.88 ppm and the pattern partially overlaps the pattern for 2α. The assignment of the 8β axial proton (*vs.* the 8α equitorial proton) was based on the presence of the coupling pattern showing large coupling with three protons (9α, 7, and 8α) and one small coupling (9β) which would be characteristic of 8β in contrast to 8α. Additional evidence for the assignment of 8β relative to 8α was derived from the presence of a very strong cross peak in the 2-D NOESY spectra between 8α and H-5 (which were separated by 1.87Å using molecular modeling calculations). The equitorial proton 9β was assigned to the signal at 1.63 ppm that appeared as a doublet of quartets which would be expected for the large geminal coupling with one proton ($J_{9\alpha,9\beta}$ = 13.1 Hz) and coupling with three vicinal protons (J = 3.3 Hz). Proton 9β was also found to show a very strong cross peak with the C-14 methyl group in the NOESY spectra which would be consistent with the close proximity of the group.

TABLE 1

^{1}H-NMR Chemical S'.ifts and Coupling Constants Observed for Arteether in Deuterochloroform.

Chemical Shifts		Coupling Constants	
Proton	δ	Groups	J (Hz)
5	5.41	1, 2α	6.4
12	4.79	1, 2β	11.2
16a	3.86	2α, 2β	12.7
16b	3.47	2α, 3α	3.9
11	2.62	2α, 3β	3.0
3α	2.37	2β, 3α	13.5
3β	2.03	2β, 3β	4.4
2α	1.88	3α, 3β	14.6
8β	1.83	7, 8α	3.3
8α	1.74	7, 8β	14.4
9β	1.63	7, 11	4.7
2β	1.51	8α, 8β	13.7
15	1.43	8α, 9α	3.3
7	1.41	8α, 9β	3.3
10	1.33	8β, 9α	14.3
1	1.25	8β, 9β	3.3
17	1.18	9α, 9β	13.1
14	0.95	9β, 10	3.3
9α	0.91	10, 14	6.3
13	0.90	11, 12	3.4
		11, 13	7.4
		16a, 16b	9.9
		16a, 17	7.1
		16b, 17	7.0

Dihedral Angles and Bond Twisting.

The original Karplus equation[5] derived from the molecular orbital calculations of the vicinal proton coupling in ethane has been used frequently to qualitatively determine bond angles, but it has also been found that the magnitude of the coupling is also strongly influenced by the substitution of electronegative atoms on either carbon. Recently a semi-empirical approach to the estimation of $^3J_{HH}$ coupling was developed that takes into consideration the dihedral angle between to two protons, the electronegativity of each of the substituents, and the angle between the substituent and

the individual protons.[6,7] In this approach to the problem (Equation 1) the dependence of J on the dihedral angle between the two protons (θ) was similar to the original Karplus equation, but the equation had three experimentally derived coefficients (A = 8.37, B = -2.83, C = 7.44) and experimentally derived coefficients for each of the four possible substituents (ΔS_i; where $\Delta S_H = 0$ by definition). Using Equation 1 for a series of 46 compounds, it was found that the J-values could be estimated with a standard deviation of ± 0.76 Hz.[6]

$$J = A + B\cos\theta + C\cos 2\theta + \cos\theta\,[(\Delta S_1 + \Delta S_4)\cos(\theta - 120) + (\Delta S_2 + \Delta S_3)\cos(\theta + 120)] \quad (1)$$

For the present study of the solution conformation of arteether, Equation 1 was used to calculate values of θ (results shown in the second column of Table 2) from the experimentally observed $^3J_{HH}$ values. Using values of θ derived from molecular modeling calculations, Equation 1 was also used to estimate the $^3J_{HH}$ values (last column in Table 2).

With regard to the chair *vs.* boat conformation of the D-ring of β-arteether, the chair conformation would have placed the ethoxy group in a rather crowded axial position; while a boat conformation would have extended the ethoxy group away from the ring system, but that would have produced considerable steric crowding between the 13-methyl group and H_8. The estimated coupling constants shown in the last column of Table 2 were based on the D-ring with a chair conformation and it showed a generally good agreement with the experimental data. However the more critical distinction between the chair and boat D-ring conformations was noted for the coupling between H_7 and H_{11}. Using the calculated angles (for the chair, θ = +51°; for the boat, θ = -8°) the coupling constant were estimated using Equation 1 ($^3J_{7,11}$: chair = 3.1 Hz, boat = 7.9 Hz) and it was found that the experimental value (observed J = 4.7 Hz) indicated that the D-ring was in a conformation that would more closely resemble a slightly distorted chair conformation. This conformation has also been reported for artemether[8] and deoxyarteether[1] in the solid state as determined by x-ray.

The differences between the observed and calculated θ-values (Table 2) could perhaps have been attributed to deficiencies in Equation 1 (ie. errors in $J \rightarrow \theta$) or perhaps due to deficiencies in the molecular modeling estimates of θ. Given the precision that has been reported[6] for Equation 1, one could estimate that the observed θ-values (Table 2, first column) would be ± 5° of the true value. While most of the differences in the observed and calculated θ-values were within this error limit, the values for the protons attached to C_2-C_3 and to C_{11}-C_{12} were not in agreement. As will be discussed more fully below, the disagreement in these two cases were more likely due to deficiencies in the calculation of the θ-values in the molecular modeling rather than due to inaccuracies in θ observed by NMR coupling measurements.

Rather than analyzing the conformation of arteether relative to the individual θ-values in Table 2, it was found to be more convenient to examine these angles as an average of the individual

TABLE 2.
Dihedral Angles and $^3J_{HH}$ Coupling Constants Observed and Calculated for Arteether.

	Dihedral Angle (θ)		Coupling Constant	
Group	Observed[a]	Calculated[b]	Observed	Calculated[c]
1, 2α	40°	38°	6.4 Hz	6.7 Hz
1, 2β	143	158	11.2	13.9
2α, 3α	−52	−74	3.9	1.1
2α, 3β	57	46	3.0	5.0
2β, 3α	161	166	13.5	14.2
2β, 3β	−49	−74	4.4	1.1
7, 8α	−177	178	14.4	15.0
7, 8β	60	58	3.3	3.5
7, 11	40	51	4.7	3.1
8α, 9α	−56	−61	3.3	2.5
8α, 9β	44	59	3.3	0.9
8β, 9α	171	179	14.3	14.9
8β, 9β	−67	−61	3.3	4.3
9β, 10	60	62	3.3	3.0
11, 12	-40	-61	3.4	1.4

[a]The "observed" θ was obtained from the observed coupling constant through Equation 1.
[b]The calculated θ was obtained from the molecular modeling *de novo* constructs of arteether.
[c]The calculated θ which was obtained from the molecular modeling *de novo* constructs of arteether was transposed to J-values using Equation 1.

dihedral angles expressed as a twist of the bond from the 60°, 120°, 180° that would have been expected from a simple model. These bond distortions measured by NMR (Table 3) showed that the A-ring (*ie.* the C_7-C_8 twist and the C_8-C_9 twist) was in an undistorted chair conformation and that the results obtained by NMR were essentially in agreement with those obtained by molecular modeling calculation as well as those obtained for artemether in the solid state. The +3° twist observed for C_2-C_3 was identical to the twist measured by x-ray, but it was significantly different that the -14° twist estimated by molecular modeling. Thus it appeared that the individual θ's obtained by NMR for the C_2-C_3 protons (listed in Table 2) were probably more reliable that the θ's obtained through molecular modeling.

TABLE 3.
Extent of Bond Twisting from the Typical 60°, 120°, and 180° Dihedral Angles

Bond	Measured by NMR[a]	Calculated, Modeling	X-ray, Artemether[b]
C_1-C_2	-28°	-22°	-16°
C_2-C_3	+3°	-14°	+3°
C_7-C_8	+4°	+2°	+0.2°
C_8-C_9	-3°	-0.8°	-0.2°
C_{11}-C_{12}	+20°	-0.8°	+5°

[a]Using θ obtained by NMR (presented in Table 2), the displacement of θ-value from the typical staggered conformation was determine, then the average of the twist angles for all of the bonded protons was calculated.
[b]Angles determined from a molecular modeling reconstruction of the previously published[8] x-ray crystallographic coordinates of artemether.

The positioning of the ethoxy group was of particular interest and it was examined in some detail. Given the easily observed coupling for H_{12} ($^3J_{11,12}$ = 3.4 Hz), the dihedral angle was determined to be -40° using Equation 1 which is also plotted in Figure 2 for $^3J_{11,12}$ using the appropriate electronegativity coefficients (ΔS_i) for the groups attached to the two carbons. Using molecular modeling, an estimate of θ for H_{11}-H_{12} of -61° was obtained which would correspond to J = 1.4 Hz (Fig. 2). Given the observed coupling constant of 3.4 Hz, it could be seen that θ was actually closer to -40° which would correspond to rotatating the ethoxy group 20° away from a typical axial orientation. Rotating the C_{11}-C_{12} bond by 20° would have reduced the strong steric interaction of the ethoxy group with both H_5 and $H_{8\beta}$. Substituting a methoxy group for the ethoxy group in arteether apparently reduces this steric interaction in that the methoxy group is rotated only 5° outward (x-ray data for artemether, Table 3).

NOESY Measurements of Proton Distances.

Estimates of the proton-proton distances in arteether were obtained from an analysis of the phase-sensitive 2-D nuclear Overhauser enhancement spectroscopy where the integral of the volume of each proton-proton cross peak was taken to be inversely related to the sixth power of the distance between the two protons.[9] Though such measurements can be complicated by the lack of a common nuclear correlation time (τ_c) or by spin diffusion effects, the results can be quite useful if used with caution.

The distances obtained through the NOESY measurements (Table 4) were in good agreement with the distances obtained through molecular modeling. On the average, a ±0.21 Å difference was found between the two types of measurements. In comparing the distance for arteether in solution to the corresponding value obtained for artemether in the solid state, on the average a ±0.18 Å difference was found between the two types of measurements.

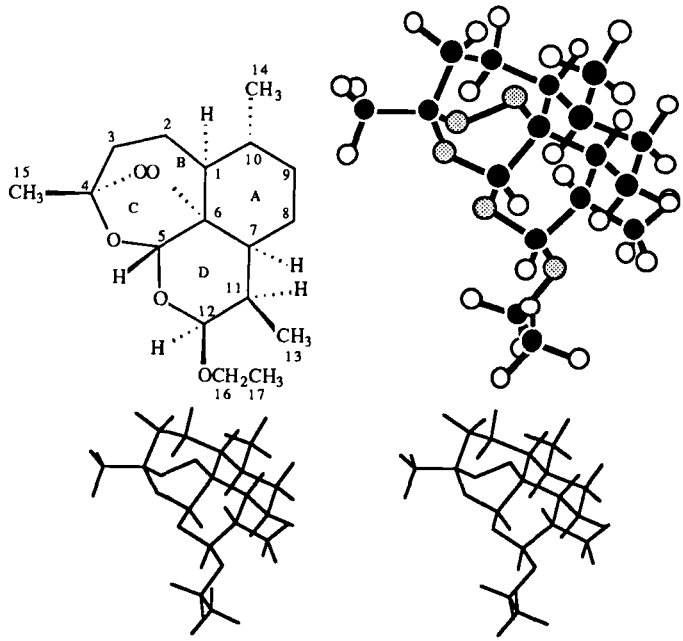

FIG. 1. Molecular Modeling Construct of Arteether after Energy Minimizations Using the Experimental (NOESY) Proton- Proton distances of H_5-H_{16a} and H_{12}-H_{16b} as Constraints During the Conformation Search.

The signal for H_5 proved to be particularly useful in that it was centrally located and could be used to measure distances to protons on the A-ring, the B-ring, and the ethoxy group. H_5 was equidistant from both of the axial protons on the A-ring (8β and 10) and slightly more distance from the "axial" proton on the B-ring (2β). H_5 was also fairly close (2.63 Å) to one of the methylene protons (16a) of the ethoxy group.

The distance obtained between the methyl groups (13 or 17) and the various protons shown in Table 4 were difficult to evaluate critically given the ambiguity of selecting specific protons on each of the methyl groups or using some type of weighted average of the possible distances. Because of the inherent errors associated using NOESY spectra to measure the distances for the methyl groups,[9] it could be anticipated that the observed distances might be shorter than the true distances. In a qualitative comparison of the distances obtained for the methyl groups by NOESY and by molecular modeling, indeed the NOESY distance gave shorter distance estimates. Thus the distances list for the 13- and 17-methyl groups in Table 4 should be used with caution and they probably underestimate the true distances.

TABLE 4.
Proton- Proton Distances Measure by Two-Dimensional Nuclear Overhauser Effect Spectroscopy

Groups	Observed, NMR	Modeling	X-ray, Artemether
2α, 3α	2.60 Å	2.59 Å	2.44 Å
2β, 5	2.52	3.01	2.99
5, 8β	2.26	1.87	2.25
5, 10	2.27	2.01	2.35
5, 16a	2.63	2.55	
7, 11	2.14	2.44	2.32
11, 12	2.30	2.51	2.47
12, 16a	2.58	2.66	
12, 16b	2.32	2.35	
5, 17	2.58		
11, 13	2.06		
12, 13	2.47		
16b, 17	2.04		
16a, 17	2.51		

TABLE 5
Minimum Energy Conformation of the Ethoxy Group of Arteether in Solution Determined using Molecular Modeling with the Experimental (NOESY) Proton- Proton distances of H_5-H_{16a} and H_{12}-H_{16b} as Constraints During the Conformation Search.

Group	Dihedral Angle
C_{11}-C_{12}-O-C_{16}	+170°
C_{12}-O-C_{16}-C_{17}	+169°

The NOESY distance data in conjunction with molecular modeling energy minimimizations was used to to determine the orientation of the ethoxy group of arteether. Using two NOESY determined distances (5,16a = 2.55 Å to 2.68 Å; 12, 16b = 2.27 Å to 2.37 Å) as search constraints, the steric and torsional energy of arteether was calculated for each conformation obtained by rotating each of the three bonds of the ethoxy group in 2° increments in order to determine the minimum energy for the molecule within the limits of the search constraints. The results of this search (Table 5, Fig. 1) indicated that the ethoxy group was essentially in a fully trans extended conformation with a slight twisting of the ethoxy group away from the ring system. The model for arteether shown in Figure 1 is the result of the minimum energy search with the two NOESY distance constraints.

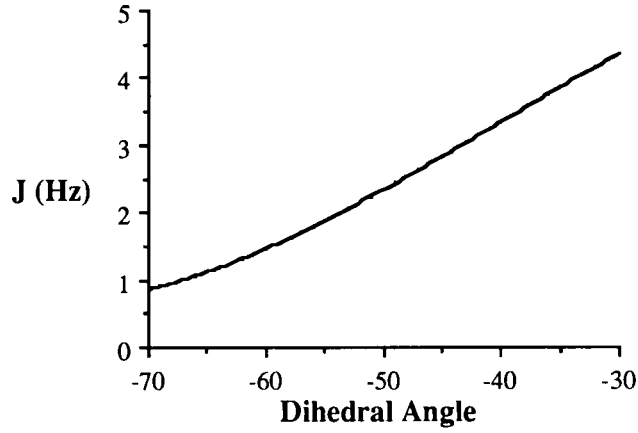

FIG. 2. $^{3}J_{H11,H12}$ Calculated from Equation 1 as a Function of θ.

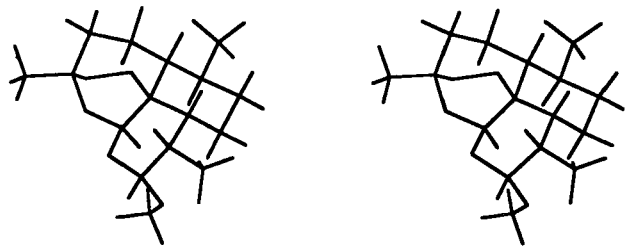

FIG 3. Molecular Modeling Reconstruction of the previously published[8] x-ray crystallographic coordinates of artemether.

In a second minimum conformational energy search, the two distance constraints were removed from the search process and the resulting structure was found to have dihedral angles for the ethoxy group that were within 2° of the search using the distance constraints. It appeared then that the placement of the ethoxy group as determined by the NOESY data was essentially the same as determined by the molecular modeling energy minimimizations.

As seen in the comparison in the conformation of arteether in solution with the conformation of artemether in the solid state (Tables 3, 4), there did not appear to be any large differences in the ring systems except in the areas of attachment of the ethoxy group. The x-ray structure of artemether

(Fig. 3) shows that the methoxy group in the solid state is twisted slightly more inward than the ethoxy group in solution. For arteether in solution C_{11}-C_{12}-O-C_{16} = +170°, while for artemether in the solid state C_{11}-C_{12}-O-C_{16} = -152° giving a 38° difference in the rotation of the alkoxy groups.

EXPERIMENTAL

β-arteether was synthesized from artemisinin using a previously reported procedure[1] and the product was found to be identical by spectral analysis to an authentic standard kindly supplied by Dr. Arnold Brossi of the National Institutes of Health. A 6.2 mg sample of the arteether was dissolved in slightly less than 1.0 mL of deuterochloroform then deoxygenated with a stream of helium gas until the volume of the sample reached 0.5 mL, the sample was then sealed and was immediately used to obtain 1-D proton spectra, 2-D COSY spectra, and 2D- NOESY spectra.

Spectra were obtained on a Varian VXR-300 spectrometer operating at 300 MHz using the standard pulse sequences supplied by the manufacturer. The 1-D spectra was obtained over a narrowed spectral width (2330 Hz) using 32 K data points, a 45° pulse angle, a 6.44 sec acquisition time, a 2.0 sec delay, and a total of 75 transients while the sample was maintained at 35°C. Proton-proton coupling constants were corrected for second order effects using computer software[10] supplied with the VXR-300 instrument in which the initial estimates of the chemical shifts and coupling constants are iteratively converged to a solution that was in agreement with the experimental data.

The NOESY spectra were obtained using a spectral width of 2330 Hz, a 0.22 sec acquisition time, a 3.0 sec relaxation delay, a 0.80 sec mixing time, a 90° pulse angle, 256 increments in the second frequency domain, using 32 repetitions at each increment, and the sample was not spun during the 34 hour (35°C) data acquisition. The phase sensitive NOESY pulse sequence was used which suppressed cross peaks due to J-coupling and the absorption peaks along the diagonal of the 2-D plot were negative (and thus do not appear on the contour plot) and the NOE peaks were phased as positive values. NOESY spectra were also obtained using mixing time both larger and smaller than 0.80 sec, but only the data from the 0.80 sec mixing time spectra which gave the stronger NOE peaks was used to calculate proton-proton distances.

The volume integrals of the NOE cross peaks were measured using the standard VXR-300 software. The volume integral of the cross peak between protons 3α and 3β used to calibrate the other proton distance measurements using 1.80 Å as the distance between 3α and 3β and the NOE effect was taken to be inversely related to the sixth power of the distance between the pairs of protons.[9]

Molecular modeling energy calculations and energy minimizations were conducted using Microchem V2.5- Organic modeling subunit computer software obtained from Intersoft, Inc., 4711 Golf Road, Skokie, IL, 60076 and the utility and limitations of this software has been recently reviewed[11]. The construct for arteether was *de novo* and it did not use the x-ray data for artemether

or any other specific compound as a starting point. When rotating bonds during the energy minimizations searches, the dispersive-repulsive forces were represented using a Lennard-Jones potential function and a torsional energy potential function for each bond was also included.

The bond angles, proton-proton distances, and the stereo projection figures for artemether were reconstructed from the x-ray crystallographic coordinate data previously published for artemether.[8] The Microchem V2.5 software was then used to transform this data into a 3-D structure to facilitate the angle and distance measurements.

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