NMR and conformational studies of the antimalarial drug arte-ether

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The ^{1}H and ^{13}C NMR spectra of the antimalarial drug arte-ether (a derivative of qinghaosu) have been obtained and stereospecific assignments made using standard one- and two-dimensional techniques. Proton homonuclear spin-coupling constants were quantified from *J*-resolved and E.COSY experiments and confirmed that the solution conformation of arte-ether is similar to that of the reported crystal structure of artmether. A conformational model was generated using AM1 semi-empirical energy calculations, and was consistent with both the measured spin-coupling constants and experimental NOE data.

Introduction

Qinghaosu (**1**) is a natural product used in the treatment of malaria.[1] It is isolated from the plant *Artemisia annua* L. which grows widely in Southern China. An aqueous decoction from this herb has been used in traditional Chinese medicine for the treatment of fevers for many hundreds of years. When it became apparent that some modern antimalarial drugs, such as chloroquine, were no longer universally effective, the active principle was isolated from *Artemisia annua* and found to be highly effective in killing malarial parasites, particularly *Plasmodium falciparum*. The structure was deduced using regular spectroscopic techniques and confirmed by X-ray crystallographic studies.[2] The correct naming of this compound, and therefore the correct numbering, is a matter of some complexity. In a previous publication[3] we favoured 3,6,9-trimethyl-9,10b-epidioxyperhydropyrano[4,3,2-*jk*]benzoxepin-2-one and this conforms to IUPAC conventions. It gives the numbering system indicated in Fig. 1. Several systems have been used in other publications and, in most cases, they are incorrect. However, the numbering used by Blaskó *et al.*[4] also conforms to IUPAC conventions. In this paper we will use the numbering of ref. 3 and make the necessary conversion when referring to other work. The name used by *Chemical Abstracts* is artemisinin, derived from the name of the plant, but the drug is more generally referred to by the Chinese name of qinghaosu.

Qinghaosu and its derivatives appear to act by generation of carbon-centred radicals, formed by interaction between iron(II) ions and the peroxide bridge, which damage the DNA of *Plasmodium*. The nature of the alkyl group at the rather remote 2-position in the ether derivatives of qinghaosu has been shown[5] to influence the nature of the radical formed. In order to understand this effect we are studying the influence of chemical modification of the qinghaosu nucleus upon radical formation. Knowledge of the stereochemistry of the molecule in solution should help us to understand the influence of structure upon the radicals obtained.

An interesting feature of the chemistry of qinghaosu is reduction using sodium borohydride. This leaves the peroxide bridge intact but converts the keto group at position 2 into a lactol.[6] From this a number of derivatives have been prepared, including the methyl and ethyl ethers. Strictly they are acetals rather than ethers, but the names artemether and arte-ether are widely used. Both of these also show antimalarial activity.[7] Our interest has been to contribute to an understanding of the mode of action of qinghaosu and its derivatives as antiparasitic agents, particularly in the role of iron.[8,9] Whereas most previous studies have used mainly qinghaosu we chose to use arte-ether (**2**). For subsequent mechanistic studies we propose to modify the structure of **2** and also to characterise the conformation of products obtained from reactions involving **2**. To this end we needed an unambiguous NMR and conformational characterisation of **2** and this we now report. The numbering system for the arte-ether shown in Fig. 2 will be used throughout this paper.

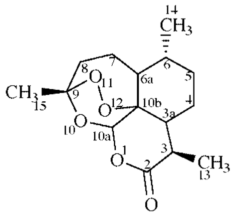

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Fig. 1 Structure and atom numbering of qinghaosu.

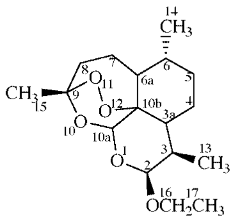

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Fig. 2 Structure and atom numbering of arte-ether.

Qinghaosu itself has been the subject of several NMR spectroscopic studies. Three early studies[10–12] produced conflicting assignments of the ^{13}C NMR spectrum, made more confusing by different numbering. A more recent study[13] used selective heteronuclear decoupling experiments for assigning the carbon signals but the proton data were reported without the stereospecific assignments of geminal protons. A substantial study by

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Table 1 Proton and carbon resonance assignments (in ppm for internal TMS at 0.00 ppm) of arte-ether (**2**) in DMSO-d_6 solution at 30 °C

	^{1}H Chemical shift (ppm)		^{13}C Chemical shift (ppm)	
Position [a]	DMSO-d_6	$CDCl_3$ [b]	DMSO-d_6	$CDCl_3$ [b]
2	4.69	4.80	100.4	101.6
3	2.40	2.61	30.3	30.8
3a	1.37	1.43	43.7	44.5
4	1.75 (*pro-R*), 1.64 (*pro-S*)	1.76, 1.84	23.9	24.6
5	0.87 (*pro-R*), 1.51 (*pro-S*)	0.90, 1.62	34.0	34.2
6	1.33	1.33	36.6	37.7
6a	1.14	1.24	52.0	52.6
7	1.38 (*pro-R*), 1.81 (*pro-S*)	1.52, 1.88	24.1	24.6
8	2.19 (*pro-R*), 2.00 (*pro-S*)	2.02, 2.38	36.0	36.4
9	—	—	103.8	103.9
10a	5.32	5.40	86.8	87.8
10b	—	—	81.1	81.1
13	0.85	0.90	12.7	12.8
14	0.90	0.95	20.0	20.2
15	1.29	1.44	25.5	26.1
16	3.72, 3.39	3.87, 3.46	62.7	63.6
17	1.13	1.18	15.0	15.1

[a] For each of the prochiral centres the *pro-R* hydrogen is axial and the *pro-S* hydrogen is equatorial to the ring. [b] As reported by Prakash *et al.*,[14] with their assignment of C-6 and C-6a reversed.[15]

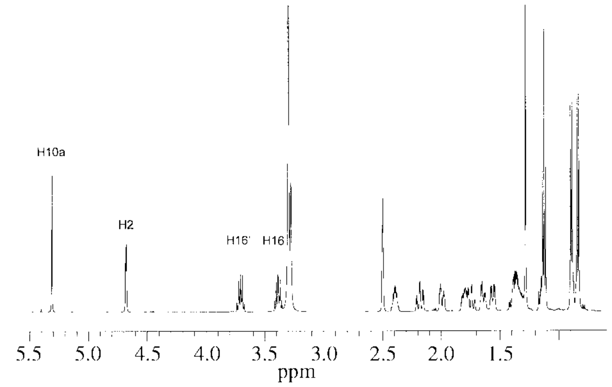

Fig. 3 ^{1}H NMR spectrum of arte-ether in DMSO-d_6 solution.

Blaskó *et al.*[4] used ^{1}H–^{1}H COSY and NOESY experiments to assign the proton resonances. We wanted to examine changes in the NMR spectra of qinghaosu on conversion to arte-ether and to assign the stereochemistry of the ethoxy group. We will compare our assignments with those from the one previously reported NMR study of arte-ether.[14]

Results and discussion

Initially, arte-ether in deuterochloroform solution was analysed, giving identical chemical shift assignments for all proton resonances to those previously reported.[14] The ^{13}C resonance assignments were also in agreement with the corrected assignment.[15] In NOE experiments, the chloroform solution produced excessive artefacts from zero-quantum coherence, and despite the use of pulse sequences modified for zero-quantum filtration the anti-phase components of the cross-peaks could not be eliminated. So the sample in DMSO-d_6 solution was analysed.

The ^{1}H NMR spectrum of arte-ether in DMSO-d_6 (Fig. 3) showed, as expected, several multiplets of intensity 1H, some of which were sufficiently resolved to permit assignment. However, in the region between 1.44 and 1.60 ppm several of the multiplets corresponding to single protons could be resolved only by complementary use of two-dimensional COSY, and ^{1}H–^{13}C correlation through one-bond (HSQC) and multiple bonds (HMBC).

The only pseudo-triplet (double doublet) in the proton spectrum is at 1.13 ppm, and this was assigned to the protons attached to C-17. In the COSY spectrum these protons correlate to the proton resonances at 3.39 and 3.72 ppm, both of which are quartets of doublets, indicating that the protons of the methylene group are non-equivalent and that each quartet is further split by *J*-coupling with the geminal proton.

The proton spectrum was assigned in a straightforward manner, by tracing the coupling path in the COSY spectrum sequentially from the C-2 proton to the non-equivalent methylene protons attached to C-8. The starting point for this assignment was the doublet (integrating to 1H) at 4.69 ppm, which was the characteristic signal from the C-2 proton. This signal is absent in the proton spectrum of 2-deuterioarte-ether. Stereospecific assignments for the prochiral protons of the four methylene groups were obtained with reference to the magnitude of spin-coupling constants, in the expectation that a diaxial orientation of vicinal protons is characterised by a large (≥10 Hz) coupling constant. For the constrained seven-membered ring system only one geometry could give rise to the observed pattern of spin-coupling constants, and from this it was possible to obtain unambiguous stereospecific assignments, as only one proton at C-7 and one at C-8 are expected to exhibit a large vicinal coupling constant. The six-membered ring that incorporates C-6 can potentially adopt low energy conformations corresponding to either $^{3a}C_6$ chair or two boat forms ($^{3a,6}B$ and $B_{5,10b}$). In the boat conformations H-6 is synclinal to both C-5 protons, thus a significant population of boat conformations can be excluded by the observation of a $^3J_{\mathrm{H5}proR\text{-}\mathrm{H6}}$ value of 11.9 Hz. This observation also confirms the stereospecific assignments of H-5$_{proR}$ and (from $^3J_{\mathrm{H5}proR\text{-}\mathrm{H4}proR} \approx 13.0$ Hz) H-4$_{proR}$.

The ^{13}C resonance assignment was obtained in a straightforward manner, primarily from an HSQC spectrum, correlating each carbon signal with the assigned resonance frequencies of the directly-bonded protons. Two- and three-bond ^{1}H–^{13}C correlations from an HMBC spectrum provided unambiguous assignments of the quaternary carbon resonances (C-9 and C10b), and was consistent with the assignments presented in Table 1. The ^{13}C resonances in DMSO-d_6 exhibited only minor shift differences from those recorded in deuteriochloroform,[15] from which we infer that there is no significant difference in conformational preference in these two solvents.

^{1}H–^{1}H spin-coupling constants were quantified from a *J*-resolved spectrum, and the assignments were verified from the pattern of in-phase and anti-phase peak splittings in a high resolution (0.3 Hz per point) E.COSY experiment. By filtering

Table 2 Two- and three-bond ^{1}H–^{1}H *J*-coupling constants[a] and approxiamate bond dihedral angles (θ) for arte-ether (**2**) in DMSO-d_6 solution at 30 °C, and in the AM1-optimised model

	Experiment		Back-calculated from AM1 model	
Assignment	*J*/Hz	θ (°)[c]	*J*/Hz	θ (°)
H2–H3	3.6	48	3.4	50
H3–H3a	5.4[b]	−45	4.3	−52
H3–H13 (methyl)	7.3	—	—	—
H3a–H4$_{proR}$	11.5	−163	12.0	−169
H3a–H4$_{proS}$	4.7	−50	4.1	−54
H4$_{proR}$–H4$_{proS}$	−14.1	—	—	—
H4$_{proR}$–H5$_{proR}$	~13.0[b]	180	11.8	161
H4$_{proR}$–H5$_{proS}$	3.6[b]	58	5.4	45
H4$_{proS}$–H5$_{proR}$	3.7[b]	57	5.6	44
H4$_{proS}$–H5$_{proS}$	3.4[b]	−60	2.2	−72
H5$_{proR}$–H5$_{proS}$	−13.2[b]	—	—	—
H5$_{proR}$–H6	11.9[b]	−167	11.8	−166
H5$_{proS}$–H6	3.5	−58	4.8	−50
H6–H6a	10.7	158	12.1	176
H6–H14 (methyl)	6.5	—	—	—
H6a–H7$_{proR}$	12.0	−169	9.9	−151
H6a–H7$_{proS}$	6.5	−40	7.3	−35
H7$_{proR}$–H7$_{proS}$	−13.8	—	—	—
H7$_{proR}$–H8$_{proR}$	13.4	180	12.2	165
H7$_{proR}$–H8$_{proS}$	5.1	47	4.7	50
H7$_{proS}$–H8$_{proR}$	2.8	65	4.8	49
H7$_{proS}$–H8$_{proS}$	4.1	−54	2.8	−66
H8$_{proR}$–H8$_{proS}$	−13.4	—	—	—
H16–H16′	−10.0	—	—	—
H16–H17 (methyl)	7.1	—	—	—
H16′–H17 (methyl)	7.0	—	—	—

[a] Measured from a homonuclear *J*-resolved spectrum with 0.1 Hz per point digital resolution, unless indicated otherwise. [b] Measured from an E.COSY spectrum with 0.3 Hz per point digital resolution. [c] Only one of the four possible dihedral angles between 0 and 360° is given.

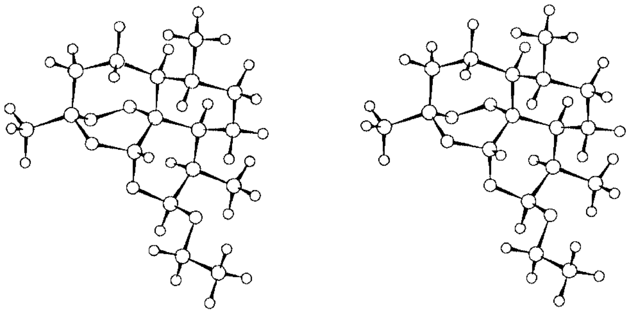

Fig. 4 Stereoview of the AM1-optimised structure of (2*S*)-arte-ether.

Table 3 ^{1}H–^{1}H relative NOE and internuclear distances for arte-ether (**2**) from a NOESY experiment with 400 ms mixing time

			Distance/Å	
From	To	Relative NOE	Experiment	AM1 Model
H-2	H-3	1.04	2.3	2.43
H-2	H-3a	0.04	4.1	4.31
H-2	H-10a	0.08	3.6	3.81
H-2	H-13[b]	0.55	2.6	2.63
H-2	H-16	0.28	2.9	3.00
H-2	H-16′	0.76	2.5	2.21
H-2	H-17[b]	0.14	3.3	4.04
H-3	H-3a	1.00[a]	—	2.36
H-3	H-10a	0.05	3.9	4.06
H-3	H-13[b]	1.25	2.3	2.11
H-4$_{proR}$	H-4$_{proS}$	2.35	2.1	1.80
H-4$_{proR}$	H-5$_{proS}$	0.97	2.4	2.39
H-4$_{proR}$	H-6	1.31	2.3	2.70
H-4$_{proR}$	H-6a	0.17	3.2	4.07
H-4$_{proR}$	H-10a	0.72	2.5	2.62
H-4$_{proR}$	H-13[b]	0.33	2.8	2.68
H-4$_{proS}$	H-3a	0.79	2.5	2.40
H-4$_{proS}$	H-5$_{proR}$	0.37	2.8	2.40
H-4$_{proS}$	H-10a	0.04	4.0	4.17
H-4$_{proS}$	H-13[b]	1.22	2.3	2.29
H-5$_{proR}$	H-5$_{proS}$	5.22	1.8	1.81
H-5$_{proS}$	H-6	0.70	2.5	2.41
H-5$_{proR}$	H10-a	0.07	3.7	4.47
H-5$_{proR}$	H-6a	1.44	2.2	2.69
H-6 + H-7$_{proR}$	H-6a	0.49	2.7	3.09, 3.05
H-6 + H-7$_{proR}$	H-10a	1.31	2.3	2.12, 3.00
H-6a	H-7$_{proS}$	0.96	2.4	2.31
H-6a	H-8$_{proR}$	0.39	2.8	3.05
H-6a	H-8$_{proS}$	0.09	3.5	4.04
H-6a	H-10a	0.18	3.1	3.89
H-7$_{proR}$	H-7$_{proS}$	5.06	1.8	1.81
H-7$_{proR}$	H-8$_{proR}$	0.04	4.0	3.07
H-7$_{proR}$	H-8$_{proS}$	0.79	2.5	2.35
H-7$_{proS}$	H-8$_{proS}$	0.49	2.7	2.54
H-7$_{proS}$	H-14[b]	1.05	2.3	2.36
H-7$_{proS}$	H-8$_{proR}$	0.60	2.6	2.42
H-8$_{proR}$	H-8$_{proS}$	3.97	1.9	1.82
H-8$_{proR}$	H-15[b]	0.32	2.9	2.75
H-8$_{proS}$	H-15[b]	0.87	2.4	2.41
H-10a	H-16	0.22	3.0	2.97
H-10a	H-16′	0.06	3.8	4.25

[a] Reference NOE. [b] For methyl groups the shortest of the three H–H distances is reported.

half of the lines in the multiplets of the ^{1}H–^{1}H shift-correlation cross-peaks E.COSY provides exceptional clarity for the assignment of spin-coupling constants, including those for strongly-coupled multiplets. The two- and three-bond spin-coupling constants given in Table 2 are largely consistent with the previous partial assignments,[16] and additionally there is a small (2.0 Hz) four-bond *J*-coupling constant resolved for $^{4}J_{\text{H2-H3a}}$. The $^{3}J_{\text{H2-H3}}$ value of 3.5 Hz confirmed the structure as having exclusively the 2*S* stereochemistry[14] ("arte-ether-β"). Using a form of the generalised Karplus approximation with a compensation for the differing electronegativities of substituents on the coupling path,[17] dihedral angles (θ) were estimated from each of the three-bond spin-coupling constants ($^{3}J_{\text{H-H}}$), and these are also given in Table 2. A model of arte-ether (Fig. 4) was constructed, with the geometry optimised using AM1 semi-empirical energy calculations, implemented within the MOPAC software package. Bond torsion angles were measured from the AM1-optimised structure, for comparison with the experimentally-derived values. Each of the AM1-derived torsion angles show at least satisfactory agreement with the experimentally approximated values; the largest discrepancy was for torsion angle H-6–C-6–C-6a–H-6a ($\Delta\theta = 18°$), and this is very satisfactory agreement within the limitations of a Karplus approximation.

To further test the validity of the AM1 structure, NOEs were measured from a NOESY experiment with 400 ms mixing time, in order to derive ^{1}H–^{1}H internuclear distance estimates from an experimental source. Positive NOEs were measured from NOESY cross-peak volumes (taking the mean value from two symmetry-related cross-peaks), and the intensities are expressed in Table 3, relative to the intensity of the H3–H3a NOE. Estimates of internuclear distances were taken from the relative NOEs using the isolated spin-pair approximation, which neglects any possible influence of anisotropic reorientation, internal flexibility or indirect NOEs. The H-3–H-3a distance (2.36 Å) obtained from the AM1 model was used as the reference distance for the NOE ratio calculations.

Comparisons of the experimental and AM1-derived H–H distances in Table 3 show generally satisfactory agreement. Of 41 NOEs quantified from the NOESY experiment none indicated that the model has a significant discrepancy from experimental data. For all medium and strong NOEs (with an experimentally estimated distance less than 3.2 Å) the NOE derived distance agreed with the AM1-derived distance to within 0.5 Å. Some slightly larger discrepancies for the longer distances were to be expected, due to the difficulty in accurately quantifying weak NOEs. The solution conformation of arte-ether has, therefore, been shown to correspond to the crystal structure previously reported for artmether.[16]

Conclusion

We conclude from this study that the solution state NMR parameters show an excellent correlation with the lowest energy structure found for arte-ether-β. The conformation in DMSO-d_6 solution is identical to that in deuteriochloroform.

Experimental

NMR spectra were acquired with a Varian Unity+ spectrometer operating at 500.2 MHz (^{1}H), with a nominal probe temperature of 30 °C. Standard pulse programs were used for all experiments except E.COSY, which was programmed in-house according to the published sequence and phase cycle.[18] The one-dimensional proton spectrum was acquired with a recycle delay of 20 seconds, to allow complete relaxation between individual transients for the accurate quantification from peak integrals. The *J*-resolved spectrum was acquired with a spectral width of 60 Hz in f_1 and was transformed to give a final digital resolution of 0.1 Hz per point.

Sodium borodeuteride (1 g; 23 mmol) was added slowly to a solution of qinghaosu (0.8 g; 3 mmol) in dry methanol (45 ml) with stirring in an ice bath. After stirring for 1 h the pH of the mixture was neutralised by addition of acetic acid with cooling. After removal of the solvent by evaporation, water was added to the resulting slurry with stirring. The precipitate of 2-deuteriodihydroqinghaosu was filtered, dried and recrystallised from dichloromethane: 77% yield, mp 148–151 °C (lit.[6] 149–153 °C). This material was converted into 2-deuterioarte-ether by the method of Brossi *et al.*:[6] 0.27 g (36%), mp 77–79 °C (lit.[6] 80–82 °C).

Semi-empirical energy calculations were performed using the AM1 hamiltonian implemented within the MOPAC software package, running on a Silicon Graphics O2 computer with an R5000 processor. Structures were constructed and analysed using the Insight molecular modelling software (MSI, San Diego). IR frequencies calculated from the second derivative of the potential energy surface for the final structure were all positive, indicating that the structure adopted is in a true energy minimum.

Acknowledgements

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References

1 D. L. Klayman, *Science*, 1985, **228**, 1049.
2 Qinghao Antimalarial Coordinating Research Group, *Chin. Med.*, 1979, **92**, 811.
3 A. R. Butler and Y.-L. Wu, *Chem. Soc. Rev.*, 1992, 85.
4 G. Blaskó, G. A. Cordell and D. C. Lankin, *J. Nat. Prod.*, 1988, **51**, 1273.
5 A. R. Butler, L. Conforti, B. C. Gilbert and A. C. Whitwood, unpublished data.
6 A. Brossi, B. Venugolplan, L. Dominguez Gerpe, H. J. C. Yeh, J. L. Flippen-Anderson, P. Bucks, X. D. Luo, W. Milhous and W. Peters, *J. Med. Chem.*, 1988, **31**, 645.
7 M. Jung, X. Li, D. A. Bustros, H. N. El-Sohly, J. D. McChesney and W. K. Milhous, *J. Med. Chem.*, 1990, **33**, 1516.
8 W. M. Wu, Z. L. Yao, Y. L. Wu, K. Jiang, Y. F. Wang, H. B. Chen, F. Shan and Y. Li, *Chem. Commun.*, 1996, 2213.
9 A. R. Butler, B. C. Gilbert, P. Hulme, L. R. Irvine, L. Renton and A. C. Whitwood, *Free Radical Res.*, 1998, **28**, 471.
10 N. Acton and D. L. Klayman, *Planta Med.*, 1985, 441.
11 F. S. El-Feraly, M. M. El-Sherei, C. D. Hufford, E. M. Croom and T. J. Mashier, *Spectrosc. Lett.*, 1985, **18**, 843.
12 W. Zhongshan, T. T. Nakashima, K. R. Kopecky and J. Molina, *Can. J. Chem.*, 1985, **63**, 3070.
13 J.-J. Huang, K. M. Nicholls, C.-H. Chang and Y. Wang, *Acta Chem. Sin.*, 1987, **45**, 305.
14 O. Prakash, R. Roy, R. S. Kapil and R. A. Vishwakarma, *Ind. J. Chem.*, 1990, **29B**, 103.
15 L. N. Misra, A. Ahmad, R. S. Thakur, H. Lotter and H. Wagner, *J. Nat. Prod.*, 1993, **56**, 215.
16 X.-d. Luo, H. J. C. Yeh, A. Brossi, J. L. Flippen-Anderson and R. Gilardi, *Helv. Chim. Acta*, 1984, **67**, 1515.
17 C. A. G. Haasnoot, F. A. A. M. de-Leeuw and C. Altona, *Bull. Soc. Chim. Belg.*, 1980, **89**, 125.
18 C. Griesinger, O. W. Sørensen and R. R. Ernst, *J. Am. Chem. Soc.*, 1985, **107**, 6394.

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