NMR Study of the Conformations Adopted by 1,2,3-Trimethoxybenzene when Dissolved in a Nematic Liquid Crystalline Solvent

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The proton, proton–{deuterium} and deuterium NMR spectra of partially deuteriated samples of 1,2,3-trimethoxybenzene dissolved in a nematic liquid crystalline solvent, I32, have been recorded and analysed to yield a set of dipolar couplings which have been used to test models for the conformational distribution. It is shown that the molecule must exist in more than one conformation. A conformational distribution has been obtained which is in agreement with the data, and which is based on the assumption that each methoxy group can occupy positions either orthogonal to, or coplanar with, the phenyl ring plane.

A single methoxy group when substituted into a benzene ring can rotate about the ring–oxygen bond subject to a rotational potential, $V(\theta)$, which has an absolute minimum when the heavy atoms are coplanar. This is true for gas, solid, and for both isotropic and liquid crystalline phases.[1] It has also been established that there is some fourfold character to the potential in both gas and liquid phases, so that there is a shallow, second minimum in $V(\theta)$ at $\theta = 90°$, corresponding to the C–O–C and ring planes being mutually orthogonal.[2,3]

Polymethoxylated benzene derivatives are of considerable importance because of their therapeutic properties, and in these compounds there are a large number of possible conformations which can be populated in the gas or liquid phases, even with the assumption that each C–O–C and the ring plane can only be either coplanar or orthogonal [which we shall refer to as an example of the rotational isomeric state (RIS) model]. We have shown recently that a relatively new method of determining the shapes adopted by flexible molecules in a liquid crystalline phase can provide what is essentially a liquid state conformational distribution for these kinds of molecule.[4] The method relies on the sensitivity to molecular shape of the dipolar coupling constants, D_{ij}, which can be obtained by analysing the NMR spectra of samples dissolved in a liquid crystalline solvent. We have shown, for example, that such data supports the cooperative nature of the rotations about the ring–oxygen bond (through an angle θ) and about the O–C–H_3 bond (through an angle φ) in anisole, in agreement with *ab initio* molecular orbital calculations.[3] We have applied this liquid crystal NMR method (LCNMR) to study the severely hindered motion in 1,2-dimethoxybenzene, and again the results are in good agreement with other attempts at characterizing the conformational distribution in this molecule.[4]

It would appear therefore that this LCNMR method can be used to determine the conformational distributions of methoxylated benzenes. There is, however, one serious drawback to the widespread application of this method, which stems from the extremely complex spectra that occur when the number of interacting nuclei increases. This problem can be overcome by such spectral simplifying methods as multiple quantum excitation, deuterium decoupling, spin-echo refocussing, or combinations of these techniques. To illustrate what can be achieved we present here the results of a study of 1,2,3-trimethoxybenzene (TMB). The 20 symmetry-related sets of conformations which this molecule can adopt, based on the RIS model, are shown in Fig. 1. In the solid state, the structure is entirely the conformation **1**, and this is also the case for this molecular fragment in the important anti-folate agent trimethoprim.‡ In rationalizing the action of trimethoprim in solution it is often assumed that **1** is the only conformation adopted, however, this is unlikely to be the case. Thus, for TMB the dipole moment obtained for a solution in benzene is not in accord with **1** as the only form,[5] and calculations by the MM2 molecular mechanics method found that **1** is only slightly lower in free energy than **2**, so that these two conformers should be almost equally populated in solution.[6]

Experimental

We have not succeeded in obtaining a well-resolved, analysable proton spectrum of TMB as a solute in a liquid crystalline solvent. We have, however, obtained all the inter-proton dipolar couplings except those between the methyl protons, by recording and analysing the proton, proton–{deuterium} (*i.e.* deuterium decoupling from protons) and deuterium spectra of partially deuteriated TMB molecules. The solvent used was I32 (Merck), which was chosen because samples containing approximately 20% by weight of TMB are nematic at ambient temperature (295 K), are strongly oriented, and gave well-resolved NMR spectra.

The partially deuteriated molecules were each synthesized using the method first introduced by Williamson.[7] Thus, [$^2H_9$]-1,2,3-trimethoxybenzene (**A**) was obtained by the reaction between 1,2,3-trihydroxybenzene with CD_3I and the same reaction with 1,3-dihydroxy-2-methoxybenzene gave 1,3-di-[$^2H_3$]methoxy-2-methoxybenzene (**B**) (all the starting materials were obtained from Aldrich). The third compound synthesized was 2,3-di[$^2H_3$]methoxy-1-methoxybenzene (**C**), from 1,2-dihydroxy-3-methoxybenzene (purchased from Apin).

The proton, proton–{deuterium} and deuterium spectra were recorded at the same temperature on a Bruker MSL 200 spectrometer using samples of approximately 10 mm length in 4 mm i.d. and 5 mm o.d. tubes in a double tuned solenoid coil.

Analysis of Spectra.—The conformational analysis used the set of dipolar couplings, $D_{ij}^{HH}(A)$, given in Table 1, which refer to compound **A** dissolved in I32 at a constant temperature of

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‡ 5-(3,4,5-Trimethoxybenzyl)pyrimidine-2,4-diamine.

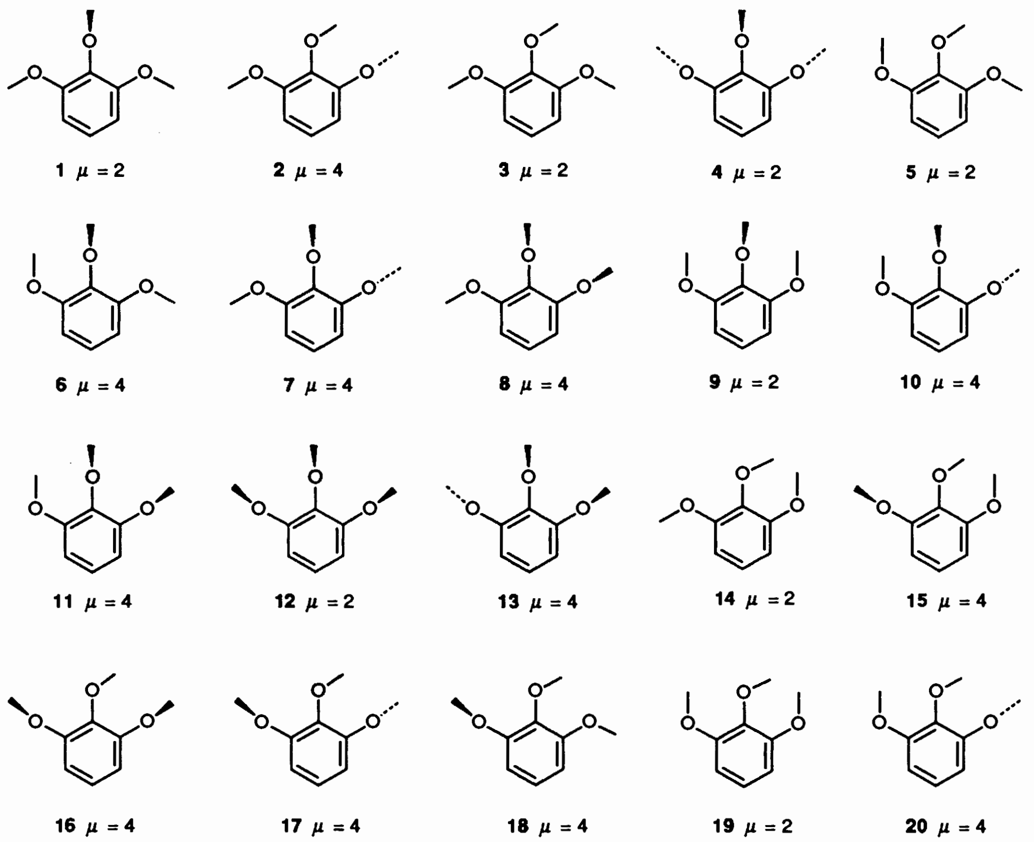


Fig. 1 Conformations possible for 1,2,3-trimethoxybenzene with the assumption that the C–O–C planes are either orthogonal to or coplanar with the phenyl ring; μ is the conformer degeneracy

Table 1 Dipolar couplings, D_{ij}, chemical shifts, δ_{ij}, and quadrupolar splittings, $\Delta\nu_i$, obtained from the analysis of the proton and deuterium spectra of a sample of [$^2H_9$]1,2,3-trimethoxybenzene dissolved in the nematic solvent I32; the complete set of inter-proton dipolar couplings, D_{ij}^{HH}(A), are given (derived as discussed in the text)[a]

i,j	D_{ij}/Hz[b]	D_{ij}^{HH}(A)/Hz[b]	J_{ij}(Hz)[c]
4,5	-1110.9 ± 0.1	-1110.9 ± 0.1	8.0
4,6	-280.8 ± 0.3	-280.8 ± 0.3	2.0
1,4	-10.7 ± 0.2	-69.7 ± 1.3	0.0
1,5	-15.2 ± 0.06	-99.0 ± 0.4	0.0
1,6	-66.0 ± 0.2	-429.9 ± 1.3	0.0
1,1		1158.0 ± 0.2	0.0
2,4		-12.4 ± 0.3	0.0
2,5		2.0 ± 0.9	0.0
2,2		-950.0 ± 0.4	0.0

[a] Chemical shift: $\delta_{4,5} = -47.1 \pm 0.2$ Hz. Quadrupolar splittings/Hz $|\Delta\nu_1| = 8397 \pm 50$, $|\Delta\nu_2| = 7223 \pm 50$. [b] The errors quoted are the standard deviations obtained from the iterative analysis and do not include the systematic errors in measuring the line positions, which stem mainly from the digitisation precision of ± 0.5 Hz. [c] Taken from the spectrum in the isotropic phase, and kept constant.

295 K. To obtain all the magnitudes and signs of these dipolar couplings it was necessary to combine data obtained from the spectra of all three compounds, **A**, **B** and **C**, and using a variety of experiments.

The proton–{deuterium} spectrum of **A** is shown in Fig. 2, and its analysis gave D_{45}^{HH}(**A**) and D_{46}^{HH}(**A**), and the former was assumed to have a negative sign. The labelling of the nuclei is given in Fig. 3. The orientational ordering of TMB in I32 is such that the absolute signs of the D_{ij} cannot be established, but this is unimportant when testing conformational models. Knowing these two D_{ij}(**A**) values it proved a relatively easy task to analyse the proton spectrum of **A**, which is shown in Fig. 2, to give the magnitudes of D_{14}^{HD}(**A**), D_{34}^{HD}(**A**), D_{24}^{HD}(**A**), D_{15}^{HD}(**A**), but not D_{25}^{HD}(**A**), which was not resolved in the spectrum. The corresponding H–H couplings were obtained by multiplying these H–D couplings by γ_H/γ_D, where γ_H and γ_D are magnetogyric ratios for hydrogen and deuterium nuclei. Note that the relative signs of these couplings were not obtained from the analysis of the proton spectrum. The deuterium spectrum of **A**, shown in Fig. 4, yields the magnitudes of the two quadrupolar splittings, $\Delta\nu_1$(**A**) and $\Delta\nu_2$(**A**) given in Table 1.

The proton–{deuterium} spectrum of **B** is shown in Fig. 5, and its analysis gave the magnitudes and signs of D_{24}^{HH}(**B**), D_{25}^{HH}(**B**) and D_{22}^{HH}(**B**). The deuterium spectrum of **B** (not shown) gave the magnitude of $\Delta\nu_1$(**B**) for the temperature and concentration of sample **B**, which differed only slightly from those used for sample **A**. This meant that it was possible to obtain $\Delta\nu_2$(**B**) as $\Delta\nu_2(\mathbf{A}) \times \Delta\nu_1(\mathbf{B})/\Delta\nu_1(\mathbf{A})$, and hence to determine the ratio $\Delta\nu_2(\mathbf{B})/D_{22}^{HH}(\mathbf{B})$. This ratio was then used to obtain the magnitude and sign of D_{22}^{HH}(**A**) from $\Delta\nu_2$(**A**), which is the origin of the value given in Table 1.

Analysis of the proton–{deuterium} spectrum of **C**, which is shown in Fig. 6, gave the magnitudes and signs of D_{16}^{HH}(**C**), D_{15}^{HH}(**C**), D_{14}^{HH}(**C**) and D_{11}^{HH}(**C**). The deuterium spectrum of **C** (not shown) gave $\Delta\nu_3(\mathbf{C}) = \Delta\nu_1(\mathbf{C})$, and hence it was possible to obtain $\Delta\nu_1(\mathbf{C})/\mathbf{D}_{11}^{HH}(\mathbf{C})$. With this data obtained from the spectra of **C** it was then possible to obtain the signs of D_{34}^{HH}(**A**), D_{35}^{HH}(**A**), D_{14}^{HH}(**A**), and both the magnitude and sign of D_{11}^{HH}(**A**).

Results and Discussion

Is the Central Methoxy Group C–O–C Plane always Perpendicular to the Phenyl Ring Plane?—This question can be answered by comparing observed and calculated values for the

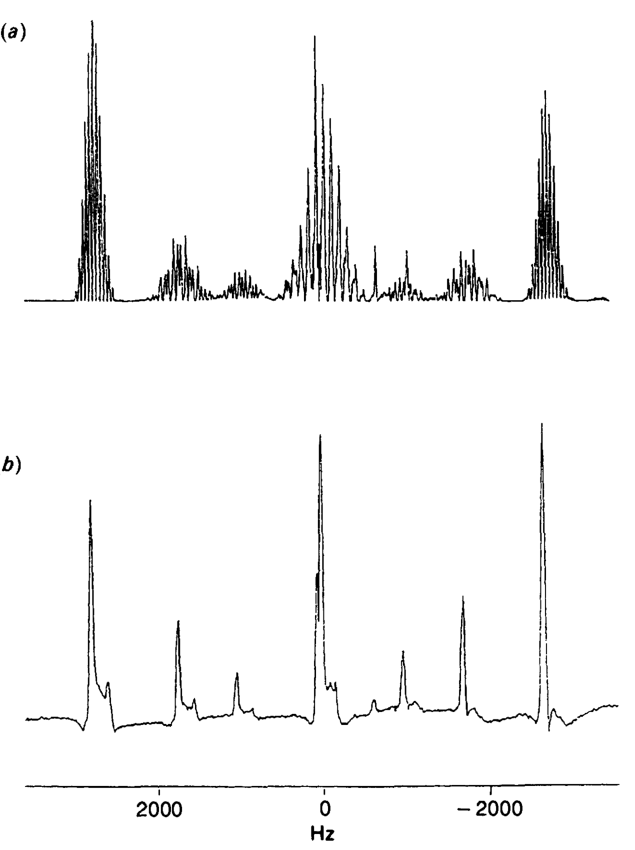


Fig. 2 200 MHz spectra of the protons in a sample of [$^2H_9$]-1,2,3-trimethoxybenzene dissolved in the nematic solvent I32: (*a*) normal spectrum [the free induction decays were acquired into 16 k words of computer memory, followed by Gaussian deconvolution, Fourier transformed with the same memory size, and then a base line correction routine (spline fitting) applied]; (*b*) deuterium-decoupled spectrum obtained and processed with the same parameters

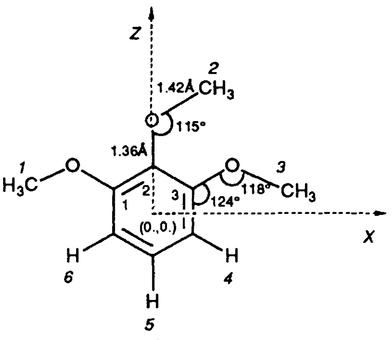


Fig. 3 Structure, atomic labelling, axes and geometry used for 1,2,3-trimethoxybenzene

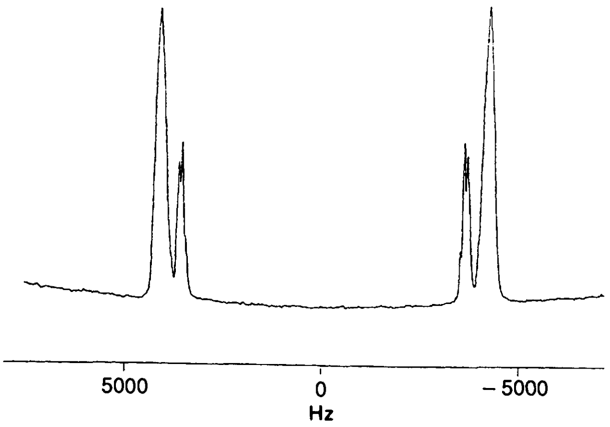


Fig. 4 30.7 MHz spectrum of the deuterons in a sample of [$^2H_9$]-1,2,3-trimethoxybenzene dissolved in the nematic solvent I32 (the free induction decays were acquired into 16 k of computer memory, and Fourier transformed with the same memory size)

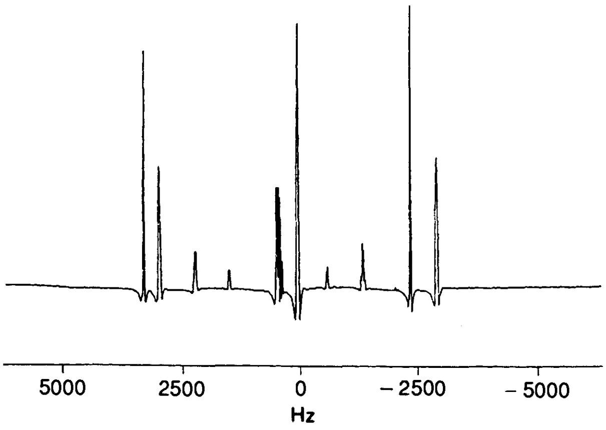


Fig. 5 200 MHz proton-{deuterium} spectrum of a sample of 1,3-di[$^2H_3$]methoxy-2-methoxybenzene dissolved in the nematic solvent I32 [The free induction decays were acquired into 16 k words of computer memory, followed by Gaussian deconvolution, Fourier transformed with the same memory size, and then a base line correction routine (spline fitting) applied]

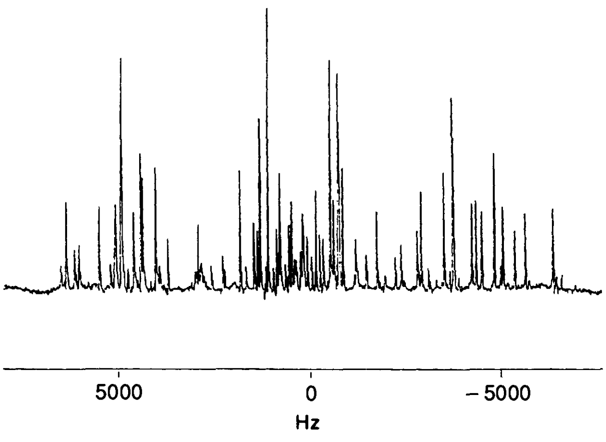
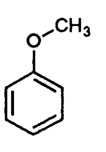


Fig. 6 200 MHz proton-{deuterium} spectrum of 2,3-di[$^2H_3$]methoxy-1-methoxybenzene dissolved in the nematic liquid crystalline solvent I32 [the free induction decays were obtained by acquisition into 16 k words of computer memory, followed by Gaussian deconvolution, Fourier transformed with the same memory size, and then a base line correction routine (spline fitting) applied]

dipolar couplings between the six protons in fragment **I**. If the central methoxy group is restricted to positions with $\theta = 90°$ or $270°$, then the values of the dipolar couplings in **I** are unchanged by a jump motion between these two forms, and are averaged only by the rotation of the methyl protons about the C(2)–O bond. If this motion is also assumed to be jumps between three equivalent positions for the methyl protons, corresponding to values of φ of 0°, 120° and 240°, then the dipolar couplings D_{24}, D_{25}, D_{22}, D_{45} and D_{46} can be calculated from eqn. (1), where $\theta_{ij\alpha}$ is the angle between the internuclear



I

$$D_{ij}/\mathrm{Hz} = \gamma_i\gamma_j h\Sigma S_{\alpha\beta}{}^1\cos\theta_{ij\alpha}\cos\theta_{ij\beta}/4\pi^2 r_{ij}{}^3 \qquad (1)$$

vector r_{ij} and axis α, $S_{\alpha\beta}{}^1$ is an element of the local Saupe ordering matrix for **I**, and in the summation the axes α,β are in turn x, y and z, which are fixed in the molecule. The dipolar couplings between the protons in **I** depend only on $S_{zz}{}^1$, $S_{xx}{}^1 - S_{yy}{}^1$ and $S_{yz}{}^1$. This is because the symmetry of the molecule dictates that whatever the conformational preferences of the two outer methoxy groups, the terms in eqn. (1) involving $S_{xy}{}^1$ and $S_{xz}{}^1$ vanish because of the creation of yz as an effective mirror plane by internal motion.

The bond lengths and angles for **I**, which are shown in Fig. 3, are based on those determined from a neutron diffraction study of trimethoprim.[8] All other angles in the benzene ring are assumed to be 120°, and all the C–H bond lengths are assigned a value of 1.09 Å. The angles within the C–CH_3 fragment are assumed to be 109.47°, the value appropriate to a regular tetrahedron.

For a jump motion of the methyl protons it is necessary to specify the structure corresponding to $\varphi = 0°$, and two such structures were tested: one with the apex of the proton triangle towards the ring, and the other with apex rotated through 180°. Both geometries gave an error function [eqn. (2)], which is

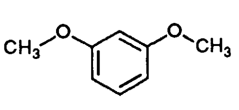
$$R = \{F^{-1}\Sigma_{ij}\,[D_{ij}(\text{calculated}) - D_{ij}(\text{observed})]^2\}^{\frac{1}{2}} \qquad (2)$$

unacceptably large compared to the errors on the experimental dipolar couplings when only the three order parameters are varied. F is the number of independent dipolar couplings in the summation, in this case 5. The value of R cannot be reduced for reasonable changes in the geometry, and so this model for the structure of **I** is rejected.

The second, conformational model tested for fragment **I** has the same geometry, but the methyl group is allowed to rotate freely about the O–C bond, *i.e.*, $V(\varphi)$ is assumed constant and independent of φ. In testing this model it was assumed that the D_{ij} in I can still be calculated with a single local order matrix, $S_{\alpha\beta}{}^1$. This is tantamount to assuming that the positions of the protons has a negligible effect on the orientational ordering. Again R is found to be unacceptably large, and the model is rejected.

It can be safely concluded that the central methoxy group cannot exist only in conformations where the C–O–C plane is perpendicular to the phenyl ring plane.

Do the Two Outer Methoxy Groups Occupy only the Planar Positions found for Solid Samples?—To answer this question the dipolar couplings between protons in fragment **II** are considered. This is because whatever the conformations adopted



II

by the central methoxy group, the molecular symmetry dictates that if the two outer methoxy groups occupy only positions which are related by yz as a mirror plane, then the dipolar couplings between the protons in **II** are given by eqn. (1) with only two non-zero local order parameters, $S_{zz}{}^1$ and $S_{xx}{}^1 - S_{yy}{}^1$. The geometry of these groups was assumed to be that found for the solid sample of trimethoprim, and R was minimized by changing the two order parameters. R was found to be very large and again cannot be reduced appreciably by reasonable changes in bond lengths and angles, and clearly the outer methoxy groups are not restricted to being in the positions found for solid samples. It is also possible to test structures in which the two outer methoxy groups are each rotated out of the plane through an angle θ, such as to preserve the mirror plane, and single structures of this type with any value of θ also do not fit the data.

We can safely conclude that with the RIS model, TMB must exist in conformations in which the outer methyl groups are in orthogonal, as well as planar, positions with respect to the ring plane.

Determination of the Conformational Distribution.—The dipolar couplings for a system of n interconverting conformers, which are not necessarily related by symmetry, and which have probabilities p_n, are given by eqn. (3), where $S_{\alpha\beta}{}^n$ is an element of an ordering matrix for the nth conformation. The crucial step in using eqn. (3) to test models of the conformational distribution

$$D_{ij} = -\gamma_i\gamma_j h\Sigma_n p_n \Sigma_{\alpha.\beta} S_{\alpha\beta}{}^n\cos\theta_{ij\alpha}{}^n\cos\theta_{ij\beta}{}^n/4\pi^2 r_{ijn}{}^3 \qquad (3)$$

is to formulate a model for how the order parameters $S_{\alpha\beta}{}^n$ depend upon n. We have used a model, fully described elsewhere,[9] known as the additive potential (AP) method, in which the order parameters are given by eqns. (4) and (5). Here, ω is the set of angles which defines the orientation of the mesophase director in the molecular frame, and $C_{2.m}(\omega)$ is a reduced spherical harmonic. The conformationally dependent potential of mean torque, $U_{\text{ext}}(\omega,n)$, is given by eqn. (6) in which the interaction parameters are given in eqn. (7). The $\varepsilon_{2.p}{}^j$ are interaction parameters for the jth rigid molecular sub-unit, and $D_{p,m}{}^2(\Omega_{jn})$ is the second rank Wigner rotation matrix describing the orientation of the jth sub-unit in a molecular reference frame.

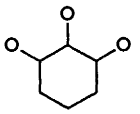
$$S_{zz}{}^n = Q^{-1}\int C_{2,0}(\omega)\exp\{-U_{\text{ext}}(\omega,n)/kT\}d\omega, \qquad (4)$$

$$S_{xx}{}^n - S_{yy}{}^n = Q^{-1}\sqrt{6}\int C_{22}(\omega)\exp\{-U_{\text{ext}}(\omega,n)/kT\}d\omega \qquad (5)$$

$$U_{\text{ext}}(\omega,n) = -\varepsilon_{2.0}{}^n C_{2.0}(\omega) - 2\varepsilon_{2.2}{}^n ReC_{2.2}(\omega), \qquad (6)$$

$$\varepsilon_{2.m}{}^n = \Sigma_j\Sigma_p\varepsilon_{2.p}{}^j D_{p.m}{}^2(\Omega_{jn}) \qquad (7)$$

Only two kinds of sub-unit need be considered for TMB:

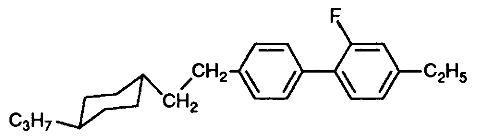


which requires $\varepsilon_{2.0}{}^R$ and $\varepsilon_{2.2}{}^R$; and O–CH_3, which requires only $\varepsilon_{2.0}{}^{OC}$. The value of R in eqn. (2) is now minimized by varying $\varepsilon_{2.0}{}^R$, $\varepsilon_{2.2}{}^R$, $\varepsilon_{2.0}{}^{OC}$ and p_n with the full set of dipolar couplings. $D_{ij}(A)$ given in Table 1.

The nine independent dipolar couplings are insufficient to test for the relative amounts of all 20 conformations, and we have restricted the conformations considered to the minimum set which will give an acceptable fit to the data, including those conformations which we expect to be the lowest in energy first. Thus we have considered just the four conformers **1**–**4** in Fig. 1. The result is that an acceptably small value of R can be obtained, as shown in Table 2, when there is 74% of **1** and 26% of **2**. The value of R is decreased by adding **3** and **4** to the conformational distribution, but not sufficiently to conclude that their presence is necessary to fit the data. Changes in the geometry, or relaxing the restriction to orthogonal and planar positions of the methoxy groups, would also undoubtedly reduce R, but we prefer to defer exploring these possibilities until a larger data set is available.

Table 2 The difference, $\Delta D_{ij}^{HH}(A)$, between observed and calculated dipolar couplings, the conformational weights, p_n, and the interaction parameters, $\varepsilon_{2,m}^{j}$, obtained for 1,2,3-trimethoxybenzene with a minimum conformational set

ij	$\nabla D_{ij}^{HH}(A)$/Hz	
4,5	−2.3	
4,6	2.8	
1,4	−4.7	
1,5	−5.1	
1,6	3.3	
1,1	−0.6	
2,4	−3.0	
2,5	−1.8	
2,2	−0.9	
R = 3.1 Hz		
Interaction parameters $\varepsilon_{2,m}^{j}$/kJ mol^{-1}		
$\varepsilon_{2,0}^{R}$	0.54 ± 0.01	
$\varepsilon_{2,2}^{R}$	2.63 ± 0.01	
$\varepsilon_{2,0}^{OC}$	−0.18 ± 0.01	
Conformer probabilities p_n (%)		
n	**1**	**2**
p_n	74.0 ± 0.1	26.0 ± 0.1



Conclusions

The important conclusion to be drawn from these calculations is that the central methoxy group cannot exist solely in the orthogonal position, as found in the solid state in trimethoprim. With the assumption that only planar or orthogonal positions are occupied by the methoxy groups, then the central methoxy group must occupy planar as well as orthogonal positions, and the outer methoxy groups must spend an appreciable amount of time (approximately 25%) in different positions to those occupied in the solid state.

These results refer to a sample dissolved in a liquid crystalline phase, and this raises the question of their generality. In the AP method the effect on the conformer probabilities of the orientational ordering is taken into account, and the distribution quoted here strictly refers to the TMB molecule in the mesogenic solvent at the temperature and density appropriate to the experiments done on the nematic phase but independent of the orientational ordering of the solute or solvent molecules. The distribution is therefore essentially that for the solvent in an isotropic phase.

The question remains as to whether the conformational distribution would be different in another solvent. The structure of I32 does not contain any groups which would be expected to interact strongly and specifically with TMB. It is probable therefore that a very similar conformational distribution to that found here should occur in solvents of similar chemical type, *i.e.*, hydrocarbons of low polarity.

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