

# High precision NOEs as a probe for low level conformers—a second conformation of strychnine†

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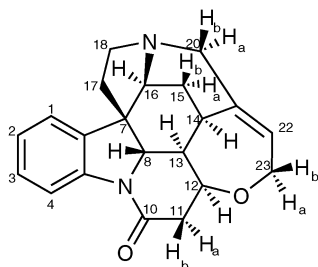
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**A second conformer of strychnine, with a very low population (2.5%), has been identified experimentally, and confirmed by computation, demonstrating the ability of NOE measurements to discriminate minute contributions to dynamic structure ensembles in solution.**

The application of the Nuclear Overhauser Effect (NOE) in NMR spectroscopy is a powerful tool for establishing stereochemical and conformational detail of chemical structure. In small molecules, such data are generally used in a qualitative (NOE/no NOE) or semi-quantitative (strong/medium/weak) fashion to characterise gross differences between conformations<sup>1</sup> but employing quantitative NOE interpretations is generally discounted and indeed discouraged. However, we have recently reported<sup>2</sup> that NOEs can quantify interproton distances in the rigid organic molecule strychnine (Fig. 1) to surprisingly high levels of accuracy. A total of 55 interproton distances for strychnine were measured in  $d_6$ -benzene and  $CDCl_3$ , with a mean error of  $\pm 3.3\%$  [ $0.09 \text{ \AA}$ ] and standard deviation of  $3.1\%$  [ $0.11 \text{ \AA}$ ] from their 'true' values as assessed by Density Functional Theory (DFT) calculations and X-ray crystallography. Only one of the distance (H11b–H23b, see Fig. 1 for numbering) was relatively poorly described, *vide infra*, with the remaining distances all falling within three standard deviations of the expected values.

With high precision evaluation of distances in hand, it should be possible to elucidate more precise conformational details from molecular structures than is typically proposed. Surprisingly, we are able to demonstrate this by identifying a new, very low population, conformer of strychnine which also accounts for the problematic H11b–H23b distance observed in our previous report.



**Fig. 1** Labelled molecular structure of strychnine.

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The interproton distance between H11b and H23b was determined based on NOE which led to a value significantly smaller than expected in both  $d_6$ -benzene and  $CDCl_3$ . In  $d_6$ -benzene, the NOE-determined distance was  $3.49 \text{ \AA}$  compared to DFT ( $4.10 \text{ \AA}$ )<sup>3</sup> and X-ray crystallography ( $4.12 \text{ \AA}$ ).<sup>4</sup> The relative error in this measurement (14.9%) is markedly higher than  $3\times$  the standard deviation (2.8%) found for the full dataset and significantly higher than the next greatest error (6.0%) in any distance determined in that solvent. In  $CDCl_3$ , where the standard deviations on all determined distances were slightly greater (3.5%), the determined distance ( $3.67 \text{ \AA}$ ) was still significantly short (10.6%) and further from its expected value than any other distance determined in that solvent. As no spectroscopic reason could be determined for the aberrant H11b–H23b interproton distance, it seems sensible to re-examine the structural model against which it was compared.

As highlighted above, all of the distances determined by NOE were compared with both DFT and X-ray structures of strychnine. These models provide only rigid single structures for comparison with the dynamic solution-state ensemble NOE measurements. A shorter than expected interproton distance measured by NOE methods, such as that for H11b–H23b, could arise from conformational dynamics in the solution-state measurements. Specifically, this will arise if some conformer exists with a reduced H11b–H23b distance and this second conformer contributes significantly to the NOE intensity. Given that H23b is bonded to the seven-membered ring of strychnine it seemed conceivable that such a conformer might indeed exist, although we have seen no report of this to date.

In order to measure the H11b–H23b distance, the previous study had assumed a direct relationship between NOE intensity and interproton distance. While this is detailed in our previous report, it is useful to reiterate the method employed here and examine the impact of multiple contributing conformers. The distances were obtained from the relative intensities of resonances in 1D-NOE spectra that were measured using mixing times for which the ratio of NOE intensities in a given experiment remain constant as a function of mixing time. This constant ratio of peak intensities implies that the initial rate (and isolated spin pair) approximations are valid for these mixing times and interproton distances could thus be determined without the need for a full cross-relaxation analysis. Under conditions where the initial rate approximation holds, then in transient NOE experiments the intensity of an NOE between two spins  $I$  and  $S$ ,  $\eta_{IS}$ , is proportional to: the cross-relaxation rate,  $\sigma_{IS}$ , between these spins; the initial  $z$ -magnetisation on spin  $S$ ,  $S_z^0$ ; and the mixing time,  $\tau_m$ , of the NOE experiment (eqn (1)).<sup>5</sup> The cross-relaxation rate

between  $I$  and  $S$  is, in turn, proportional to the internuclear distance between  $I$  and  $S$  ( $r_{IS}^{-6}$ ) as described in eqn (2).

$$\eta_{IS} \propto \sigma_{IS} \tau_m S_z^0 \quad (1)$$

$$\sigma_{IS} = k r_{IS}^{-6} \quad (2)$$

where

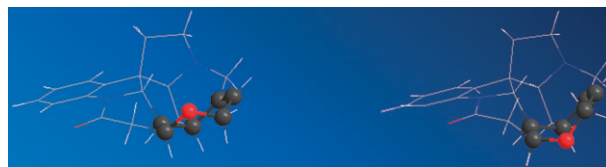
$$k = \left( \frac{\mu_0}{4\pi} \right) \frac{2h^2 \gamma^4}{10} \left( \frac{6\tau_c}{1 + 4\omega^2 \tau_c^2} - \tau_c \right)$$

Because of this strong  $r_{IS}^{-6}$  relationship between distance and NOE intensity, any contributing conformer with shorter  $r_{IS}$  will have a disproportionately large effect on  $\eta_{IS}$ , even if the conformer is present in low quantities. Conversely, contributions from conformers with significantly longer  $r_{IS}$  will have little or no impact on  $\eta_{IS}$ . In the case of strychnine, the NOE data therefore suggest the presence of a second conformation of strychnine, with a shorter H11b–H23b distance. However, given the very good fit for all of the other interproton distances in  $d_6$ -benzene, the putative second conformer must have similar or longer distances for all other measured proton pairs in strychnine.

**Experimental:** NMR samples were prepared in 5 mm tubes with 0.7 ml  $C_6D_6$  and 7 mg strychnine, under air without degassing. NMR data were all collected on a 500 MHz Varian VNMRS DirectDrive spectrometer equipped with an indirect observe probe. 1-Dimensional selective transient NOESY spectra (32 k data points, 10 kHz sweep width, 500 ms mixing time, 4.096 s acquisition time, 1 s relaxation delay, 1024 scans (90 minutes/irradiation)) were obtained using the Varian Chempack NOESY1D sequence which is based on the DPFGENOE (double-pulse field gradient spin-echo NOE) excitation sculpted selective sequence reported by Stott *et al.*<sup>6</sup> and a zero-quantum filter element.<sup>7</sup>

Geometry optimization at the B3LYP/6-31G(d) level was carried out using the Spartan 06 and Gaussian 03 program packages and frequencies were computed to characterize the minima. Single point energies were then calculated at the two minima (a) in Gaussian, with B3LYP/6-31G\* and a polarisable continuum model (IEF-PCM, parameters for benzene solvent,  $\epsilon = 2.247$ ) (b) at the LCCSD(T0)/cc-pVTZ level of theory, and (c) at the LMP2-F12/cc-pVTZ level of theory. Full details of the calculations (b) and (c), carried out using the MOLPRO program package<sup>8</sup> can be found in the ESI.†

**Results:** A B3LYP/6-31G(d) conformational search in Spartan led to two low-energy conformers for strychnine. The first, with the seven-membered ring in a boat-chair arrangement **1**, with carbons 21–14–13–12–(O)24–23 in a chair, while 12–(O)24–23–22–21–14 form a boat, closely matches the structure reported previously by both DFT and X-ray crystallography.<sup>3,4</sup> The second, **2**, to our knowledge not reported previously, was found to lie 11.6 kJ mol<sup>−1</sup> higher in energy at this level of theory. After correction for zero-point energy and thermal and entropic corrections, the calculated  $\Delta G$  at 298 K is of 12.9 kJ mol<sup>−1</sup>. Including the solvation free energies for both species leads to a predicted free energy difference of 11.8 kJ mol<sup>−1</sup> and suggests a minor population ( $\sim 1$ –2%) of conformer **2**. The very accurate LCCSD(T0) and LMP2-F12 correlated calculations provide a refined estimate



**Fig. 2** Structures of conformers **1** (left) and **2** (right) with 7-membered rings highlighted for clarity.

of the gas-phase energy difference *in vacuo* of 8.9 kJ mol<sup>−1</sup>, slightly lower than that found with B3LYP. In turn, this leads to a predicted free energy difference in solution of 9.4 kJ mol<sup>−1</sup> which corresponds to a population of  $\sim 2.2\%$  for the minor isomer. Favourable error cancellation together with the very similar electronic structure of the two conformers suggests that the error on this quantity is small, perhaps as small as 0.5 kJ mol<sup>−1</sup>, or  $\pm 0.5\%$  on the minor species relative population.

The structures of each conformer are shown in Fig. 2 for comparison. In the minor conformer **2**, the seven-membered ring occupies a *pseudo*-boat conformation, with carbons 14,21,22,23 and (O)24 being effectively co-planar. Conformers **1** and **2** essentially differ only in the torsion angles around the C22–C23 bond (C21–C22–C23–O24 =  $-62.4^\circ$  and  $8.5^\circ$  respectively) and C11–C12 bond (C13–C12–C11–O24 =  $55.7^\circ$  and  $17.7^\circ$  respectively).

Of particular interest, in conformer **2** the H11b–H23b distance was found to be substantially different, and crucially lower (2.11 Å) than in conformer **1** (4.10 Å). Since the H11b–H23b distance in conformer **2** is nearly half that of conformer **1**, then the H11b–H23b NOE in conformer **2** will build-up approximately  $50\times$  faster than in conformer **1**, hence the small population of conformer **2** should make a non-trivial contribution to the observed H11b–H23b NOE, which could account for the observed deviation for this quantity. On the other hand, the next greatest difference in proton distances between the conformers was in the H8–H22 distance (3.99 Å in conformer **1** vs. 3.42 Å in conformer **2**) which would give rise to a difference in NOE build-up rate of only two-fold. Hence, the H11b–H23b distance will be a substantially more sensitive probe for conformer **2** than any other distance measured.<sup>9</sup>

In quantitative terms, the calculated population of conformer **2** in  $C_6D_6$  of  $\sim 2.2\%$  ( $\pm 0.5\%$ ), together with the relevant static H11b–H23b distances at the two minima (4.10 Å and 2.11 Å respectively), can be used to predict an ensemble-averaged NOE which can be converted into an effective ‘distance’ for the sample, for comparison with the NOE-derived value. Note that full averaging of this type would also require that one accounts for zero-point and thermal fluctuations away from the two equilibrium geometries, but this correction should be significantly smaller than that for the conformational averaging used here. Averaging the equilibrium geometries yields a time- or ensemble-averaged ‘distance’ of 3.60 Å (with estimated error  $\pm 0.07$  Å using the estimated error of 0.5 kJ mol<sup>−1</sup> on the relative free energies) which now compares remarkably well with the experimentally determined distance of 3.49 Å and is well within the standard deviation (2.8%, 0.11 Å) for the experimental set of interproton distances in  $d_6$ -benzene.<sup>2</sup> Indeed by correcting the H11b–H23b distance for this

conformational flexibility we also further improve the accuracy of the entire NOE-distance dataset for strychnine in benzene, with the standard deviation of errors reducing from 2.8% to 2.0%, and the average error from 3.0% to 2.7%. It should be noted at this stage, that we have assumed the validity of the  $r^{-6}$  distance averaging for this conformationally flexible system. This applies for fast conformational exchange in fast tumbling molecules, although in slow tumbling systems, such as macromolecules, the effect of very fast conformational exchange, *e.g.* methyl groups, can give rise to  $r^{-3}$  averaging. For a more complete discussion of this, see ref. 1.

To confirm that no other interproton distances would be perturbed by the presence of conformer **2**, the average values for all the observed (NOE) interproton distances were determined using the same approach (Table S1, ESI†). It was gratifying to find that the H11b–H23b distance was the only value which changed significantly (0.5 Å, 12.2%). The next greatest correction is to the H22–H23a distance (0.02 Å, 0.7%), which is negligible relative to the standard deviation of the experimental measurements.

In summary, the data presented herein demonstrate that high-precision assessment of interproton distances in solution by a simple analysis of NOE spectra can be used to identify even fairly low level conformational contributions. In the case of strychnine, a previously unrecognised low-population conformer has been characterised and confirmed by NOE measurements and computation. Such a conformer could not have been recognised, let alone characterised, by traditional probes of conformational behaviour such as *J*-coupling or the qualitative use of NOEs. Indeed it is

interesting to note that the NOE measurements were substantially more accurate in determining conformer populations than standard computational approaches, such as DFT, and only the most demanding levels of calculation could reproduce the experimental data.

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