Solving the powder pattern of Verapamil hydrochloride

Aim

To apply knowledge of intra- and inter- molecular geometry to solving the powder pattern of a complex and problematic structure.

Introduction

Verapamil hydrochloride is a drug used to treat angina and hypertension by inhibiting the movement of calcium ions across cell membranes and hence depressing the contraction of myocardial and vascular smooth muscle. It is a complex and flexible molecule with 13 torsion angles. Solving the structure of such a flexible molecule from powder X-ray diffraction data using only the information contained in the powder pattern will always be a significant challenge, and utilizing additional sources of knowledge is crucial to success.

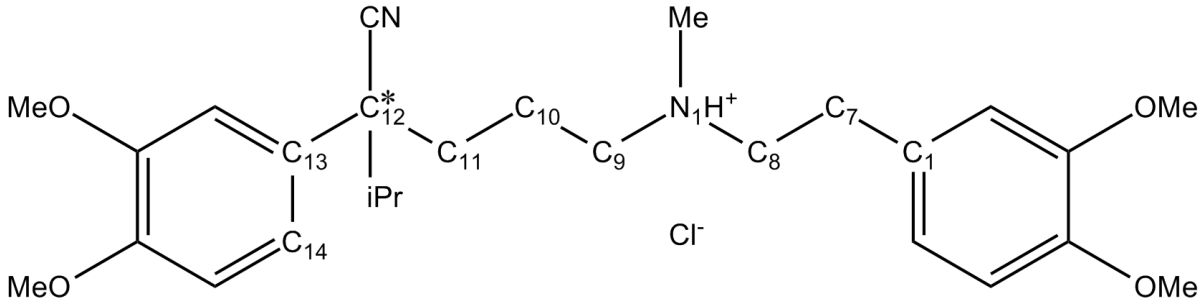

Figure 1. 2D chemical diagram of Verapamil hydrochloride. The chiral centre, C_{12}, is indicated with a "*".

Method

One of the keys to solving complex structures such as Verapamil hydrochloride is to collect good data. The powder pattern of Verapamil hydrochloride, collected in the lab of Alastair Florence, at the University of Strathclyde, is an example of good data:[1] clearly resolved, sharp peaks to relatively high 2Θ values. The pattern was indexed using DICVOL06 and the cell parameters returned were then refined along with background, zero point, peak shape parameters and intensities in a Pawley fit[2], using DASH.[3] Structure solution attempts using an unrestrained simulated annealing protocol have been shown to have a very low rate of success.[1] Therefore, other sources of knowledge, which can be used to restrain the simulated annealing are valuable. The search space can be significantly

reduced by using knowledge derived from the Cambridge Structural Database (CSD):[4] intramolecular geometry, in particular torsion angle space can be restricted to populated areas of conformational space and intermolecular geometry can be restrained using tethers between molecules or ions of multicomponent systems. In the case of Verapamil there are two features, in addition to torsional space, which are worth investigating for their potential to reduce search space. Firstly there is a chiral carbon in the molecule, which may allow torsion space to be reduced further from the usual +/- boundary ranges. Secondly, the chloride ion can be tethered to the positively charged NH^+ group.

Results

There are 13 torsion angles in Verapamil, 6 positional and orientational degrees of freedom, plus a choride ion, resulting in a hugely complex search space with 22 degrees of freedom. Clearly targeting the search of the solution space towards areas where correct answers are likely to be found is vital: an unconstrained simulated annealing of 50 trials, taking approximately 11 hours of CPU time (on a desktop PC of moderate specifications), yielded no solutions. A quick and easy method to reduce the search space is to use torsion angle distributions returned from the CSD to target the most likely conformational space. Therefore we applied the Mogul Distribution Bias (MDB) methodology to the torsion angles of Verapamil. Each flexible torsion angle fragment identified by DASH was automatically submitted to Mogul[5], and the returned distribution of torsion angles was used to bias the trial structures generated by the simulated annealing protocol. The methodology ensures that more trial structures are generated with torsion angles from densely populated areas of the torsion angle histogram and fewer structures are generated with torsion angles representative of sparsely populated areas. However, no area of torsional space is forbidden.

Verapamil contains a chiral centre, indicated with an asterisk in Figure 1, which offers the potential for further reduction of search space. A search in Mogul for the fragment shown in Figure 2 (left) returned the histogram given (Figure 2, right).

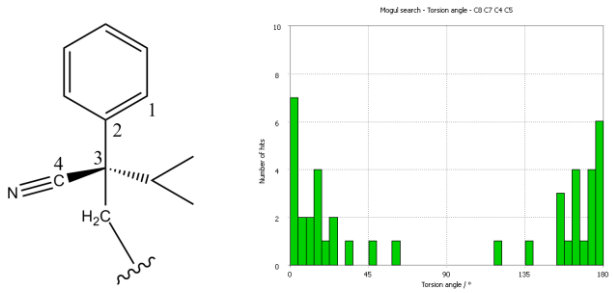

Figure 2. A fragment of Verapamil (left) is shown with the torsion angle of interest indicated with the labels 1-4. On the right is an histogram of torsion angles found in the CSD, using Mogul.

It is clear from the distribution that the cyano group is frequently found in the plane of the phenyl ring, either at 0° or 180°. Therefore, the handedness of the carbon atom, combined with the planar cyano group constrains the placement of the backbone carbon of Verapamil. Placing the cyano group at approximately 0° means the backbone carbon has to reside at 120°, whereas placing the cyano group at 180° means that backbone carbon resides at -60° (Figure 3).

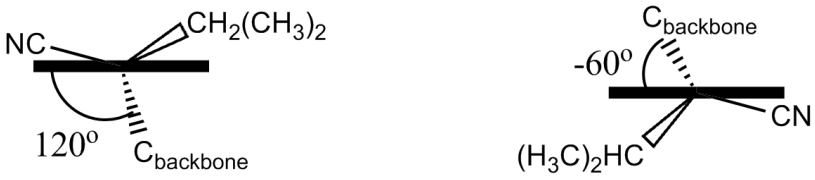

Figure 3. A diagram illustrating the possible orientations of the backbone carbon, with respect to the substituted phenyl ring (indicated by a thick line). From the Mogul distribution, the cyano group resides in the plane of ring, resulting in a $C_{backbone}$ position of +120° or -60°.

Thus, the torsional degree of freedom has been reduced from a trimodal distribution to either 120° or -60°, assuming the chiral configuration shown in Figure 3. In order to establish which position the backbone of Verapamil adopted, simulated annealing runs were performed with data truncated to about 30° 2Θ (3 Å resolution). The use of a lower resolution pattern, with fewer reflections, allowed the simulated annealing to progress faster whilst maintaining enough information content to allow the correct geometry to be established. Simulated annealing runs of 50 trials were set up, applying bounds of +/- 15° to the chiral carbon at either 120° or -60°, and using the MDB protocol for the remaining torsion angles. Solutions were found for the chiral torsion angle ($C_{14}C_{13}C_{12}C_{11}$) at values of around 120°, thereby identifying the correct backbone geometry. The CPU time taken to identify the correct geometry was approximately an hour: the low resolution pattern combined with MDB made these experiments to identify the correct geometry at the chiral carbon comparatively quick and efficacious to perform. It should be noted that a simulated annealing run of 50 trials, with the MDB protocol in place for all 13 torsion angles, using the truncated, lower resolution pattern did not yield any solutions – thus the additional, database-derived tight constraints for the chiral carbon was the only rapid method of establishing the correct geometry available.

Solutions were found from the low resolution experiments: the best solution had an RMSD in atomic positions, from the single crystal structure of 0.1055 Å.[6] This solution could be improved with Rietveld refinement using the whole powder pattern. However, it is worth investigating structure solution using a higher resolution pattern, in order to yield the best starting point for subsequent refinement. To this end, the best solution from the low resolution experiments was taken and analysed using Mogul, to identify any areas of the molecule which might have an unusual intramolecular geometry which is not well represented by the MDB method. Firstly, a torsion angle for one of the methoxy groups was identified as having an unusual geometry. Examination of the histogram showed that the torsion angle was marginally out of acceptable bounds: the torsion was found at approximately 30° from planar in the structure and the distribution showed a very tight cluster around planarity. This “unusual” torsion angle would most likely be corrected by the use of the higher resolution data. The second outlying torsion angle was $C_{11}C_{10}C_{9}N_{1}$ (Figure 1). The angle found during simulated annealing for this torsion was -75°, a value that corresponds to a sparsely populated mode of a trimodal distribution (Figure 4). Despite being in a sparsely populated area of the distribution there is no reason to doubt this torsion angle value: the solution it was obtained from showed a good fit to the experimental data ($\chi^2_{profile}/\ \chi^2_{Pawley}$ = 2.8), and no unusually short contacts were observed in the resulting structure.

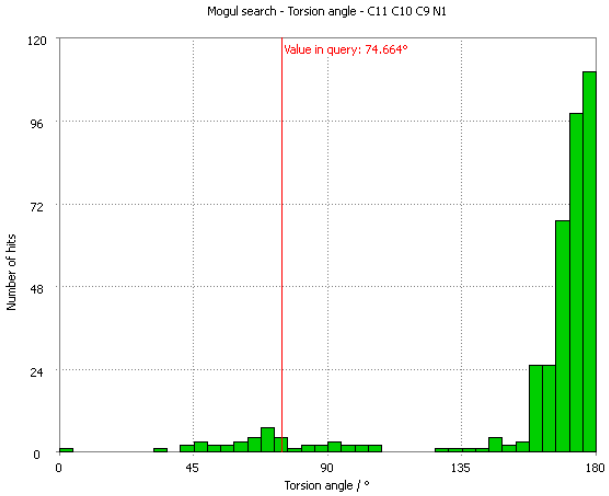

Figure 4. Distribution of torsion angle values for the fragment $C_{11}C_{10}C_9N_1$ accessed using Mogul. The value of the torsion angle found in the solution is indicated in red.

However, for this torsion angle, the MDB protocol will significantly bias the search space away from the "correct" geometry and towards the more usual planar geometry. Thus, the low resolution solution has provided some very useful information: applying manually derived bounds to this torsion angle will significantly improve the structure solution process over and above the MDB protocol. In order to demonstrate the gain in efficacy of the simulated annealing protocol, torsion angle bounds were applied manually to all 13 torsion angles in Verapamil using Mogul distributions invoked through the DASH interface. The bounds applied are presented in Table 1.

Torsion	Modality	Ranges in degrees (°)		
N1:C9:C10:C11	Trimodal	-30 to -90	30 to 90	150 to -150
C12:C11:C10:C9	Unimodal	160- to -160		
C8:N1:C9:C10	Trimodal	-30 to -90	30 to 90	150 to -150
C13:C12:C11:C10	Bimodal	-30 to -90	30 to 90	
C7:C8:N1:C9	Trimodal	-30 to -90	30 to 90	150 to -150
C14:C13:C12:C11	Unimodal	110 to 130		
C23:C22:C12:C11	Trimodal	-30 to -90	30 to 90	150 to -150
C1:C7:C8:N1	Trimodal	-30 to -90	30 to 90	150 to -150
C2:C1:C7:C8	Bimodal	45 to 135	-45 to -135	
C27:O4:C17:C18	Unimodal	-20 to 20		
C26:O3:C16:C15	Unimodal	-20 to 20		
C20:O2:C5:C6	Unimodal	-20 to 20		
C19:O1:C4:C3	Unimodal	-20 to 20		

Table 1 – Modal torsion angle bounds applied to Verapamil. Atom numbering taken from the single crystal structure of Verapamil[6] (refcode: CURHOM), as visualised in Mercury.[6]

Applying these bounds to a powder pattern truncated to 42° 2Θ (2.1 Å resolution) resulted in 24/50 correct solutions being found. An overlay of the best solution with structure obtained from single crystal diffraction results in an RMSD in atomic positions, of 0.0800 Å[6] (see Figure 5).

In all of the above runs, an additional constraint was placed on the structure solution run: the chloride ion was tethered to be within 3 +/- 0.3Å of the NH^+ group, using database-derived knowledge to provide the distance range. In this case, the powder pattern is good quality and so the tethering of these two species was not vital to successful structure solution. However this kind of constrained simulated annealing run will certainly, for more complex problems, yield an increase in the rate of success.

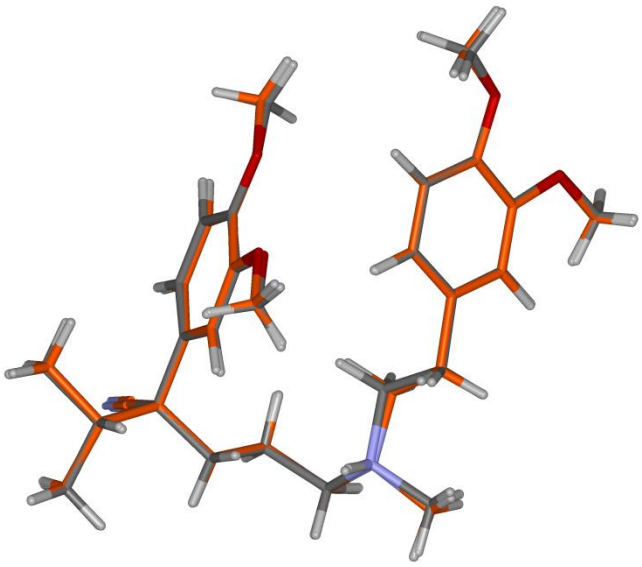

Figure 5. Overlay of Verapamil hydrochloride determined by single crystal diffraction[7] (indicated with orange carbons) and powder diffraction. RMSD in atoms positions calculated to be 0.008Å. The chloride ions have been hidden for clarity.

Conclusions

Structure solution from powder diffraction patterns can tackle increasingly complex problems with the help of additional sources of knowledge at the structure solution stage. Torsion angle data has been shown to be crucial in turning the highly flexible molecule Verapamil from an intractable problem into an easily solvable structure. We have also shown that applying the Mogul Distribution Bias protocol with a low resolution pattern is a very effective method of establishing a "rough" molecular geometry, which can be used a starting point for further constrained simulated annealing runs using higher resolution data.

References

1. A.J. Florence, N. Shankland, K. Shankland, W.I.F. David, E. Pidcock, X. Xu. A. Johnston, A.R. Kennedy, P.J. Cox, J.S.O. Evans, G. Steele. S.D. Cosgrove, C.S. Frampton. *J.Appl. Cryst*., 2005, **38**, 249-259

2. G.S. Pawley. *J. Appl. Cryst*. 1981, **14**, 357–361

3. W.I.F. David, K.Shankland, J.van de Streek, E. Pidcock, W.D. Motherwell, J.C. Cole. *J. Appl. Cryst.*, 2006, **39**, 910-915

4. F.H. Allen, *Acta Cryst*. 2002, **B58**, 380-388

5. I.J. Bruno, J.C. Cole, M. Kessler, J. Luo, W.D.S. Motherwell, L.H. Purkis, B.R. Smith, R. Taylor, R.I. Cooper, S.E. Harris, A.G. Orpen. *J.Chem.Inf. Comput. Sci*. 2004, **44**, 2133-2144

6. C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek and P. A. Wood, *J. Appl. Cryst.*, 2008, **41**, 466-470

7. A. Carpy, J.-M. Leger, C. Melchiorre, *Acta Cryst*. 1985, **C41**, 624-627

Products

CSD – the world's only comprehensive, fully curated database of crystal structures, containing over 500,000 entries

DASH – an easy to use tool for structure solution from powder diffraction data

Mogul – a knowledge base of CSD-derived molecular geometries which provides a quick route to validated and trustworthy molecular models

Mercury – a versatile and feature-rich visualisation tool for molecular structures

For further information please contact Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK. Tel: +44 1223 336408, Fax: +44 1223 336033, Email: admin@ccdc.cam.ac.uk