Structure solution and refinement of tetracaine hydrochloride from X-ray powder diffraction data

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The previously unknown crystal structure of the local anaesthetic tetracaine hydrochloride ($C_{15}H_{25}N_2O_2{}^+Cl^-$) has been solved from synchrotron X-ray powder diffraction data using a direct-space global optimisation method in which the position and conformation of a structural model is adjusted. The $C_{15}H_{25}N_2O_2{}^+$ moiety and the chloride counterion were treated as independent units during the structure solution process. This complex structure has been solved despite the nine torsional degrees of freedom in the cation. A restrained Rietveld refinement of the initial solution gives an excellent fit to the data, with $R_{wp} = 0.0956$ and $R_{F^2} = 0.1638$, and reveals the presence of a significant degree of preferred crystallite orientation in the sample. The crystal structure shows efficient packing involving parallel sheets made up of molecules lying parallel to one another, head-to-tail, in chains. Hydrogen bonds are observed between adjacent molecules and an extensive pattern of N^+–Cl^- contacts is apparent.

Introduction

Tetracaine hydrochloride, 2-(dimethylamino)ethyl *p*-butylaminobenzoate hydrochloride (Fig. 1), also known as amethocaine hydrochloride, is used as a topical local anaesthetic in ophthalmic preparations. Synthesis is achieved by treating sodium *p*-butylaminobenzoate with dimethylaminoethyl chloride and converting the product to the hydrochloride.[1] The crystal structure of this compound has not been determined, nor have there been any reports of polymorphism. Attempts to grow a single crystal of sufficient quality to allow single crystal X-ray structure determination to be carried out have proved unsuccessful, but for relatively small molecules such as this, powder X-ray diffraction now offers a viable alternative route to traditional single crystal structure determination.

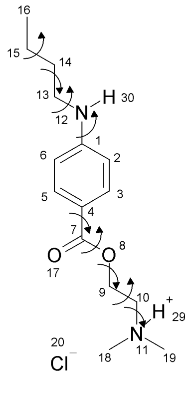

Fig. 1 The tetracaine hydrochloride ion pair. The 9 torsion angles optimised during simulated annealing in the cation are indicated by arrows. The atomic numbering is shown; hydrogen atoms are numbered 21 to 45, only those bonded to N are shown.

There are two major variants on powder diffraction approaches to solving structures. The first involves the extraction of individual reflection intensities from the diffraction data, followed by the application of traditional methods of structure solution, such as direct or Patterson methods. One of the earliest uses of this approach was by Zachariasen[2] in 1948, and in 1986 it was used for the first time with synchrotron powder X-ray data by Attfield *et al.*[3] to solve the structure of α-$CrPO_4$. Algorithmic developments, both in the intensity extraction stage and the structure solution stage have ensured its continued use; see, for example, Brunelli *et al.*[4] for a recent application of direct methods and Bénard-Rocherullé and Louër[5] for a recent Patterson methods example. The second approach has emerged over the last decade and is sometimes referred to as a "direct space" strategy. It involves the comparison of diffraction data calculated from a trial structure against the measured diffraction data. The level of agreement between the two sets of data is quantified by some figure of merit (typically, a goodness-of-fit, χ^2, or a profile *R*-factor) and the trial structure is then adjusted in order to maximise agreement between calculated and observed data until the crystal structure is solved. The problem is thus one of global optimisation, and methods for adjusting the trial crystal structure range from the simple grid search approach through to Monte Carlo methods,[6,7] one example of which is simulated annealing,[8–15] and genetic algorithms.[16–21] The majority of variants on the basic global optimisation strategy exploit the known chemical connectivity of the structure under study, making this method ideal for solving the unknown packings of molecules whose internal structures are largely predictable.

The direct space solution process used here is based upon the approach described by David *et al.*[10] that was subsequently implemented in the DASH[22] computer program. The key features of this approach are that it uses the known chemical connectivity, implements a simulated annealing methodology and

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uses the correlated integrated intensities figure of merit χ^2 for assessing the goodness-of-fit to the data.

Data collection

A sample of tetracaine hydrochloride was obtained from Aldrich Chemical Company. X-Ray diffraction data were collected at room temperature on station 9.1 of the CLRC Daresbury Synchrotron Radiation Source. The sample was contained in a 0.7 mm diameter capillary and the incident wavelength was 1.000446 Å. Data were collected using a step size of 0.01°, to a maximum of 40° in 2θ, corresponding to a maximum resolution of 1.46 Å.

Crystal data: $C_{15}H_{25}ClN_2O_2$, $M = 300.82$, triclinic, space group $P\bar{1}$ (no. 2), $a = 7.4002(1)$, $b = 8.5669(1)$, $c = 13.6923(3)$ Å, $\alpha = 106.211(1)$, $\beta = 90.853(1)$ $\gamma = 98.778(1)°$, $V = 822.26(4)$ Å^3, $T = 298$ K, $Z = 2$. Data were collected in the range $3 \leq 2\theta \leq 40°$ [step size 0.01° (2θ)]. Indexing was carried out using Dicvol.[23] Rietveld refinement was carried out using GSAS.[24] A global isotropic temperature factor was refined for all non H-atoms. At convergence, $R_{wp} = 0.0956$, $R_p = 0.0728$ and $R_{F^2} = 0.1638$ for 4019 profile points, 113 restraints, and 160 variables.

CCDC reference number 177247. See http://www.rsc.org/suppdata/nj/b1/b109494g/ for crystallographic data in CIF or other electronic format.

Structure solution and refinement

Initial indexing of the powder pattern using the program Dicvol[23] returned a triclinic unit cell with lattice parameters $a = 7.391$, $b = 8.559$, $c = 13.671$ Å, $\alpha = 106.21$, $\beta = 90.81$ and $\gamma = 98.78°$ and a volume of 819.2 Å^3. Consideration of molecular volumes suggested the presence of two tetracaine hydrochloride units within the unit cell, and a Pawley fit[25] in space group $P\bar{1}$ gave a reasonable fit to the data, with a reduced χ^2 of 13.9.

An internal co-ordinate description of tetracaine was constructed using standard bond lengths, bond angles and torsion angles. The possible conformations of the molecule are represented by nine torsion angles, indicated in Fig. 1, which were treated as variables to be optimised by the DASH program. Treating the position of the chloride ion separately gave a total of 18 variable parameters to be optimised in the crystal structure solution; three describing position, three for molecular orientation (expressed as four quaternion numbers), and nine describing torsion angles for the cation, plus three describing the position of the chloride. Given the complexity of this search space, an attempt was made to simplify the search *via* the application of chemically sensible bounds (derived from the examination of related structures found in the Crystal Structure Database, CSD[26]) to a particular torsion angle within the tetracaine molecule. Using the observation that the vast majority of torsion angles of the type defined by atoms C3–C4–C7–O8 lie in the ranges 0 ± 20° or 180 ± 20°, this torsion angle was restricted to lie between −20 and 20°. The C13–N12–C1–C2 torsion angle was allowed to vary between −180 and 0°, this allowed the molecule sufficient flexibility. The remaining seven torsion angles were allowed to vary fully in a 360° range. Atomic numbering is represented in Fig. 1.

A promising solution with a final reduced χ^2 of ~130 was reached after ~4.5 million simulated annealing moves. Whilst this value is high compared to the Pawley χ^2, the lack of any unrealistic close contacts and the presence of a realistic network of hydrogen bonds suggested that the solution could be correct. This solution was reached in approximately half the runs when the simulated annealing procedure was repeated several times, the other runs gave chemically unrealistic solutions with higher χ^2 values. The atomic co-ordinates from the promising DASH solution were then input into the program GSAS[24] in order to carry out a Rietveld profile refinement of the structure. Initially, only the background (a twelve-term linear interpolation function), peak shape and lattice parameters, the zero point and scale factor were refined. However, introduction of a March Dollase correction for preferred crystallite orientation[27,28] along the [001] direction gave a significant improvement in the fit to the data and χ^2 fell to ~63, with an R_{wp} of 0.175. The March Dollase preferred orientation ratio refined to 1.24(1); the value of > 1 indicates that the crystallites have a platy habit.

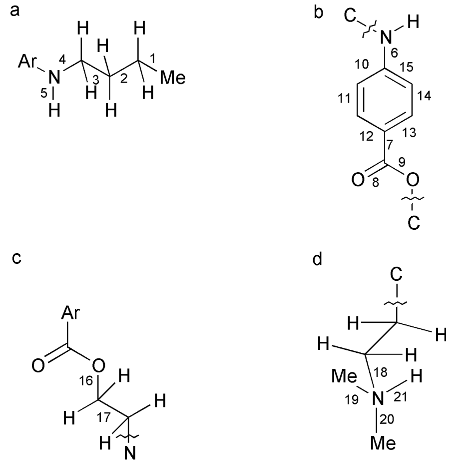

Fig. 2 The four fragments sought in the CSD to obtain ideal distances for the numbered bonds to use in the restrained refinement of tetracaine hydrochloride. The number of independent fragments (and the number of independent crystal structures they were found in) were as follows: (a) 7 (6), (b) 12 (11), (c) 21 (19) and (d) 172 (123).

A series of restraints were then used to carry out a Rietveld refinement in which the positions of all 45 atoms were refined, in addition to the aforementioned variables. All 44 bond lengths were restrained to 'ideal' values within a deviation of ±0.02 Å (the restraints are weighted so that the standard deviation in the distribution of values around the mean is 0.02 Å). The ideal C–C/N/O and N–H interatomic distances were determined by searching the CSD for four fragments of the tetracaine molecule, shown in Fig. 2, and averaging the results for each fragment. This brings out subtle effects such as the alternation of short and long C–C bonds in the C13–C16 chain. C–H bond distances were restrained to 1.08 or 1.10 Å for aromatic or aliphatic carbons, respectively. 67 Bond angles were restrained to ideal values (109.4° for tetrahedral angles and 120° for trigonal angles) within a deviation of ±2°. Ideal values for the C–O–C angle and angles around N11 were determined from comparable molecules in the CSD, using the fragments shown in Fig. 3. In addition, the aromatic ring and all directly connected atoms were restrained to lie in a plane. In order to maintain a chemically sensible geometry around N12, atoms N12, C13, C14 and H30 were restrained to lie in a plane. The shifts in the atom positions during the refinement were found to be quite small with atoms

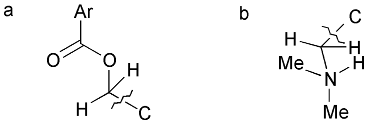

Fig. 3 The two fragments sought in the CSD to obtain ideal values for bond angles to use in the restrained refinement of tetracaine hydrochloride: (a) was used for the C7–O8–C9 angle from 681 fragments (in 496 structures) and (b) was used for the C10–N11–C18, C10–N11–C19 and C18–N11–C19 angles from 250 fragments (196 structures).

Table 1 Refined and ideal bond lengths in the tetracaine hydrochloride molecule

Bond	Bond number (in Fig. 2)	Length/Å	"Ideal" length/Å
C1–C2	15	1.401(7)	1.39
C2–C3	14	1.394(8)	1.39
C3–C4	13	1.395(8)	1.39
C4–C5	12	1.406(8)	1.39
C5–C6	11	1.394(8)	1.39
C6–C1	10	1.402(8)	1.39
C1–N12	6	1.412(7)	1.40
N12–C13	4	1.478(8)	1.45
C13–C14	3	1.545(8)	1.52
C14–C15	2	1.514(8)	1.50
C15–C16	1	1.534(8)	1.51
C4–C7	7	1.501(7)	1.48
C7–O17	8	1.202(8)	1.21
C7–O8	9	1.342(8)	1.34
O8–C9	16	1.440(8)	1.45
C9–C10	17	1.502(8)	1.49
C10–N11	1	1.503(8)	1.49
N11–C18	2	1.503(8)	1.49
N11–C19	3	1.498(8)	1.49
C2–H24		1.082(9)	1.08
C3–H23		1.082(9)	1.08
C5–H22		1.083(9)	1.08
C6–H21		1.079(9)	1.08
C9–H25		1.099(9)	1.10
C9–H26		1.099(9)	1.10
C10–H27		1.098(9)	1.10
C10–H28		1.091(9)	1.10
N11–H29	4	0.944(9)	0.94
C18–H40		1.098(9)	1.10
C18–H41		1.098(9)	1.10
C18–H42		1.100(9)	1.10
C19–H43		1.093(9)	1.10
C19–H44		1.099(9)	1.10
C19–H45		1.102(9)	1.10
N12–H30	5	0.883(9)	0.88
C13–H31		1.108(9)	1.10
C13–H32		1.103(9)	1.10
C14–H33		1.108(9)	1.10
C14–H34		1.101(9)	1.10
C15–H35		1.102(9)	1.10
C15–H36		1.103(9)	1.10
C16–H37		1.103(9)	1.10
C16–H38		1.106(9)	1.10
C16–H39		1.100(9)	1.10

typically moving by ~0.25 Å from their starting positions. Temperature factors were fixed at 0.01 and 0.025 Å^2 for non-hydrogen and hydrogen atoms respectively—refinement of these variables did not improve the fit significantly. The final reduced χ^2 was 21.1, to which the contribution of the profile fit is 19.7. Bond lengths, together with their ideal values, are in Table 1, other results are summarised in the crystal data above. The final profile fit is shown in Fig. 4.

Packing and intermolecular contacts

The intermolecular packing and contacts derived from this type of structure determination are an important check on the correctness of the solution. Virtually all of the intramolecular distances and angles are constrained in the structure solution and are restrained in the subsequent refinement. However, the intermolecular contacts are determined only by the fit to the diffraction data and can be considered as true experimental results for comparison with freely refined single crystal structures.

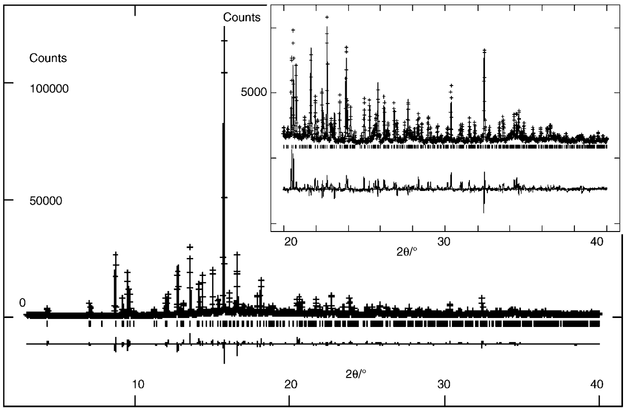

Fig. 4 The final Rietveld fit to the powder X-ray diffraction profile of tetracaine hydrochloride. The inset shows the 20 to 40° region of 2θ in greater detail.

The crystal structure consists of parallel sheets of molecules, shown in Fig. 5, stacked approximately normal to the *a*-axis, with an average separation distance of approximately *a*/2. Within the sheets, the molecules lie parallel to one another, head-to-tail, in chains. Hydrogen bonding is apparent between the carbonyl oxygen and the N–H group next to the aromatic ring on an adjacent molecule, as shown in Fig. 6. The efficiency of the hydrogen bonding system is characterised by the N–H···O angle of 172° which compares to an average of 161° for 7 comparable systems in the CSD. The O···H and N–H···O distances of 2.11 and 2.99 Å are comparable to those in the latter systems.

Weakly bound pairs of N^+ and Cl^- ions are present in the structure with a separation distance of 2.94 Å. A search of the CSD for short N^+–Cl^- contact distances gave a range of

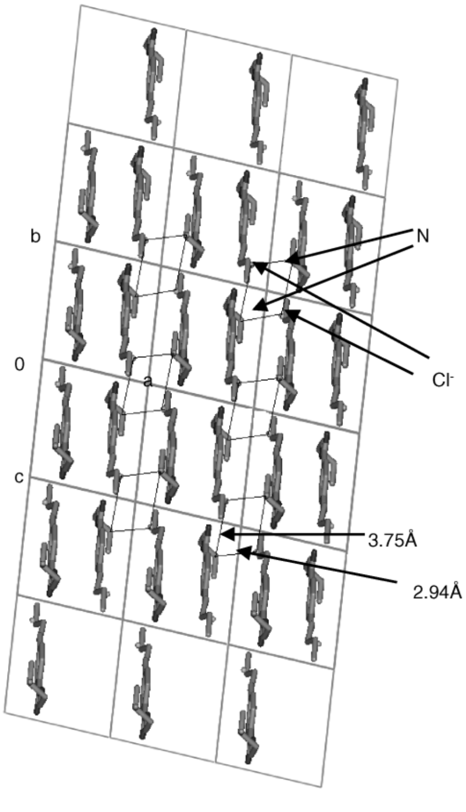

Fig. 5 Stacked sheets of tetracaine hydrochloride molecules and the pattern of N^+–Cl^- contacts. The view is normal to the [011] plane and hydrogen atoms have been omitted for clarity.

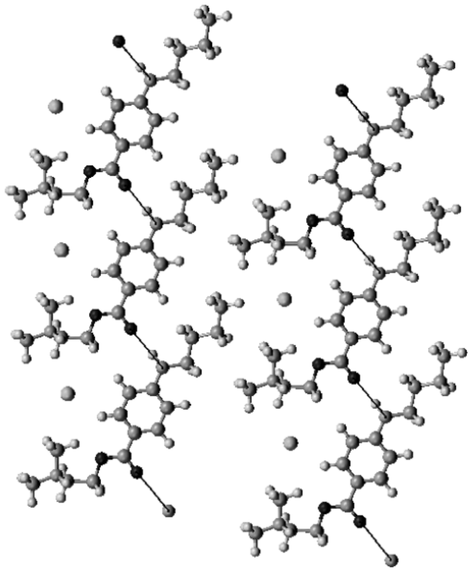

Fig. 6 [100] view of the hydrogen bonds formed between the N–H group adjacent to the aromatic ring and the carbonyl oxygen on a neighbouring molecule.

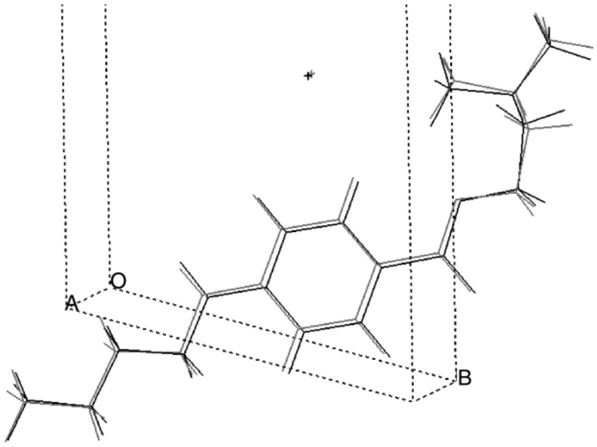

Fig. 7 The DASH structure solution for tetracaine hydrochloride (grey) superimposed upon the final refined structure (black). The principle effect of the refinement is to move the entire molecule 0.16 Å along the direction of the *b*-axis. It is likely that the overall misplacement of the molecule in the DASH solution is a consequence of the preferred orientation present in the sample.

distances from 2.85 to 3.30 Å, with a mean value of 3.07 Å. A more extensive pattern of N^+–Cl^- contacts is also apparent, as illustrated in Fig. 5. The ion pairs are weakly bound into dimers with a N^+–Cl^- distance of 3.75 Å between the pairs.

Conclusions

The crystal structure of tetracaine hydrochloride has been solved directly from powder diffraction data using a global optimisation approach. The relatively large number of variables that were optimised in order to solve the crystal structure made this a challenging problem. Nevertheless, the structure was solved to a high degree of accuracy, in spite of the presence of a significant degree of preferred orientation in the sample, which introduces systematic errors into the solution process. The subsequent restrained Rietveld refinement made only small changes to the initial solution, see Fig. 7. Nevertheless, it produced a significant improvement to the final fit to the data and, therefore, a demonstrable improvement in the quality of the final reported crystal structure. This emphasises the importance of performing a final profile refinement following such direct space structure solutions, using the best available estimates for the intramolecular distances and angles. These are easily obtained from the CSD, geometry optimisation calculations would provide an alternative approach. The crystal structure shows realistic patterns of intermolecular contacts and efficient packing involving chains of molecules aligned head-to-tail, stacking to form sheets.

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