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Structure determination of a steroid directly from powder diffraction data†

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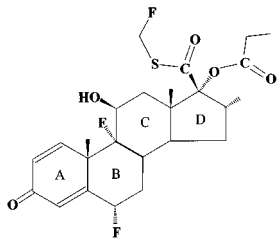
We report the determination of the crystal structure of a new polymorph of the pharmaceutical material fluticasone propionate, which is obtained as a microcrystalline powder by a supercritical crystallization procedure; the structure was solved directly from powder diffraction data using our Genetic Algorithm technique (in which a population of trial structures evolves through well-defined procedures for mating, mutation and natural selection) and refined using Rietveld refinement techniques.

Single crystal X-ray diffraction is without question the most powerful tool for elucidating crystal and molecular structures. However, the requirement for single crystal samples imposes a natural limitation on the scope of this technique, as many materials of interest cannot be prepared as single crystals of sufficient size and quality, but are instead available only as microcrystalline powders. Such materials include many industrially important solids, such as pigments, pharmaceuticals and catalysts, as well as many materials of biological importance. Here we report progress in the structure determination of one such material—a new polymorph of the steroid fluticasone propionate, which is important with regard to its pharmaceutical applications—using a Genetic Algorithm technique developed recently for solving crystal structures directly from *powder* diffraction data.

Fluticasone propionate ($C_{25}H_{31}F_3O_5S$; FP) is a potent synthetic anti-inflammatory steroid which suppresses inflammation of the bronchial passages in the lungs. When formulated as an inhaled product (under the trademark‡ Flixotide™ or



Flovent™), the anti-inflammatory action of FP treats the underlying inflammatory component of asthma. FP also has an indication in an intra-nasal form for rhinitis, where it is marketed under the trademark‡ Flixonase™. It has a superior therapeutic index to beclomethasone dipropionate (BDP).

FP is known to exist in two polymorphic forms. Form 1 can be obtained by crystallization from a variety of solvents (typically acetone), and the crystal structure of form 1 has been determined previously[1] from single crystal X-ray diffraction (and also confirmed as part of our current research by structure determination from powder diffraction data using the techniques discussed below). In attempts to produce crystals of FP of controlled size and morphology for pharmaceutical applications, crystallization in a supercritical fluid medium (with EtOH or acetone as solvent) was carried out, and was found to yield a new polymorph (form 2). As form 2 was obtained only by the supercritical crystallization method, yielding polycrystalline powder samples, structural characterization of form 2 could not be carried out by single crystal X-ray diffraction. Here we report the determination of the crystal structure of form 2 of FP directly from powder X-ray diffraction data.

Although traditional techniques[2–4] for structure solution from powder diffraction data have been applied successfully in several cases, these techniques have certain intrinsic limitations[3] and organic molecular crystals represent a particularly challenging case. For this reason, there has been much recent interest in the development of new methods for solving crystal structures directly from powder diffraction data, leading *inter alia* to a new generation of 'direct-space' approaches that are particularly suited for molecular crystals. The direct-space strategy[5,6,3] is based on sampling trial crystal structures in direct space, with the 'quality' of each trial structure assessed by comparing the powder diffraction pattern calculated for the trial structure and the experimental powder diffraction pattern. In our work, this comparison is made using the profile *R*-factor R_{wp},[3,5] which considers the whole digitized intensity profile and thus implicitly takes care of peak overlap. In effect, the structure solution process involves searching a hypersurface $R_{wp}(\mathbf{X})$ to find the best structure solution (lowest R_{wp}), where $\{\mathbf{X}\}$ represents the variables that define the trial structures. In the case of one molecule in the asymmetric unit, the variables in $\{\mathbf{X}\}$ represent the position $\{x, y, z\}$, orientation $\{\theta, \phi, \psi\}$ and intramolecular geometry (specified by variable torsion angles $\{\tau_1, \tau_2, \ldots \tau_n\}$) of the molecule. In general, the bond lengths and bond angles (and any known torsion angles) are fixed in the calculation, and are taken either from standard values for the type of molecule under study or from the known geometries of similar molecules. Methods used to search *R*-factor hypersurfaces to locate the global minimum (structure solution) within direct-space structure solution strategies have included Monte Carlo,[5,7,8] simulated annealing[6,9,10] and Genetic Algorithm (GA)[11–15] techniques. Here we focus on the application of our GA method, details of which are described in ref. 12. In this approach, a population of trial structures is allowed to evolve subject to the normal rules and operations (mating, mutation and natural selection) that govern evolutionary systems.

The powder X-ray diffraction pattern of form 2 of FP was recorded at 22 °C in transmission mode on a Siemens D5000 diffractometer, using Ge-monochromated Cu-Kα_1 radiation and a linear position-sensitive detector covering 8° in 2θ. The total 2θ range was 5° to 60°, measured over 12 h in steps of 0.02°. The powder X-ray diffraction pattern was indexed by the program ITO.[16] [The lattice parameters following Rietveld refinement (see below) are: a = 23.2434(9), b = 13.9783(5), c = 7.6510(3) Å.] Systematic absences are consistent with space group $P2_12_12_1$, and density considerations suggest that there is one molecule in the asymmetric units.§

In the GA structure solution calculation (using the program GAPSS,[17,12]), all non-hydrogen atoms of the FP molecule were used (to define the asymmetric unit). The tetracyclic ring system

† Fractional coordinates for the non-hydrogen atoms in the final crystal structure of form 2 of FP are available from the RSC web site, see http://www.rsc.org/suppdata/cc/1999/1677/

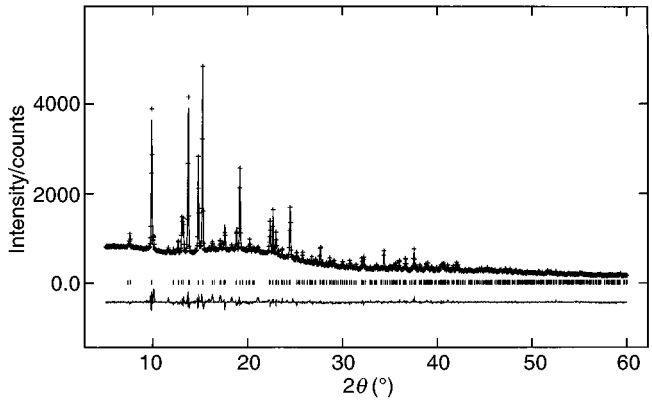


Fig. 1 Experimental (+), calculated (solid line) and difference (lower line) powder X-ray diffraction profiles for the Rietveld refinement of form 2 of FP. Reflection positions are marked. The calculated powder diffraction profile is for the final refined crystal structure, details of which are given in the supplementary material. See note †.

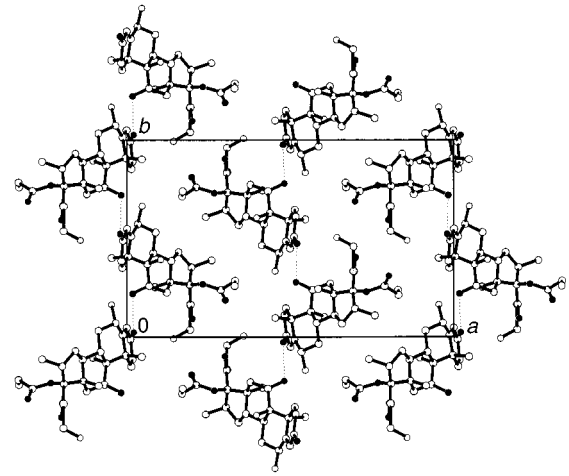


Fig. 2 Final refined crystal structure of form 2 of FP (hydrogen atoms not shown) viewed along the *c*-axis. Dashed lines indicate hydrogen bonding interactions.

was considered as a rigid unit, comprising one planar six-membered ring (designated A), two six-membered rings in chair conformation (B and C), and a five-membered ring in an envelope conformation (D). The side-groups attached to the D ring were considered as flexible units, with their conformations defined by six variable torsion angles. Thus, the GA calculation involved 12 degrees of freedom $\{x, y, z, \theta, \phi, \psi, \tau_1, \tau_2, \ldots \tau_6\}$. Bond lengths and angles were taken from known structures of other steroid molecules and from other information on standard molecular geometries.[18] The GA calculation involved the evolution of 60 generations of a population of 100 structures. In each generation, 200 offspring (involving 100 pairs of parents) and 10 mutations were considered. For mating and mutation, each of the 12 variables was considered as an independent gene. In carrying out a given mating operation between two parents to generate two offspring, the 12 variables from each parent were combined and distributed between the two offspring, with no restriction on the combination of variables allowed to pass from a given parent to a given offspring. In carrying out the mutation procedure on a selected structure, six variables were selected at random, and a new random value was assigned to each of the selected variables.

The best structure solution (lowest R_{wp} in the final generation) was taken as the starting structural model for Rietveld refinement using the GSAS program package.[19] The positions of all non-hydrogen atoms were refined, with standard geometric restraints applied to bond lengths and angles. A common isotropic displacement parameter was refined (final U_{iso} = 0.050 Å^2), and in the final stages a preferred orientation parameter was refined. The final Rietveld refinement (Fig. 1; Table 1) gave R_{wp} = 4.8% and R_p = 3.3%.

In the crystal structure of form 2 of FP (Fig. 2), the molecules form stacks along the *c*-axis with adjacent molecules related by translation. Zig-zag chains of molecules related by the 2_1 screw operation along the *b*-axis are linked by C–O–H···O=C hydrogen bonds involving the hydroxy group (C ring) and carbonyl group (A ring) of adjacent molecules (O···O, 2.8 Å; C–O···O, 110°). This structure provides interesting similarities and contrasts with the structure of form 1 of FP.[1] Both structures contain similar hydrogen-bonded chains (described above along the *b*-axis in form 2), but differ in the structural relationship between adjacent chains of this type; in form 2, adjacent chains are anti-parallel (related by a 2_1 axis), whereas in form 1, adjacent chains are parallel to each other (related by translation).

It is clear that knowledge of the structure of form 2 of FP provides a basis for understanding differences in the properties of forms 1 and 2, including those relating to pharmaceutical applications of these materials. This opportunity has arisen through the present-day ability to solve molecular crystal structures of moderate complexity directly from powder diffraction data.

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Notes and references

‡ Flixotide™, Flovent™ and Flixonase™ are trademarks of the Glaxo Wellcome group of companies.

§ *Note added in response to a referee:* Form 2 of FP transforms to form 1 between *ca.* 154 and 165 °C which then subsequently melts (with decomposition) between *ca.* 279 and 291 °C. The densities at ambient temperature are: form 1, 1.33 g cm^{-3} (measured), 1.34 g cm^{-3} (calculated); form 2, 1.34 g cm^{-3} (calculated).

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