Structural characterisation of two pharmaceutically important steroids by solid-state NMR†

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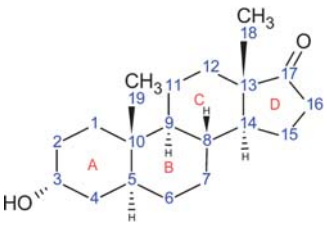
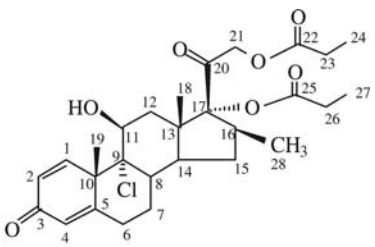
Several crystal modifications of two steroids (androsterone and beclomethasone dipropionate) have been characterised by solid-state NMR methods, and the chemical shifts between the different forms compared. The gradual loss of water from androsterone hemihydrate has been monitored by XRPD. The crystal structures are discussed in relation to the NMR data. That of the beclomethasone dipropionate ethyl acetate solvate has been determined for the first time. It is found that there are clear channels containing the ethyl acetate molecules, which are apparently not incorporated by hydrogen bonding. Carbon-13 CP MAS spectra with long contact times and MAS proton spectra are used to show the mobility of ethyl acetate molecules in the BDP solvate. The second-order effect of chlorine on the ^{13}C spectrum of beclomethasone dipropionate monohydrate has been explored by varying the applied magnetic field.

Introduction

Steroids form a highly important class of pharmaceutical compounds, with a range of biochemical and therapeutic effects. Their structural characterisation is therefore of considerable interest, especially as they are prone to occur in a number of polymorphic forms, which may differ in their bioavailability when formulated as clinical drugs. They are also known to exist in a range of solvates, which again require characterisation. Many techniques have been used in recent decades to obtain information about the various solid forms. For many chemists, the ultimate tool for such characterisation is, of course, single-crystal diffraction. However, derivation of complete crystal structures by this method is not always feasible and also suffers from a number of limitations (*e.g.* with respect to disordered or amorphous states). Therefore solid-state NMR, which can be applied to any solid system, has come to the fore as a complementary technique of considerable power. The present article discusses its application to two steroid systems, namely androsterone (**I**) and beclomethasone dipropionate (BDP, **II**).



(I)



(II)

Androsterone, 5α-androstane-3α-ol-17-one ($C_{19}H_{30}O_2$), is a powerful male sex hormone (androgen) with functional prostate property. It was proved to be a metabolite of testosterone[1] and has recently been studied as a contributory factor to prostate cancer.[2] Structurally, it is a very simple steroid, with no sidechains other than methyl and hydroxyl. Literature references suggest the possibility of different solid forms.

Form 1 is anhydrous and information about its crystal structure has been reported in the literature a number of times. First, the unit cell parameters were given (CSD code ANDOON03) by Bernal and Crowfoot[3] in 1936. In 1966 the full crystal structure was determined (CSD code ANDOON) by High and Kraut.[4] Recently, the crystal structure was redetermined by Shikii *et al.*[5] (CSD code ANDOON04) and by

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† Electronic supplementary information (ESI) available: Fig. 1: Rietveld fitting of the observed diffractogram for the hemihydrate of androsterone. Fig. 2: Variable-time XRPD monitoring of dehydration for the hemihydrate form of androsterone. Fig. 3: Androsterone solution-state ^{13}C NMR spectrum. Fig. 4: Androsterone HSQC spectrum. Fig. 5: Androsterone NOESY spectrum. Fig. 6 and 7: Androsterone HMBC spectra. CCDC reference number 685681. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b803586e

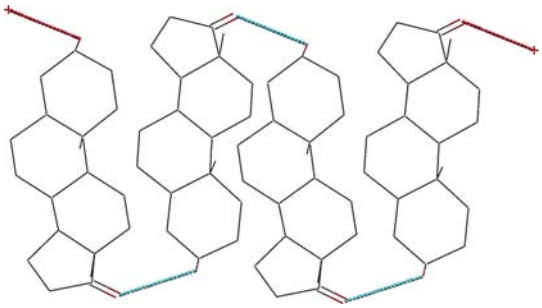

Fig. 1 Androsterone Form I crystal structure, showing the head-to-tail hydrogen bonding. The O–O distance is 2.956 Å.

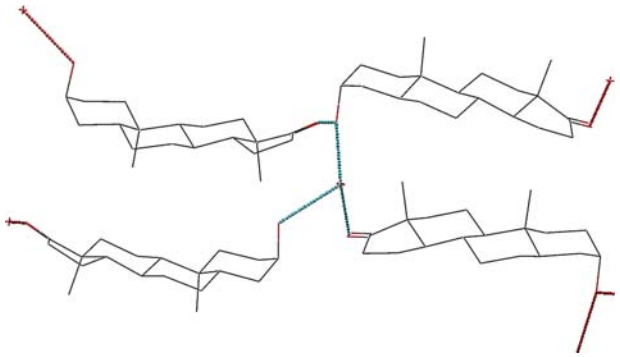

Fig. 2 Hydrogen bonds in the androsterone hemihydrate crystal structure.[6] The upper two molecules constitute a dimer, mutually linked by a hydrogen bond between O51″ and O2″, but otherwise only hydrogen-bonded to water molecules. O90 represents water oxygen, which is hydrogen bonded to three androsterone molecules, one *via* a carbonyl oxygen (O52) two *via* hydroxyl oxygens. The atom numbering mentioned here is that of the crystal structure determination, not that displayed in structure (**II**) (see the CSD entry).

Hulme *et al.*[6] (ANDOON06) using low-temperature single-crystal XRD. Whilst High and Kraut[4] noted several unusual geometrical features present in their structure, all of these take more conventional values in the redetermination by Hulme *et al.*[6] The differences between those two structural determinations were attributed[6] to the different temperatures at which the data sets were collected, the superior nature of the determination based on the use of a modern diffractometer, and the availability of more powerful computing capability.

Form 1 crystals were grown by slow evaporation from a solution of diethyl ether for the work of the first two references,[3,4] while Hulme *et al.*[6] reported that their crystals were obtained by slow evaporation from a solution of absolute ethanol. Form 1 has a melting point of 186 °C and it has two symmetry-related molecules ($Z = 2$) in the unit cell, linked head-to-tail by hydrogen bonding (O–O distance 2.956 Å) connecting the hydroxyl group (ring A) with the carbonyl group (ring D) of a second molecule, see Fig. 1. The crystal structure has hydrogen-bonded chains propagated parallel to the *b* crystallographic axis by the 2_1 screw axis.

Kuhnert-Brandstätter and Grimm[7] identified a hydrate of androsterone by thermal microscopy and reported that it desolvated at 80 °C. Bouché and Draguet-Brughmans (B & D-B)[8] replicated the desolvation using crystals grown by evaporation from 70% aqueous ethanol at room temperature and reported the unit cell parameters (CSD code ANDOON01). Banerjee[9] postulated the existence of another form of androsterone, and reported a unit cell (CSD code ANDOON02) different from that of Form 1. The crystals that gave this unit cell were grown from a solution of diethyl ether. B & D-B[8] showed that crystals grown from diethyl ether and then dried exhibited a weak endotherm in the DSC trace at approximately 165 °C, which was followed by melting at 183–186 °C, the latter event attributed to Form 1. They identified the former event as a phase change from a new Form 2 to Form 1. They also reported that this change could be initiated by grinding. Furthermore, they suggested that the change occurs spontaneously, but slowly, when Form 2 crystals are left to stand for a period of time. Because this phase change occurs well above the desolvation temperature of the hemihydrate (as it is now known to be—see below), there is no suggestion that a transformation related to the hydrate is involved. This subtle phase change was not observed by either thermal microscopy or infrared spectroscopy. The thermal behaviour of the new form did not change even after drying the crystals under vacuum for 24 h. Powder diffraction was used to provide a unit cell reported for Form 2, which on the one hand is different from that of Form 1 and the hydrate identified by thermal microscopy[7] but on the other hand concurred with the unit cell published by Banerjee[9] for his proposed form (though they did not find the water). Recent work by Hulme *et al.*[6] shows that the form obtained by B & D-B following the phase change at *ca.* 165 °C is difficult to reproduce, and that the unit cell parameters reported by both Banerjee and B & D-B correspond to a hemihydrate (structurally characterised under CSD code ANDOON05). Hulme *et al.* conclude that the high-temperature form "is a subtle relaxation in the crystal structure of the newly dehydrated phase". Their crystal structure of the hemihydrate shows that each water molecule forms three hydrogen bonds with androsterone molecules (Fig. 2), being a donor to ring D carbonyl groups for two of them and an acceptor from the hydroxyl group for the third. The two independent androsterone molecules form dimers, connected head-to-tail (ring A to ring D) by intermolecular hydrogen bonds. The water molecules were found to lie inside a channel along the *a* crystallographic axis (Fig. 3).

Beclomethasone dipropionate (BDP) is an important substance for pharmaceutical industry, both as an anti-inflammatory agent and as a component in a number of inhalation products. It is structurally more complex than androsterone since it has two sidechains, as well as a methyl group at position 16 and a chlorine atom at C9. It has four carbonyl and carboxyl groups available for hydrogen bonding. Like androsterone it has a hydroxyl group, but in this case at C11 rather than C3. It was identified by infrared spectroscopy as existing in three main forms: an anhydrate, a monohydrate, and numerous organic solvates. Many solvents can be incorporated into the crystal structure[10] including alkanes (5–8 carbon atoms long), alcohols (2–5 carbon atoms long), ethers and ketones (each 2–5 carbon atoms long), and halo-alkanes (1 to 2 carbon atoms and containing various combinations of hydrogen, fluorine, chlorine and bromine). The Arcton

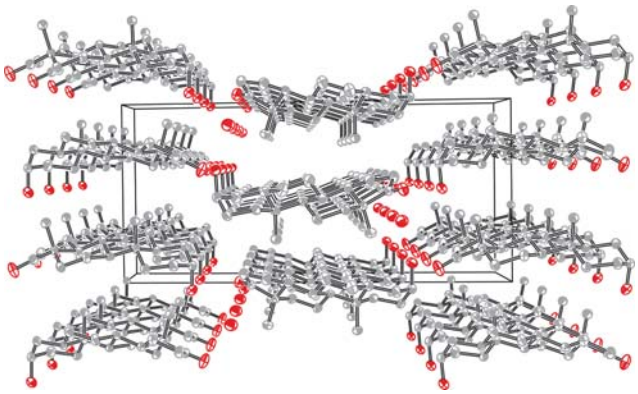

Fig. 3 Crystal packing for androsterone hemihydrate, with the view along the *a* crystallographic axis showing water molecules inside channels.

($CFCl_3$) solvate of BDP was formerly used in aerosol formulations of Becotide™, Becloforte™ and Ventide™ inhalers for the treatment of asthma. Perhaps the most interesting aspect of BDP solvates is the fact that non-stoichiometric amounts of the solvents can be incorporated into the solid and that they can be lost progressively (as measured by elemental microanalysis and quantitative gas chromatography). All forms of BDP can be readily identified and characterised by IR spectroscopy.[11] Similarities in the IR spectra have lead to the proposal that the solvates with organic solvents share a common crystal structure incorporating solvent-containing channels. Accordingly, single-crystal X-ray studies of the anhydrate, monohydrate, ethanolate solvate and diethanol 1,1,2,2-tetrafluoroethane-134a solvate have been performed to determine the crystal structures (CSD codes WOYPAB,[12] BCLMSN,[13] INECOT[14] and VATMOT,[15] respectively). We have now obtained the structure of the ethyl acetate solvate (see below). We have also been informed about unpublished structures of both the anhydrate and the ethyl acetate solvate.[10]

Experimental

Samples

A sample of androsterone was obtained from Sigma–Aldrich. It was examined by XRPD and found to be pure Form 1. The sample as received contained large crystallites, which gave rise to substantial differences between the observed and computed XRPD patterns due to preferred orientation effects. Crushing a sample produced some improvement, but some preferred orientation prevailed. The sample was examined on a glass slide, which caused a broad hump in the base line of the observed pattern. Samples of androsterone hemihydrate were prepared as described in the literature.[6]

BDP was supplied by Glaxo Group Research Ltd. (now GSK) in the form of anhydrate and monohydrate samples. The solvates were prepared by dissolving the anhydrate in the appropriate solvents and recrystallising. For instance, for the ethyl acetate sample, 1 g of BDP anhydrate was dissolved in hot, reagent-grade ethyl acetate. The solution was cooled, filtered and washed with cold solvent. The sample was then dried using only a water vacuum pump in an attempt to remove excess solvent from the surface of the crystals without taking it out of the channels in the crystal structure.

NMR spectroscopy

The ^{13}C CP MAS NMR spectra of androsterone Form 1 and of the hemihydrate were measured at ambient temperature using a Varian Inova-300 spectrometer, operating at 75.40 MHz for ^{13}C and 299.18 MHz for ^{1}H. Spectra of Form 1 were also obtained at ambient probe temperature using a Varian InfinityPlus spectrometer, which operates at 125.65 MHz for ^{13}C and 499.70 MHz for ^{1}H. Samples were packed, with light grinding, into 5.0 mm o.d. rotors. For the androsterone samples, the contact times were in the region of 1 ms, with spin rates of 5 and 9 kHz (for the hemihydrate and Form 1, respectively), recycle delays of 60 s and acquisition times of 40 and 75 ms (for Form 1 and the hemihydrate, respectively). The duration of the proton 90° pulse was 4.3 μs. Proton decoupling was carried out with the TPPM pulse sequence[16] using a proton decoupling power equivalent of about 60 kHz. The spectrum of the hemihydrate was acquired over 240 transients and for Form 1 1900 transients were recorded.

All the solid-state spectra of BDP were acquired using a Bruker CXP-200 spectrometer unless noted otherwise. The spectrometer was equipped with a 4.7 T, superconducting magnet operating at 50.32 MHz for carbon-13 and 200.13 MHz for protons. The rotors were 7 mm in diameter and were made of zirconia with Kel-F end-caps. Magic-angle spinning speeds were normally in the region of 4 kHz. Some ^{13}C solid-state BDP work was also carried out at higher fields, on Varian VXR-300 and Bruker AMX-500 spectrometers (operating at 75 and 125 MHz, respectively). The shielding tensor components were determined from spectra obtained using slow MAS rates. The spinning sideband manifolds were analysed by iterative fitting using an in-house computer program[17] based on the equations given by Maricq and Waugh.[18] Generally the experiments required 10 000 to 20 000 transients to achieve acceptable S/N and therefore they took 14–28 h of spectrometer time. Three different spin rates were used in each case and the average parameters are presented herein. The measurements were only feasible for the high-frequency signals. The shielding anisotropy, ζ, and shielding asymmetry, η, are defined as follows:

$$\zeta = \sigma_{ZZ} - \sigma_{\mathrm{iso}}$$

$$\eta = (\sigma_{YY} - \sigma_{XX})/\zeta$$

with the principal components ordered by $|\sigma_{ZZ} - \sigma_{\mathrm{iso}}| \geq |\sigma_{XX} - \sigma_{\mathrm{iso}}| \geq |\sigma_{YY} - \sigma_{\mathrm{iso}}|$.

A Varian Inova-500 was employed to obtain solution-state spectra of androsterone in $CDCl_3$. The spectrometer was equipped with a 9.4 T, 54 mm-bore superconducting magnet operating at 100.577 MHz for C-13 and 399.952 MHz for protons. A 5 mm Varian switchable dual-channel probe was used. Solution-state NMR spectra for BDP in $CDCl_3$ were acquired using a Varian VXR-400 spectrometer at Glaxo Group Research Ltd.

The ^{13}C chemical shifts for the solids were indirectly calibrated through the high-frequency adamantane carbon signal (at 38.4 ppm relative to the signal for the standard reference TMS). The solution-state chemical shifts were measured directly from the signal for internal TMS.

Crystal data for BDP ethyl acetate solvate

$C_{28}H_{37}ClO_7 \cdot C_4H_8O_2$, M = 609.13 g mol^{-1}, hexagonal, space group $P3_121$, $a = b$ = 13.3963(1), c = 31.0233(5) Å, U = 4821.57(9) Å^3, $F(000)$ = 1956, Z = 6, D_c = 1.259 Mg m^{-3}, μ = 0.17 mm^{-1} (Mo-Kα, λ = 0.71073 Å), T = 120(1) K. 89272 reflections ($2.19 \leq \theta \leq 29.00^\circ$) were collected on a Rigaku R-Axis SPIDER IP diffractometer (ω-scan, 3°/frame) and, after processing with CrystalClear software, yielded 8549 unique data points (R_{merg} = 0.074). The structure was solved by direct methods and refined by full-matrix least squares on F^2 for all data using SHELXTL software. The absolute configuration was established by anomalous dispersion effects in diffraction measurements; the absolute structure Flack parameter is equal to 0.03(6). All non-hydrogen atoms were refined with isotropic displacement parameters. H-atoms of the steroid frame were located on the difference map and refined isotropically, while the H-atoms of methyl groups and disordered solvent molecules were placed into calculated positions and refined in riding mode. The whole ethyl acetate molecule is disordered over two positions which were refined with a fixed site occupation factor of 0.5. Final $wR_2(F^2)$ = 0.1393 for all data (453 refined parameters); conventional $R(F)$ = 0.0499 for 7919 reflections with $I \geq 2\sigma$; GOF = 1.027.

CCDC reference number 685681.

For crystallographic data in CIF or other electronic format see DOI: 10.1039/b803586e

Results and discussion

Androsterone

Thermal analysis carried out on androsterone hemihydrate showed that dehydration takes place between 70 and 115 °C (weak endotherm) along with a mass loss of 3.2% (shown by TGA). The latter is close to the calculated mass loss expected for dehydration of a hemihydrate (3%). The DSC data indicate that dehydration results in transformation to Form 1, as was further confirmed by variable-temperature XRPD. There was no evidence of a phase transformation at 150 °C as proposed earlier by DSC work.[6]

A variable-time XRPD experiment was carried on androsterone hemihydrate at ambient temperature (25 °C). The XRPD pattern was recorded about every 14 min (*i.e.* each scan took 14 min), as illustrated in the ESI.† Transformation to Form 1 occurred over 24 h (Fig. 4), with a first-order half-life of 4.18 ± 0.02 h. These results differ from those in the literature, where it was reported that full transformation took place between 65 and 130 °C. However, the sample subjected to VT XRPD in the literature was not ground, whereas in our variable-time experiment the sample was first ground under liquid nitrogen.

The ^{13}C spectrum of androsterone was recorded for a solution in $CDCl_3$. The chemical shifts, shown Table 1, are close to literature values,[19] though Blunt and Stothers were unable to resolve the signals from C10 and C16. The assignments (see Table 2) were confirmed by HMBC, HSQC and NOESY two-dimensional experiments (see ESI†); it was found to be necessary to interchange the assignments of C4 and C10 from the earlier work.[19]

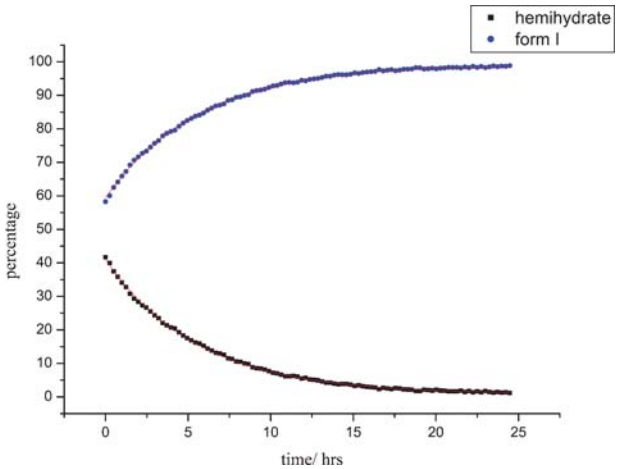

Fig. 4 Plot showing transformation of androsterone hemihydrate to Form 1 with time at 25 °C. Initially, the sample contained 41.7% of the hemihydrate form and 58.3% of Form 1.

The ^{13}C spectrum (excluding the carbonyl resonance) of Form 1 is shown in Fig. 5. The chemical shifts are listed in Table 1. An important stage in the understanding of such spectra is the assignment of the signals to specific carbon sites, and this is not always a trivial process. As is frequently the case, for the androsterone solid forms the peaks were assigned initially by comparison with the solution-state results. Many were confirmed by dipolar dephasing[20] and selective population inversion (SPI) experiments[21] (see Fig. 5). The methylene carbon peaks from C4 and C16 only show up as a shoulder on the signal for the quaternary carbon C10. However, they are revealed as a negative peak in the SPI experiment with an inversion time of 50 μs (see Fig. 5(d)).

The spectrum of the hemihydrate was also obtained (Fig. 6). The peaks observed in the CH_2 region (25–35 ppm) were quite broad because of the low dipolar decoupling power used. However, the decoupling power was not increased because the acquisition time was long (75 ms), thus potentially leading to over-heating of the probe. Fig. 6 shows that there are minor peaks which correspond to those of Form 1, indicating that the sample examined contained Form 1 as an impurity.

The chemical shifts for the hemihydrate are given in Table 1. The ^{13}C CP MAS NMR readily distinguishes between Form 1 and the hemihydrate. The spectrum of the latter was consistent with the crystal structure in that some non-equivalent carbons gave rise to two clearly-resolved peaks, indicating that the hemihydrate has two molecules in the asymmetric unit ($Z' = 2$). The relatively large linewidths for the CH_2 resonances generally obscure splittings in these cases. The observed crystallographic splittings range in magnitude up to 2.2 ppm (see Table 1).

Table 1 also lists the differences between the solution-state and solid-state chemical shifts. These appear to show general

Table 1 Solid-state and solution ^{13}C chemical shift (δ_C/ppm) assignments for androsterone forms

		Solid state		
Carbon (type)	Solution	Form 1[a]	Hemihydrate form[a]	Splitting for hemihydrate
C19 (CH_3)	11.4	12.7 (−1.3)	11.7/13.5 (−0.3/−2.1)	1.8
C18 (CH_3)	14.1	14.9 (−0.8)	14.3/15.6 (−0.2/−1.5)	1.3
C11 (CH_2)	20.3	21.2	22.0 (1.7)	
C15 (CH_2)	22.0	21.2	22.0	
C6 (CH_2)	28.5	27.9 (0.6)	28.3 (0.2)	
C2 (CH_2)	29.3	29.7 (−0.4)	29.7 (−0.4)	
C7 (CH_2)	31.1	32.1 (−1.0)	30.5 (0.6)	
C12 (CH_2)	31.8	33.0 (−1.2)	32.4 (−0.6)	
C1 (CH_2)	32.4	33.9 (−1.5)	34.0 (−1.6)	
C8 (CH)	35.3	35.7 (−0.4)	35.8/36.2 (−0.5/−0.9)	0.4
C4 (CH_2)	36.0	37.2 (−1.5)[b]	37.5 (−1.2)	
C16 (CH_2)	36.1	37.4 (−1.4)[b]	37.5 (−1.3)	
C10 (C)	36.5	37.0 (−0.5)	36.9 (−0.4)	
C5 (CH)	39.4	39.7 (−0.3)	39.3/40.0 (0.1/−0.6)	0.7
C13 (C)	48.1	48.7 (−0.6)	48.5/48.8 (−0.4/−0.7)	0.3
C14 (CH)	51.7	52.8 (−1.1)	52.8/55.0 (−1.1/−3.3)	2.2
C9 (CH)	54.7	55.7 (−1.0)	55.4/57.2 (−0.7/−2.5)	1.8
C3 (CH)	66.7	66.4 (0.3)	64.5/66.4 (2.2/0.3)	1.9
C17 (C═O)	221.0	223.9 (−2.9)	224.7/225.7 (−3.7/−4.7)	1.0

[a] The differences ($\delta_{solution} - \delta_{solid}$) between solution-state and solid-state chemical shifts are given in parentheses. [b] Observed as a negative peak in the Selective Population Inversion experiment.

deshielding for both Form 1 and the hemihydrate with respect to the solution state, although part of the change may be accounted for by small referencing differences. However, it is clear that the carbonyl carbon (C17) is deshielded in the solid state, which is characteristic for a hydrogen-bond acceptor atom. This deshielding is more marked for the hemihydrate than for Form 1, presumably because of the stronger hydrogen bonding in the former case (see Fig. 2). The shielding of the acceptor C3 is scarcely affected by the phase differences.

Androsterone is a rather simple steroid (in comparison with BDP) in the sense that there are no flexible substituents. The structure of the ring system is relatively rigid and changes little from compound to compound or between polymorphs. Thus the differences in chemical shifts between androsterone Form 1 and the hemihydrate will largely stem from intermolecular effects, *i.e.* packing, hydrogen bonding and the presence of water molecules in the hemihydrate. The hydrogen bond in Form 1 is rather long (2.956 Å) compared to those involving the carbonyl group in the two independent molecules of the hemihydrate (2.756 Å between molecules and 2.747 Å to a water molecule), which explains why the C17 chemical shift is somewhat lower for Form 1. The hydrogen bond between the hydroxyl group and the water molecule is slightly longer (2.831 Å). The 1.0 ppm crystallographic splitting for C17 in the hemihydrate probably arises from the difference in the nature of the hydrogen bonding in the two independent molecules. One COH group in the hemihydrate hydrogen-bonds to both another androsterone molecule and to water, possibly giving rise to the lower-frequency C3 signal (64.5 ppm).

Beclomethasone dipropionate crystal structures

The ethyl acetate solvate was found to crystallise in the trigonal system, space group $P3_121$. It is confirmed as a monosolvate, with six formula units in the unit cell. The asymmetric unit contains only one BDP molecule and one ethyl acetate molecule. The ethyl acetate molecule is disordered as a whole (fitting well to 50 : 50 occupancy), apparently over two positions without any overlapping atoms. The structure is presumably isomorphous with those of the ethanol and diethanol 1,1,2,2-tetrafluoroalkane-134a solvates (CSD codes INECOT and VATMOT, respectively), though it may be noted that the temperatures at which their diffraction patterns were recorded were higher. In those cases also, the solvent molecules are strongly disordered.

The unit cell information for the anhydrate, the monohydrate and the ethyl acetate solvate is summarised in Table 2.

Table 2 The crystal structures of BDP polymorphs

		Unit cell dimensions							
Form	Space group	*a*/Å	*b*/Å	*c*/Å	*α*/°	*β*/°	*γ*/°	*Z*	*Z′*
Anhydrate[10]	$P2_12_12_1$	14.116	15.542	12.013	90	90	90	4	1
Anhydrate[12a]	$P2_12_12_1$	12.124	14.129	14.838	90	90	90	4	1
Monohydrate[13]	$P2_12_12_1$	14.152	16.268	12.085	90	90	90	4	1
Ethyl acetate solvate[10]	$P3_121$	13.570	13.570	31.081	90	90	120	6	1
Ethyl acetate solvate (this work)	$P3_121$	13.396	13.396	31.023	90	90	120	6	1

[a] There is a different assignment of axes for the anhydrate in refs. 10 and 12.

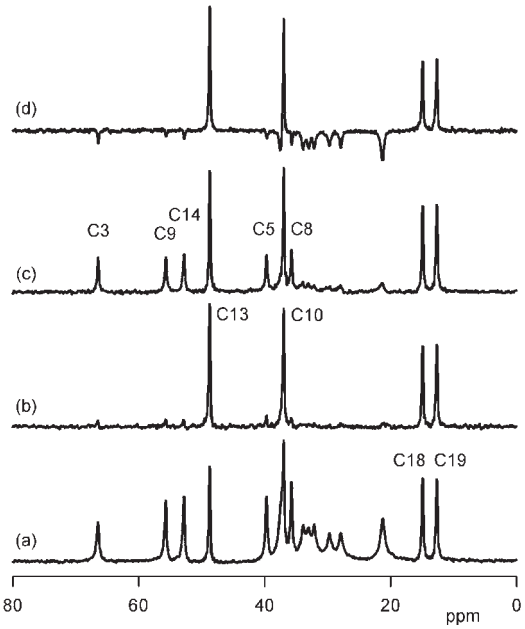

Fig. 5 Carbon-13 75 MHz CP MAS spectra (low-frequency regions only) of androsterone Form 1 obtained with different pulse sequences. (a) Full spectrum. (b) Dipolar dephased spectrum. (c) and (d) SPI spectra with inversion times 25 μs (c) and 50 μs (d) to null CH_2 signals and CH signals, respectively.

As with many steroids, the crystal structures are held together by hydrogen bonds, the natures of which are presented in Table 3 (and also shown in Fig. 7). It is interesting to note that the water molecule in the monohydrate acts both as a bridge between C11 and C22 in the same molecule, and as a bridge to a neighbouring molecule. The host structure of the ethyl acetate solvate is held together by hydrogen bonding between the hydroxyl oxygen at C11 and the carbonyl oxygen at C25 of another molecule, forming a chain structure. The structure of the solvate includes long channels containing the ethyl acetate molecules (see Fig. 8). The diameter of the channels, as estimated from a space-filling model, is about 6 Å and there is no hydrogen bonding between the guest ethyl acetate molecules and the host BDP. It is therefore not clear how the presence of the ethyl acetate molecules stabilise the solvate structure. It seems that the ethyl acetate molecules can easily leave the crystal, and they can be replaced by other solvent species. Attempts at desolvation lead to collapse of the structure, presumably because there is no hydrogen bonding between different chains of the host molecules. The volume per host molecule is significantly higher (803.6 $Å^3$) for the ethyl acetate solvate than it is (635.4 $Å^3$) for the anhydrate.

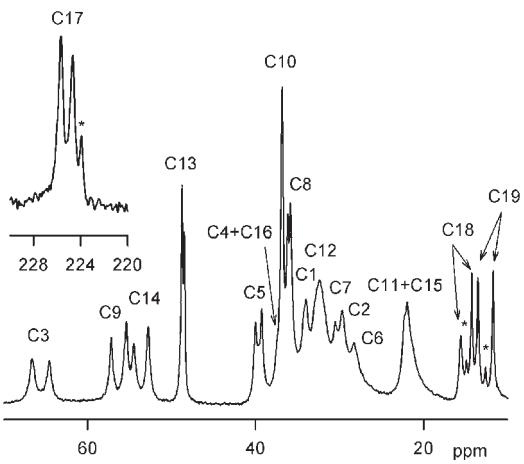

Fig. 6 The 75 MHz ^{13}C CP MAS NMR spectrum for androsterone hemihydrate. Peaks arising from a small amount of Form 1 are indicated by the asterisks.

Beclomethasone dipropionate NMR spectra

The ^{13}C spectra of the three forms of BDP are shown in Fig. 9 and the chemical shifts are listed in Table 4. The precise assignment of the signals was more problematic than in the case of androsterone. The solution-state spectrum of BDP has been assigned[22] using a variety of two-dimensional experiments; the chemical shifts are also given in Table 4 for comparison. These data were used to provide putative assignments for the solid-state spectra. Dipolar dephasing experiments identified the quaternary and methyl carbon signals. However, some assignments in the region 27–37 ppm remain uncertain.

Somewhat unusually, clarification for the assignments for some of the high-frequency signals can be obtained by considering the shielding tensor parameters, since these are known to differ significantly for different chemical types, as shown in Table 5. Thus we have measured the tensor parameters for the BDP forms by spinning sideband analysis. A summary of the results is given in Table 6. Consideration of the data in Table 6 enables us to be sure of the assignments for BDP into the four categories in Table 5.

The values of the shielding anisotropies and asymmetries agree quite well with those predicted from the literature,[23] which gives confidence in the assignments. Thus the carbonyls C20 and C3 have large, positive anisotropies, although the asymmetries are perhaps a little larger than might have been expected. The ester groups (C22 and C25) have anisotropies that are smaller than for the carbonyls and of opposite sign. Also, the asymmetries are below 0.5. The other carbon atoms in the A ring also exhibit some interesting properties. The quaternary sp^2 atom (C5) has, as expected, a large, positive anisotropy and a moderately large asymmetry. The protonated sp^2 atoms (*i.e.* C1, C2 and C4) have, generally, somewhat lower anisotropies than C5. This is to be expected from the predicted values. The sign of the anisotropy of C1 is not significant as the asymmetry is approaching unity. It is interesting to note the similarity in the values for C2 and C4, as might be expected from the similarity in structure. However, it is clear that C1 has a significantly larger anisotropy (whatever the sign) than C2 and C4, and is much closer to that of C5. Perhaps this is not unexpected as C1 and C5 are both at the termini of the conjugated system and so there is some similarity in the environment of these atoms.

However, even when the shielding tensors are known, the signals for C3 and C20 in BDP still cannot be distinguished as both are from carbonyls, and a similar problem occurs for C2 and C4, which are all sp^2-hybridized protonated carbons. The

Table 3 The hydrogen bonding present in BDP polymorphs

Form	Hydrogen bond	O···O/Å	Notes
Anhydrate (WOYPAB)[12]	C(11)–OH···O=C(3)	2.776	Bonding is between adjacent molecules in chains.
Monohydrate (BCLMSN)[13]	C(11)–OH···OH_2	2.770	The C(11) and C(22) are in the same molecule,
	H_2O···O=C(22)	2.881	whilst C(25) is in an adjacent molecule.
	H_2O···O=C(25)	2.917	Each water molecule is involved in three hydrogen bonds.
Ethyl acetate solvate	C(11)–OH···O=C(25)	2.912	Bonding is between adjacent molecules.

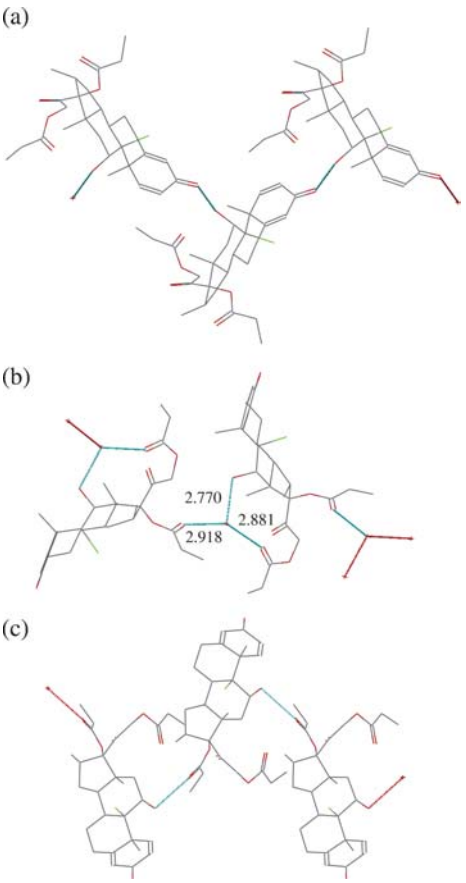

Fig. 7 Hydrogen bonds in the crystal structures of the BDP forms. (a) Anhydrate[12] (O···O distance 2.776 Å). (b) Monohydrate.[13] (c) Ethyl acetate solvate (O···O distance 2.912 Å). The O···O distances for the monohydrate are given in (b) in Å. (c) excludes the ethyl acetate molecules, which are substantially disordered.

atoms C22 and C25 cannot be distinguished, by either chemical shift or shielding anisotropy. On the other hand, a distinction between C22 and C25 is possible in the solvate, as only C25 is involved in hydrogen bonding. As the formation of hydrogen bonds causes a deshielding of the nucleus and a subsequent increase in the chemical shift by about 3 ppm, one would expect C25 to resonate at the higher frequency of the pair, *i.e.* at 179.2 ppm. Moreover, it has been noted[22,23] that the formation of hydrogen bonds causes, along with a high frequency shift, an increase in the asymmetry of the carbonyl group involved, *i.e.* a move away from the nearly axial symmetry ($\eta \sim 0$) that is often observed for carbonyl groups. This adds to the confidence in the assignment of C25 for the ethyl acetate solvate since $\eta = 0.33$ for the signal at 179.2 ppm but is only 0.09 for the one at 175.2 ppm. However, distinguishing C22 and C25 for the other two forms is still problematic.

The hydrogen bond angles are given in Table 7. The figures show that the hydrogen bonds are not at 180° to the C=O bond. Neither are the C=O···H angles 180°, where these can be found (hydrogen atoms are often not located in the crystal structures, but are added in suitable positions later). Thus, deformation of the axial symmetry of the carbonyl is to be expected. As was found for cortisone acetate,[24] the formation of hydrogen bonds has the effect of increasing the value of δ_{22}. This effect is useful in the identification of hydrogen bonds.

Variation of the contact time in a CP MAS experiment can be used to assess mobility of components of the system studied. Any mobility at the molecular level partly averages dipolar coupling, making cross polarization less efficient. Thus ^{13}C signals from carbons at mobile sites may not appear at short contact times, but may be visible under longer contact. This fact was used to assess the mobility of the ethyl acetate molecules in the BDP solvate. Under standard CP conditions (contact time 2 ms), signals from the solvate molecules are not visible at all in the spectrum (see Fig. 9). However, when suitable acquisition parameters are used (*i.e.* a long contact time of 10 ms in the cross polarization experiment or a single pulse experiment), all the peaks from the ethyl acetate molecules can be seen strongly. Clearly the solvate molecules are indeed highly mobile.

The ethyl acetate chemical shifts are given in Table 8 and compared to solution-state data. The influence of incorporation into the solvate structure is small ($\leq$0.6 ppm). As the chemical shifts are so very close, one might suggest that ethyl acetate is in a solution-like environment when in the solvate, *i.e.* having no strong interactions with the steroid and possibly with rapid motion. This conclusion is supported by the narrowness of the ethyl acetate resonances (10–15 Hz, compared to 15–20 Hz for the sharper steroid signals).

Most attempts to study the proton spectra of solid steroids produce no useful results even with magic-angle spinning unless very high spin rates are used, with (at best) very broad and ill-defined peaks. Exceptions are seen for highly mobile parts of samples. The proton spectrum of the ethyl acetate solvate of BDP was readily obtained at a modest spin rate (3.2 kHz) and can be seen to contain three clearly resolved lines, each about 80 Hz wide (see Fig. 10). The observed chemical shifts are presented in Table 9 along with those of ethyl acetate in solution. The spectrum is clearly that of ethyl acetate (with H,H indirect coupling obscured by the linewidths), which confirms that the ethyl acetate molecules have solution-like mobility within the solvate structure. This is consistent with the ethyl acetate molecules occupying the channels within the

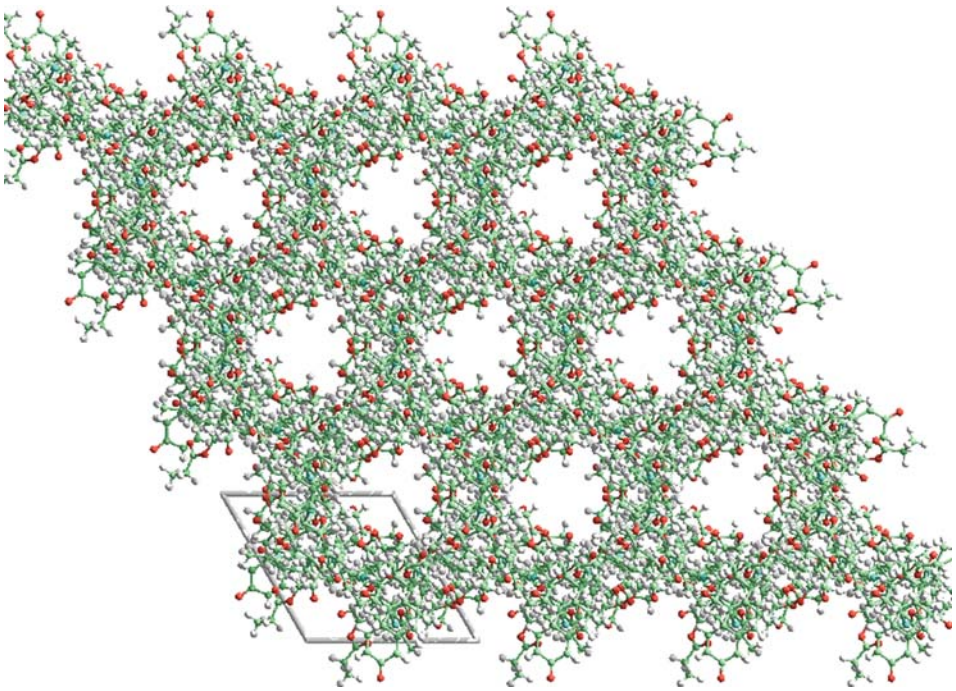

Fig. 8 Channels in the crystal structure of BDP ethyl acetate solvate. The solvent molecules are omitted.

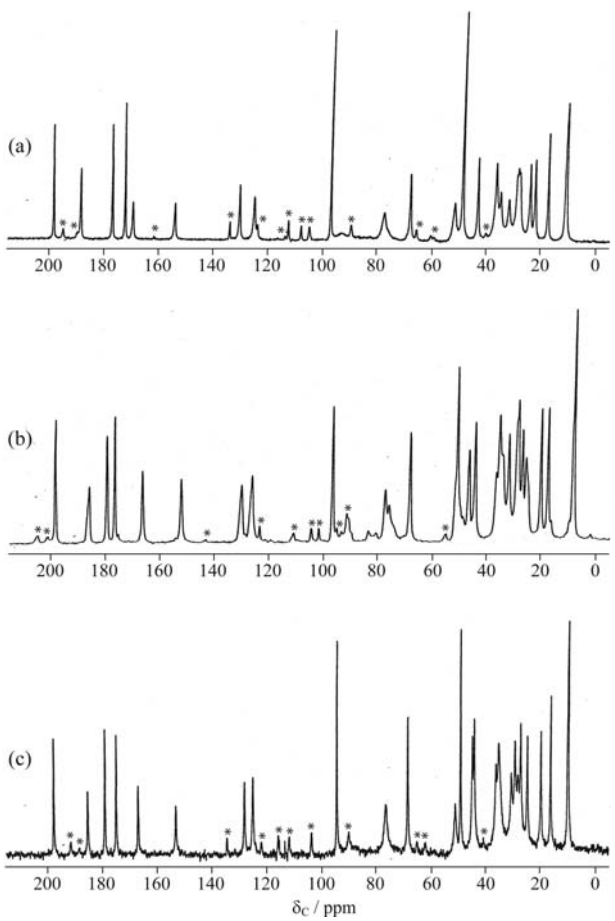

Fig. 9 Carbon-13 CP MAS spectra (obtained at 50 MHz) for the forms of BDP: (a) anhydrate, (b) monohydrate, (c) ethyl acetate solvate. The asterisks indicate spinning sidebands.

crystals and having only weak bonding to the host BDP structure.

BDP is a more complex system than androsterone, in that it contains two substantial sidechains. There are four carbonyl/carboxyl groups capable of hydrogen-bond acceptance and a single hydroxyl group to act as H-bond donor. The crystal structures of the three forms have been determined, so the modes and lengths of hydrogen bonds are known, allowing discussion of their effect on chemical shifts. Comparison of the chemical shifts in the three forms with that in solution makes it clear that there are large high-frequency changes in certain cases. The chemical shifts of atoms potentially involved in hydrogen bonds are presented in Table 10 along with other significant chemical shifts (for atoms conjugated to C3 and hence potentially affected by hydrogen bonding).

C11 is involved (as donor) in hydrogen bonding in all three forms. However, the C3 carbonyl group is involved in hydrogen bonding in only the anhydrate. In this form the chemical shift is significantly higher than for the solution and for the other (non-bonded) solid polymorphs. The chemical shifts of C1 and C5 have also moved to higher frequencies for the anhydrate, compared with the other forms. As with cortisone acetate,[24] the formation of the hydrogen bond at C3 has a significant effect on the atoms at the termini of the conjugated system, *i.e.* the electron density is withdrawn from C1 and C5 *via* the conjugated system. The ester carbons C22 and C25 are not easily distinguished even in the solution state. Therefore, one may only make solid-state assignments for their resonances by assuming that high-frequency shifts occur for the known hydrogen bonds. From the crystal structure of the ethyl acetate solvate it is known that only C25 is involved in hydrogen bonding. Therefore one may assume that this resonance has been shifted to high frequency, to 179.2 ppm for the ethyl acetate solvate. By elimination, the C22 resonance is at 175.2 ppm. This conclusion is supported by the fact that the

Table 4 Solution ($CDCl_3$) and solid-state ^{13}C chemical shifts (δ_C/ppm) for the forms of BDP

		Solid state					
Carbon	Solution	Anhydrate	NQS	Monohydrate	NQS	Ethyl acetate solvate	NQS
20	198.4	198.5	*	198.4	*	197.8	*
3	186.4	188.4	*	185.9	*	185.5	*
25	174.6	177.0	*	179.4	*	179.2	*
22	174.1	172.4	*	176.5	*	175.2	*
5	165.6	169.5	*	166.3	*	167.2	*
1	152.0	154.0		152.1		153.4	
2	129.5	130.2		130.1		128.4	
4	125.1	124.9		126.3		125.4	
17	94.5	96.8	*	96.5	*	94.7	*
9	82.6	87[a]		~83		~90	
11	75.2	77.3		76.6		76.6	
21	67.8	67.8		68.2		68.7	
10	50.0	51.3	*	50.9	*	51.2	*
13	48.2	48.7	*	50.9[b]		49.4	*
16	47.0	48.7[b]		46.5		45.0	
14	43.5	43.1		44.3		44.3	
12	36.5	36.3[a]		36.5		36.3	
8	34.4	36.1[a]		35.4		35.4[b]	
15	34.4	34.7[a]		34.3		30.8	
6	30.6	31.6[a]		32.0		29.5	
7	28.4	28.9[a]		30.0		28.4	
26	27.5	28.3[a]		28.6		27.4	
23	27.1	27.8[a]		27.0			
19	24.4	24.1	*	25.3	*	24.9	*
28	19.3	22.3	*	20.2	*	19.8	*
18	16.9	17.5	*	17.7	*	16.2	*
27	8.9	11.0[ac]	*	8.4[b]	*	9.9[c]	*
24	8.7	10.9[ac]				9.7[c]	*

[a] Data from a 175 MHz spectrum (courtesy of B. Elena and L. Emsley). [b] Double intensity. [c] Assignments to C24 and C27 could be interchanged.

Table 5 Typical shielding anisotropy and asymmetry parameters for different structural groups[23,24]

	Anisotropy/ppm	Asymmetry
Carbonyl	107	0.2
Ester	−82	0.2
sp^2 quaternary carbon	113	0.7
sp^2 protonated carbon	−88	0.8

Table 7 The hydrogen bond angles (°) for the forms of BDP

Anhydrate	C11–O11–O3	115.3
Monohydrate	C22–O22–O(water)	155.0
Monohydrate	C25–O25–O(water)	144.2
Monohydrate	C11–O11–O(water)	111.0
Ethyl acetate solvate	C25–O25–O11	133.6

Table 8 Solid and solution-state ^{13}C chemical shifts (δ_C/ppm) of ethyl acetate in the BDP solvate

Solid state	14.2	20.4	60.3	169.7
Solution[25]	13.8	20.0	60.0	170.3

asymmetry is higher for the higher frequency peak (hydrogen bonding causes deformation of the axial symmetry, thereby increasing η). By contrast, the monohydrate is known to be hydrogen bonded *via* both C22 and C25. If one compares the chemical shifts with those of the ethyl acetate solvate, it seems reasonable that C25 should again resonate at a higher frequency than C22. Note that C22 resonates to higher frequency than in the

Table 6 The average[a] values of the shielding anisotropy and asymmetry for the different forms of BDP

	Anisotropy/ppm			Asymmetry		
Atom	Anhydrate	Monohydrate	Ethyl acetate solvate	Anhydrate	Monohydrate	Ethyl acetate solvate
C20	92	95	90	0.55	0.46	0.43
C3	106	106	100	0.30	0.32	0.36
C25	−91	−88	−84	0.26	0.45	0.33
C22	−77	−86	−85	0.08	0.28	0.09
C5	126	126	116	0.66	0.71	0.66
C1	97	−130	—	0.95	0.96	—
C2	−78	−89	—	0.75	0.66	—
C4	−77	−79	—	0.37	0.52	—

[a] Of measurements at three different spinning speeds.

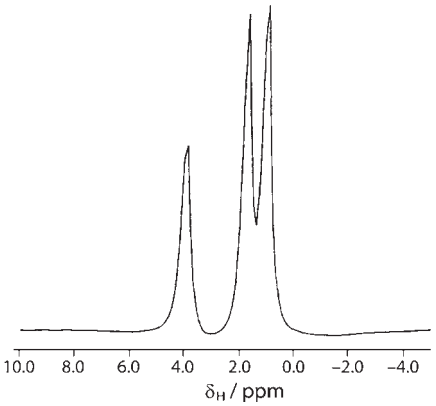

Fig. 10 The 500 MHz MAS proton spectrum of BDP ethyl acetate solvate, obtained using a 4 μs 90° pulse, 4 transients and a spin rate of 3.2 kHz.

Table 9 Solid and solution-state ^{1}H chemical shifts of ethyl acetate in the BDP solvate

Solid state	1.14	1.85	4.08
Solution[25]	1.21	1.93	4.03

Table 10 The chemical shifts (δ_C/ppm) of atoms potentially affected by hydrogen bonding

Carbon	Solution	Anhydrate	Monohydrate	Ethyl acetate solvate
C3	186.4	188.4	185.9	185.5
C20	198.4	198.5	198.4	197.8
C25	174.6	177.0	179.4	179.2
C22	174.1	172.4	176.5	175.2
C1	152.0	154.0	152.1	153.4
C5	165.6	169.5	166.3	167.2
C11	75.2	77.3	76.6	76.6

ethyl acetate solvate, and that both peaks have large asymmetries. For the anhydrate, where neither is involved in hydrogen bonding, the resonances are observed at lower frequency than for the monohydrate. As C25 resonates at the higher frequency of the pair in other solid forms, it is assumed that this is the case again here. Both peaks have small asymmetries, consistent with not being involved in hydrogen bonding. It seems then that the solution-state spectrum is unusual in that C22 and C25 resonate at about the same frequency. In the solid-state spectra, C25 resonates to high frequency of C22 in all three forms.

Residual dipolar coupling in BDP

The spectra of BDP were generally obtained at a relatively low magnetic field (4.7 T). Therefore, it may be expected that the presence of a quadrupolar chlorine atom at C9 would cause some second-order effects in the ^{13}C spectra of the solid forms arising from residual dipolar coupling (not averaged to zero by MAS).[26,27] This is indeed the case, the signals from C9 being very broad and difficult to observe clearly. Resonances of several other carbons are much broader than in general in the spectrum of anhydrous BDP, namely C11 (at *ca.* 77 ppm) which is about 80 Hz wide at half height, and C10 (at *ca.* 51 ppm) which is about 65 Hz wide, compared with the typical line width of 15–20 Hz. These effects are interpreted as arising from long-range C, Cl residual dipolar interactions. This explanation is confirmed by spectra obtained at higher magnetic field for the monohydrate, since the second-order effects are expected to diminish with increasing field. At 125 MHz, for example, the signal for C11 is considerably sharper (and so, presumably, are those for C10 and C8, though these signals are complicated by being accidentally nearly degenerate with those for C13 and C12, respectively).

Obviously the residual dipolar coupling effects on the signal for C9 are much larger than those for C8, C10 and C11, since it is C9 which is directly bonded to the chlorine, giving the maximum value for the C, Cl dipolar interactions. This situation has been investigated in more detail for the monohydrate; the relevant region of the spectrum was carefully examined at 75 and 125 MHz to complement the data at 50 MHz. The results are shown in Fig. 11 and compared to that for 50 MHz. This allowed the shape of the resonance at 50 MHz to be detected (shaded in Fig. 11). At 125 MHz, the C9 signal is a clear doublet, as expected[26–28] when scalar C,Cl coupling can be ignored, the two peaks corresponding to molecules with chlorine spin components $\pm\frac{1}{2}$ and $\pm\frac{3}{2}$ (but each is in principle a powder pattern). Recent measurements at 175 MHz at Lyon (courtesy of B. Elena and L. Emsley) show a narrower doublet (splitting *ca.* 1 ppm) and give a more reliable chemical shift (*ca.* 87 ppm) from the doublet centre. Analysis of residual dipolar coupling patterns is well known.[26] They depend on the quadrupole coupling parameters, the magnitude of the dipolar coupling constant and the mutual orientation of the quadrupole and dipole principal axes. For directly bonded C–Cl groups, it is reasonable to assume that the quadrupole tensor is axially symmetric, with its principal axis lying along the C–Cl bond (so that the two tensors are coaxial). If it is further assumed that isotropic scalar C,Cl coupling can be neglected, then perturbation theory (valid for high magnetic fields) predicts that the magnitude of the doublet splitting, Δ, is:

$$\Delta = 3\chi D'/5\nu_{Cl}$$

where χ is the quadrupole coupling constant, ν_{Cl} is the chlorine resonance frequency and D' is the effective dipolar coupling constant, *i.e.* $D - \Delta J/3$ (with D the dipolar C,Cl coupling constant and ΔJ the anisotropy in the indirect C,Cl coupling constant). Now:

$$D = \mu_o\gamma_C\gamma_{Cl}h/16\pi^3 r_{C-Cl}{}^3$$

where γ_C and γ_{Cl} are the magnetogyric ratios for carbon and chlorine, while r_{C-Cl} is the internuclear distance. If r is known, D can be calculated. In the present case, the published crystal structure for the monohydrate[13] gives r = 1.836 Å, which leads to D = 479 Hz (in magnitude) if the ^{35}Cl nucleus is considered to dominate the second-order effect. The splitting at 125 MHz is measured as 325 Hz. The quadrupole coupling constant, derived from NQR data, is 65 MHz. Use of the equation for Δ yields a value of 210 Hz for ΔJ. This is not an accurate calculation; consideration of reasonable errors in the various parameters suggests ΔJ = 210 ± 110 Hz. In principle there is also an ambiguity in the calculations since the relative signs of D and D' are unknown; however, the alternative to 210 Hz for ΔJ is far too large to be reasonable. Second-order splitting at neighbouring carbons (C8, C10 and C11) can be predicted (using the angles between the relevant internuclear distance and the C9–Cl bond)

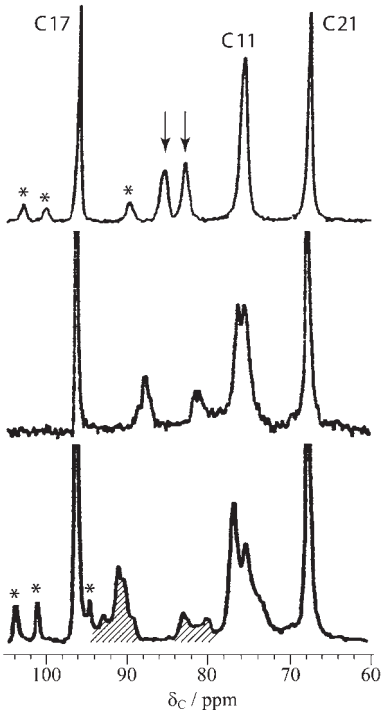

Fig. 11 Partial ^{13}C spectra of BDP monohydrate at 50 (bottom), 75 (middle) and 125 (top) MHz, showing the second-order effect of dipolar coupling of C9 to ^{35}Cl and ^{37}Cl. The hashed area is the C9 signal for 50 MHz, as is the doublet indicated by arrows for the 125 MHz spectrum. The asterisks indicate spinning sidebands.

to be *ca.* 20–25 Hz at 50 MHz, reducing to 9 Hz at 125 MHz. These values are in line with the broadenings observed for these signals. In the case of C11 for the monohydrate at 50 MHz there is an observed splitting of 33 Hz which is likely to be the effect of residual dipolar coupling from the chlorine.

Conclusions

Steroids are widely studied and show a range of activity and structural complexity. In general they tend to crystallise fairly easily, but they often show a tendency to exhibit polymorphism as well as solvation and hydration. These phenomena can have a profound effect on product performance and on the nature of the formulation employed. Single-crystal diffraction data on all forms are often not obtainable, but solid-state NMR offers a high degree of fundamental chemical understanding of both crystalline systems, and those where solvation or desolvation has occurred, the latter often inducing an element of disorder. The present paper illustrates this point for a structurally simple steroid (androsterone) and one with structural complexities introduced by sidechains (BDP). The latter case is of particular interest because of the existence of a range of solvates, with the solvent molecules incorporated into channels. This situation has been revealed by the crystal structure of the ethyl acetate solvate, reported herein. Variable-temperature XRPD measurements of the dehydration of androsterone hemihydrate show that this is a first-order process. Carbon-13 CP MAS spectra have been reported for two forms of androsterone and three of BDP. The signals have been assigned by a variety of techniques and reveal the effects of hydrogen bonding. The difficulty of clearly observing signals for carbons with directly-bonded chlorine is emphasised. Proton spectra and ^{13}C CP spectra obtained at high contact times prove the substantial mobility of the ethyl actetate molecules in the solvate, thus explaining the disorder in the structure revealed by the single-crystal X-ray experiments.

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