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Solid-state NMR study of fluorinated steroids

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ABSTRACT

Solid-state $\{^1H\}^{13}C$ cross-polarization/magic angle spinning (CP/MAS) NMR spectroscopy was performed to analyze two fluorinated steroids, i.e., betamethasone (BMS) and fludrocortisone acetate (FCA), that have fluorine attached to C9, as well as two non-fluorinated analogs, i.e., prednisolone (PRD) and hydrocortisone 21-acetate (HCA). The ^{13}C signals of BMS revealed *multiplet* patterns with splittings of 16–215 Hz, indicating multiple ring conformations, whereas the ^{13}C signals of FCA, HCA, and PRD exhibited only *singlet* patterns, implying a unique conformation. In addition, BMS and FCA exhibited substantial deviation (>3.5 ppm) in approximately half of the ^{13}C signals and significant deviation (>45 ppm) in the $^{13}C9$ signal compared to PRD and HCA, respectively. In this study, we demonstrate that fluorinated steroids, such as BMS and FCA, have steroidal ring conformation(s) that are distinct from non-fluorinated analogs, such as PRD and HCA.

1. Introduction

Fluorine uniquely affects the chemical properties of organic compounds due to strong polar interactions that arise from high electronegativity and small atomic radius [1]. The high electronegativity of fluorine contributes a more electrostatic and less covalent characteristic to C–F bonding, which causes a relatively large dipole and dipole–dipole interactions leading to preferred conformations of organofluorine compounds. Importantly, pharmaceuticals containing fluorinated aromatic groups exhibit enhanced solubility, as well as higher bioavailability and metabolic stability than their non-fluorinated analogs [2–5].

Steroid hormones regulate important physiological processes in humans including maturation, reproduction, development of gonads [6,7], maintenance of blood volume and electrolyte concentration [8], and synthesis of bone and muscle [9,10]. Corticosteroids such as betamethasone (BMS), and prednisolone are anti-inflammatory drugs, and fluorinated corticosteroids mixed with industrial cream have been studied as a potential drug for skin penetration [11,12]. For example, BMS derivatives, such as phosphate, dipropionate, and valerate esters, are commercially available in various pharmaceutical formulations, such as injectables, creams, and aerosols. In addition, it has long been recognized that the fluorination of steroidal compounds enhances its biological activities [13,14]. Therefore, we investigated the effect of fluorination of steroids using solid-state NMR spectroscopy.

The use of solid-state NMR techniques (i.e., $\{^1H\}^{13}C$ cross-polarization [15] and magic angle spinning [16] (CP/MAS)) provides high-resolution spectra comparable to those observed in the solution phase resulting in conformational information that is directly accessible from the spectra. In fact, $\{^1H\}^{13}C$ CP/MAS techniques have been applied to a variety of steroids, including testosterone, hydrocortisone, dehydroepiandrosterone (DHEA), and spironolactone (SPI) [17,18]. The high-resolution CP/MAS spectra recorded for vitamin D [19] and other steroids such as deflazacort [20] and prednisolone *tert*-butylacetate [21] reveal distinct *multiplet* patterns with splittings of 0.2–2.1 ppm (15–160 Hz), which are indicative of various steroid conformations. In addition, solid-state NMR has been applied to DHEA and SPI as well as 17α- and 17β-estradiol to probe their steroidal ring conformations in a lipid environment [18,22].

The goal of this study was to investigate the effect of fluorination upon steroidal ring conformation by solid-state NMR spectroscopy. To this end, we applied ^{13}C solid-state NMR spectroscopy to four steroids including two fluorinated steroids, i.e., BMS and fludrocortisone acetate (FCA) and two non-fluorinated steroids, i.e., prednisolone (PRD) and hydrocortisone 21-acetate (HCA). The molecular structures of these steroids are shown in Scheme 1. Unlike PRD or HCA, both BMS and FCA have fluorine attached to C9. In comparison to PRD, BMS has an extra methyl group attached to C16. Our results indicate that the ^{13}C NMR spectrum of BMS contained a *multiplet* pattern, suggesting BMS is able to adopt multiple

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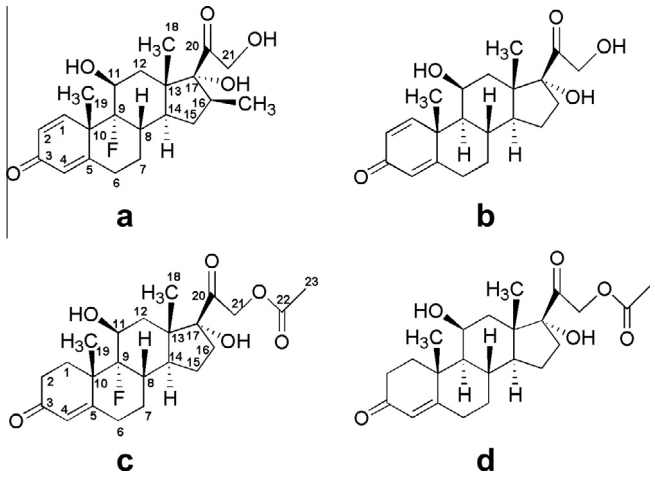

Scheme 1. Molecular structures of betamethasone (BMS, **1a**), prednisolone (PRD, **1b**), fludrocortisone acetate (FCA, **1c**), and hydrocortisone 21-acetate (HCA, **1d**).

steroidal ring conformations. However, the ^{13}C spectra of FCA, PRD, and HCA contained a *singlet* pattern for each of the carbon atoms, indicating that these steroids adopt a single ring conformation. Therefore, our chemical shift isotropy and anisotropy analyses demonstrated that the steroidal ring conformations of these fluorinated steroids are notably distinct from those of their non-fluorinated analogs.

2. Methods

BMS, PRD, FCA, and HCA, were purchased from Sigma–Aldrich (St. Louis, MO). All of the chosen compounds have a high degree of purity that is greater than 98% or nearly 99%, as determined by their ^{1}H and ^{13}C solution NMR spectra and assignments (for details see Supporting information Figs. S3–6 and Table S1). These samples were used without further purification. The ^{19}F fast MAS experiment was performed on a widebore Bruker Avance III 600 MHz NMR spectrometer (Bruker Spectrospin, Rheinstetten, Germany) operating at ^{19}F and ^{1}H Larmor frequencies of 564.76 and 600.21 MHz, respectively. The samples were spun at 20 kHz in the 2.5 mm HFX probe head. A $\pi/2$ pulse of 5 μs was applied for ^{19}F excitation, and the ^{1}H two pulse phase modulation (TPPM) [23] decoupling scheme was applied with a field strength of 70 kHz during acquisition. The FIDs were collected with a spectral width of 250 kHz and 8192 data points. $\{^{1}H\}^{13}C$ CP/MAS NMR spectra were acquired with a Bruker Avance 300 MHz NMR spectrometer equipped with a 4 mm double resonance probe operating at ^{1}H and ^{13}C Larmor frequencies of 300.13 and 75.47 MHz, respectively. The ^{1}H to ^{13}C polarization transfer was optimized to fulfill the Hartman-Hahn matching condition [15]. In the normal CP experiments, the contact-time was set to 1 ms, and *rf* field strengths of 41.0 kHz were chosen for both the ^{1}H and ^{13}C channels. In the ^{1}H filtered experiments, a 50 μs delay was added immediately prior to data acquisition, which allows the ^{1}H-coupled ^{13}C signals to be attenuated under strong $^{1}H-^{13}C$ dipolar coupling such that only the ^{13}C signals of non-protonated carbons were detected. A complementary short CP experiment was performed where a contact time of 30 μs was used for detection of only the protonated carbons. During data acquisition, ^{1}H decoupling by TPPM [23] was applied with *rf* field strength of 79.3 kHz. The powder samples were packed in double-bearing 4-mm zirconium oxide MAS rotors. Unless otherwise specified, ^{13}C spectra were acquired with a sample spinning frequency of 8 kHz regulated by a spinning controller to within ~1 Hz. All of the $\{^{1}H\}^{13}C$ CP/MAS experiments were performed at ambient temperature. The ^{13}C chemical shifts were referenced to the glycine carboxyl carbon signal at 176.4 ppm. For ^{1}H and ^{13}C solution NMR measurements, BMS, PRD, and HCA samples were dissolved in DMSO-*d6*, and FCA was dissolved in MeOD-*d4*, and measured at 25 °C.

To ensure the multiple conformations, BMS powdered sample was analyzed using the Bruker X-ray powder diffractometer (D2 Phaser) equipped with a Cu-Kα radiation (wavelength 0.15406 nm) at 30 kV and 10 mA in the 2θ range of 5–50° in a continuous scanning mode.

3. Results and discussion

3.1. ^{13}C chemical shift assignments

The ^{13}C CP/MAS NMR spectra of two fluorinated steroids, i.e., BMS and FCA, and two non-fluorinated steroids, i.e., PRD and HCA, are shown in Fig. 1a–d. These high-resolution ^{13}C spectra present as fingerprints of these steroidal compounds. A typical spectral feature of these compounds is that more than half of the ^{13}C resonances are localized in a narrow upfield zone (15–60 ppm) because of similarity in their chemical environments. Notably, the ^{13}C signals are somewhat overcrowded and less discernible. However, the remaining ^{13}C resonances in the downfield zone (60–210 ppm) are well dispersed. This spectral feature is similar to that observed in ^{13}C spectra of other previously studied steroids, such as estradiol [22], prednisolone [17], hydrocortisone [17], SPI [24], and DHEA [25–27], and other steroid-related molecules, such as deflazacort [20] and vitamin D [19].

It has been previously demonstrated that the ^{13}C chemical shift assignments obtained in solution are strongly correlated with those in the solid phase. Therefore, solid-state ^{13}C chemical shift assignments may be inferred from solution-state assignments reported in the literature [17]. Given the well-dispersed ^{13}C signals in the downfield zone, ^{13}C chemical shifts assignments were readily determined from solution NMR data. For example, the tertiary ^{13}C resonances of BMS and PRD in the downfield zone (60–210 ppm) that appeared in the order C21, C11, C17, C4, C2, C1, C5, C3, and C20 were assigned accordingly. Fluorinated C9 in both BMS and FCA were resonant at approximately 100 ppm.

In contrast to those in the downfield zone (60–210 ppm), the ^{13}C resonances in the upfield zone (15–60 ppm) overlap and are therefore less discernible. To achieve better resolution, we performed ^{1}H-filtered ^{13}C NMR spectroscopy and normal $\{^{1}H\}^{13}C$ CP/MAS NMR spectroscopy at a short contact time (30 μs). These two experiments were complementary. The ^{1}H-filtered ^{13}C NMR spectroscopy targeted "non-protonated" signals, and the short contact time CP/MAS spectroscopy targeted "protonated" signals. In contrast to those observed in the normal ^{13}C CP/MAS spectra (Fig. 1a), the non-protonated signals of BMS, such as C10 and C13, are well resolved in the resulting ^{1}H-filtered ^{13}C spectra and do not overlap (Fig. 2a–c). Signals that arose from the protonated C14 and C16 are distinct from those in the short contact time CP/MAS spectrum. The selective detection of non-protonated and protonated ^{13}C signals in FCA was also achieved. Due to the improved spectral resolution, we successfully determined the chemical shift resonances of BMS and FCA in the upfield zone with the aid of solution NMR data [28–30]. Complete ^{13}C chemical shift assignments of BMS, PRD, FCA, and HCA are shown in Table 1. The ^{13}C spectra of BMS exhibits a *doublet* pattern with splittings of 16–215 Hz for most of the carbon atoms. Due to the fluorination at C9 in FCA, a significant downfield chemical shift of more than 50 ppm was detected from the $^{13}C9$ resonance, and a $^{13}C-^{19}F$ coupling constant of 178 Hz was determined from the splitting of the $^{13}C9$ *doublet* signal. These observations are consistent with a $^{13}C-^{19}F$ coupling