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Full spectroscopic characterization of two crystal pseudopolymorphic forms of the antiandrogen cortexolone 17α-propionate for topic application☆

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ABSTRACT

Cortexolone-17α-propionate (**CP**) is a topically active antiandrogen useful in the treatment of skin disorders. In the solid state, three anhydrous forms of this drug (**CPI**, **CPII** and **CPIII**) occur, together with one hydrated crystal (**CPW**). The single crystal structure of the monohydrated phase, **CPW**, compared with that of the anhydrous form **CPIII**, shows a markedly different solid state behavior. These latter pseudopolymorphic forms have also been fully characterized by spectroscopic methods.

1. Introduction

The main androgen, testosterone **1** (Chart 1), is active on several target organs not as such but after its transformation into the more active metabolite dihydrotestosterone (DHT, **2**) through a 5α-reductase-catalyzed hydrogenation [1].

The capability of the skin to synthetize and convert androgens is well documented [2] and DHT is involved in many androgen related skin disorders such as hirsutism, alopecia, and acne [2–6]. These disorders can be treated either by inhibiting the 5 α -reductase or by antagonizing the binding of testosterone and DHT at the androgen binding sites [7].

We investigated, in the past, the antiandrogenic activity of a series of 17α-esters of cortexolone (**3**), an intermediate of glucocorticoids biosynthesis endowed only with a weak glucocorticoid activity [8]. Among the examined 17α-esters, propionate (**4**, **CP**) was the most promising in view of a possible application in the treatment of skin disorders: indeed it is topically active but it is devoid of systemic effects.

The antiandrogen activity of **4** depends on its capability to antagonize the binding of androgens at their receptors, as we reported in a recent work [9]. In the same article, the conformational characterization of **4** is described, in comparison with other androgens and antiandrogens, by means of theoretical calculations and NMR spectroscopy.

The possible application of **4** in the treatment of skin disorders, of course, requires a formulation in form of pharmaceutical compositions for topical administration (for example creams, gels, ointments…); these compositions may contain at least a portion of the active substance in solid form, suspended into the vehicle. Moreover, it is well known that one of the more crucial steps in drug substance manufacturing is the final crystallization step, since process parameters must be carefully controlled in order to obtain the desired crystalline form. Both these aspects make mandatory the full characterization of the solid state of **4**. The chemical structure of **4** shows that, apart from the quite rigid tetracyclic system, it has seven bonds that can rotate freely along the chains linked to the penta-atomic ring and this flexibility favors its crystallization into different pseudopolymorphic structures [10]. In fact, in the solid state, three anhydrous forms of this drug (**CPI**, **CPII** and **CPIII**) occur, together with one hydrated crystal (**CPW**), endowed with different features. All these forms have been described in an international patent application [11], and most of them have been patented in several countries/regions. Recently, **CP** was characterized in solution by spectroscopic and theoretical techniques [9], but a detailed investigation of the structural origin of the pseudopolymorphism of this compound is still missing and this will be accessible only by elucidation of the internal arrangement of the molecule in the crystal lattice.

For the solid 17α-propionate, it is reasonable to hypothesize the complete conversion to **CPW** when **CP** is suspended in water in the product formulations [12]. It is important to underline that different local dissolution and solubility rates could lead to remarkable

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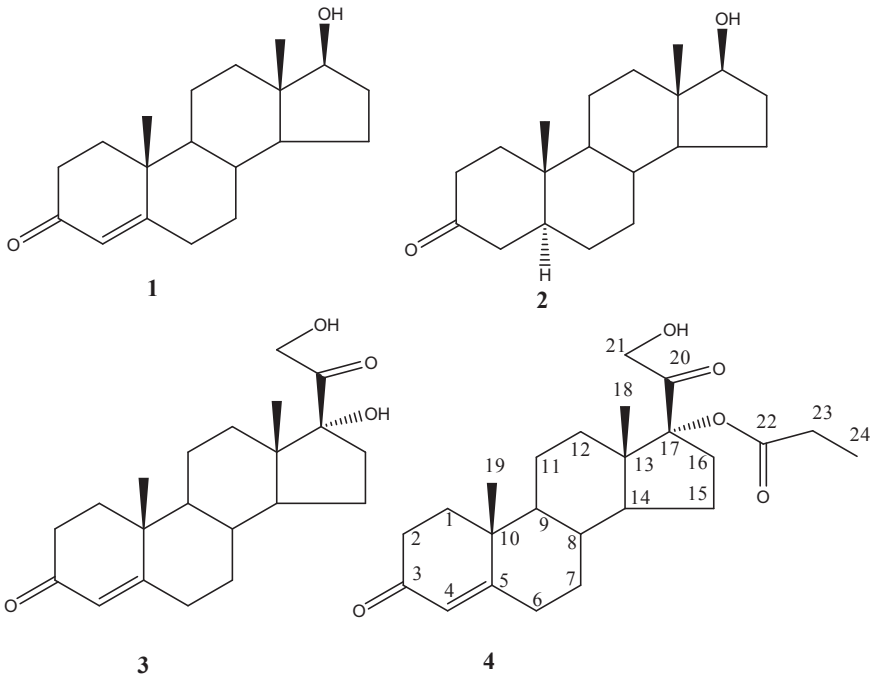

Chart 1. Scheme of testosterone (**1**), dihydrotestosterone (**2**), cortexolone (**3**), cortexolone 17α-propionate (**4**, **CP**) molecules and atomic numbering used in this study.

bioavailability changes, thus affecting the efficacy and safety of the product, especially in long-term use. As the structural changes following the hydration/dehydration process can affect considerably the physicochemical properties of an API, we deemed it useful to address for **CP** the crystallochemical features of two pseudopolymorphs, one anhydrous forms (**CPIII**) and the hydrated one (**CPW**), in order to accurately elucidate their characteristics.

Following our experience in the characterization of compounds endowed with pharmaceutical interest, such as steroids [13,14], in this paper we report a deep study of two isolated solid state forms, which afforded the best quality single crystals for X-ray studies, **CPIII** and **CPW**. We used a combination of different techniques in order to fully investigate their chemical physical features and intrinsic structural properties: Raman spectroscopy, powder X-ray diffraction (PXRD), single crystal X-ray diffraction, Fourier transform infrared (FTIR), spectroscopy thermogravimetric analysis (TGA) and solid-state nuclear magnetic resonance (SS-NMR) spectroscopy. Since the packing arrangements of the anhydrous and of the hydrate are still unknown, this study will also bridge this gap, allowing to completely investigate their crystallographic features, which notably well approximate the hormone conformations adopted in the receptor binding [15,16]. In particular, the crystal packing analysis, compared with solid-state ^{13}C NMR spectroscopy data, could help in defining the factors playing a crucial role for the pseudopolymorph solid-state properties, such as an optimized molecular orientation, and the presence of H-bonds and non H-bonds in the crystal lattice [17]. This important information is the basis for selecting the form able to confer the best product performance [18].

Atom numbering adopted in this work is shown in compound **4** of Chart 1.

2. Experimental

2.1. General

All reagents and solvents were purchased from Sigma-Aldrich.

2.2. Origin of the samples

CP was synthesized following the literature procedure [19]. The anhydrous form which gave single crystals of suitable dimensions for an X-ray analysis was **CPIII**: single crystals were obtained through slow precipitation at room temperature in an acetone/hexane 1:8 solution. The addition of water to this solution led to the obtainment of well-diffracting crystals of **CPW**.

2.3. FTIR spectroscopy

FTIR spectra were registered using a Perkin Elmer (Waltham, MA, USA) FTIR Spectrometer "Spectrum One", in a spectral region between 4000 and 600 cm^{-1} and analyzed by transmittance technique through 32 scanning and 4 cm^{-1} resolution. Solid samples were mixed in a mortar with KBr (1:100) and pressed in a hydraulic press (10 tons) to thin tablets.

2.4. RAMAN spectroscopy

A laser equipped FT-Raman Nicolet Nexus 9600 (ND:YVO4 – 1064 nm) spectrophotometer was used to measure the samples. Spectra were recorded using OMNIC in a spectral region between 98 cm^{-1} and 3700 cm^{-1}, at 4 cm^{-1} resolution and through 256 scans.

2.5. Single crystal X-ray analysis

Crystal data of **CPIII** and **CPW** were collected at room temperature on a Bruker Apex II CCD diffractometer, using graphite-monochromatized Mo-Kα radiation (λ = 0.71073 Å). Intensity data were corrected for Lorentz-polarization effects and for absorption (*SADABS* [20]). For **CPW**, intensity data were collected at room temperature on Enraf Nonius CAD4 diffractometer with Mo-Ka radiation at room temperature. The lattice parameters were determined by least-squares refinements of 25 high angle reflections.

The structures were solved by direct methods (*SIR-97* [21]) and completed by iterative cycles of full-matrix least-squares refinement on F_o^2 and Δ*F* synthesis using the *SHELXL-97* [22] program (*WinGX* suite) [23]. The positions of hydrogen atoms were introduced at calculated positions, in their described geometries and allowed to ride on the attached carbon atom with fixed isotropic thermal parameters (1.2 Ueq of the parent carbon atom).