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Crystal growth and structure of a new hormonal derived compound

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Abstract

The 17α-acetoxy-6-hydroxymethyl-3,20-dioxo-19-nor-pregna-4,6-diene ($C_{23}H_{30}O_5$), a new hormonal derived compound presenting a potential therapeutic interest for a pharmaceutical formulation, raises a problem as it precipitates spontaneously when in contact with an aqueous solution. The solubilization of this potential pharmaceutical drug was investigated to control and/or prevent crystal formation in the presence of water. The solubility limit of this potential drug, either in Cremophor® EL or RH40, was determined to be 2.6% (w/w). In addition, crystal growth of this compound solubilized in Cremophor® and in contact with aqueous environment was undertaken to improve crystal quality and size and subsequently to determine its three dimensional structure. We used a system that allows slow single crystal growth at room temperature. The crystallization system that we set-up comprised an interface between two solutions: one containing the compound solubilized either in Cremophor® RH40 or EL and the other containing water and glycine. The crystal structure was established by single crystal X-ray diffraction analysis. The structure refinement study revealed the presence of one water molecule with strong intermolecular hydrogen bonding leading to an atomic arrangement corresponding to an infinite chain in the [001] direction.

Keywords: Drug; Solubilization; Crystal growth; Single crystal X-ray diffraction; Water

1. Introduction

17α-Acetoxy-6-hydroxymethyl-3,20-dioxo-19-nor-pregna-4,6-diene ($C_{23}H_{30}O_5$) (Tchernatinsky, 1983), that we have called TX219 in the present work, is a hormonal derived product belonging to the progestogen hormone family. This promising pharmaceutical drug causes a problem as it

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recrystallizes spontaneously, as fine and small white needles, when it is in contact with an aqueous media. The dimensions of the crystals thus formed (length < 80 μm) are incompatible with the use of this drug in pharmaceutical formulations. Solubilization of the compound appeared to be a means to control and/or prevent the crystallization phenomenon in the presence of water (Lanquetin, 1993).

The objectives of this study were to find firstly a solubilizer for TX219 in order to prepare a solution which remains stable, at 37 °C, in contact with an aqueous media; secondly, to set-up a system or a method to allow single crystals to grow and reach appropriate size and quality for the determination of the three dimensional structure of TX219 by single crystal X-ray diffraction analysis. The latter part of this work could give further information on the solubility and the stability of this compound in aqueous media.

Crystallization is an important process in industry but this phenomenon can be problematic in the advanced stages of product development as for example the unexpected appearance of crystals especially in pharmaceutical solution formulations. Characterization of newly developed drug substances by single crystal X-ray diffraction is of great importance since crystal structure can determine the stability, the solubility and the dissolution kinetics which in turn may influence the bioavailability of the pharmaceutical compound. In many cases, the compound cannot be crystallized in particles of sufficient size and purity for this type of analysis and it is necessary to solve this problem and to increase the crystal quality and dimensions. This objective can be reach by the aid of crystal growth science.

2. Materials and methods

2.1. Solubilization and solubility limit

Among several potential solubilizers of TX219 tested, Cremophor®, either Cremophor® RH40 (batch number 20-2451) or Cremophor® EL (batch number 27-2030) from BASF, was selected because of its ability to solubilize TX219, its ready availability and especially its biotolerance. Different amounts of TX219 powder were added to a solution of Cremophor® magnetically agitated and maintained at about 40 °C in a water bath. The complete solubilization of the powder in Cremophor® was visually controlled by optical microscopy.

Viscosity measurements have been performed at 25 °C with a Haake Rheostress 75 rheometer using a stainless steel cone/plate geometry (cone diameter: 60 mm; angle: 1°; gap: 0.053 mm). The flowing cycle included three steps (an increase of the shear rate from 0 to 1000 s^{-1} during 200 s, a plateau at 1000 s^{-1} during 60 s and a decrease of the shear rate from 1000 to 0 s^{-1} during 200 s).

2.2. Crystal growth

The aim of this crystal growth study was to obtain a large crystal with suitable dimensions to perform single crystal X-ray diffraction analysis.

The dimensions of crystals, especially their width and thickness, formed spontaneously when TX219 was in contact with water, were too small (length < 80 μm, thickness < 20 μm) and the crystals were of poor quality to perform a single crystal X-ray analysis. Generally, larger crystals are obtained with lower crystal growth rate. In order to have a slow crystal growth rate, it was necessary to supply, as slowly as possible, water molecules, which are responsible for the recrystallization process of this compound, to the growing crystals. Thus, we set-up a system, including two compartments separated by sintered glass, that allowed crystals to grow at the interface between the two phases (see Fig. 1). One compartement contained TX219 solubilized in Cremophor® (EL or RH40) at the solubility limit (2.6% w/w) and water was added to the second compartment. This crystallization system was made of glass and presented a porous interface (sintered glass with pores size diameters from 41 to 100 μm) between the drug solution and the aqueous solution to slow down the diffusion of water towards the drug solution (see Fig. 1).

Three parameters that can influence crystal growth were tested: the temperature, the presence of glycine in the aqueous compartment and the

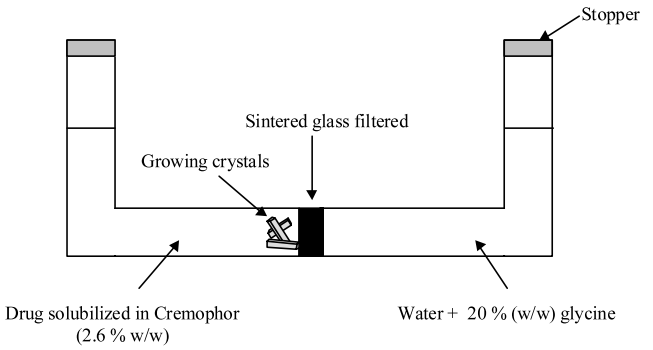

Fig. 1. Schematic representation of the crystallization system (length, 15 cm; height, 7 cm; diameter, 1.5 cm).

type of Cremophor®. The crystal growth experiments were carried out at two different temperatures: 5 and 25 °C. Moreover, it was expected that the addition of glycine in the aqueous compartment (from 0 to 25% w/w) could further slow the diffusion of water through the sintered glass filter. Finally, the viscosity of the solubilizer was tested since, at room temperature, Cremophor® RH40 was a gel whereas Cremophor® EL was a viscous liquid. We determined the viscosity of pure Cremophor® EL and RH40, at 25 °C and for a shear rate of 50 s^{-1}, to be equal to 680 and 1370 mPa s, respectively.

Observation with an optical microscope enabled measurement of crystal size, verification of its quality and eventually selection of a large single crystal for the X-ray diffraction analysis.

2.3. Single crystal X-ray diffraction analysis and 3D-structure determination

X-ray data were collected with a Enraf Kappa-CCD diffractometer. Data were processed using the *hkl* program package (Otwinowski and Minor, 1997). The structure was solved by direct methods using SIR-92 (Altomare et al., 1994). The software used for crystal structure refinement and for the preparation of material for publication was maXus (Mackay et al., 1999). The molecular graphic representation was performed using ORTEP (Johnson, 1976).

3. Results

3.1. Solubilization and solubility limit

Among the different commercial solubilizers of liposoluble substances for the phamaceutical industry, Cremophor® RH40 and EL from BASF seem to be good candidates to solubilize TX219 and we determined the solubility limit of TX219 in Cremophor®, either Cremophor® EL or RH40, to be equal to 2.6% (w/w). This value is largely sufficient to impart a therapeutic activity of this hormonal compound.

3.2. Crystal growth

Crystals were formed in the drug solution compartment and close to the interface as illustrated on Fig. 1. The addition of glycine in the crystallization environment and the use of the crystallization system facilitated the growth of single crystals whereas we obtained smaller crystals (in all three dimensions) by decreasing the temperature to 5 °C.

The largest crystals (especially with the greatest width and thickness) were obtained after 90 days with the following optimum operating conditions: presence of glycine in the aqueous compartment (about 20% of glycine in water w/w), temperature = 25 °C and with Cremophor® RH40 as the solubilizer of TX219. After 90 days, the formed crystals were needle-shaped and transparent with average dimensions of about 10 000 × 800 × 200 μm for the largest ones. Fig. 2 shows

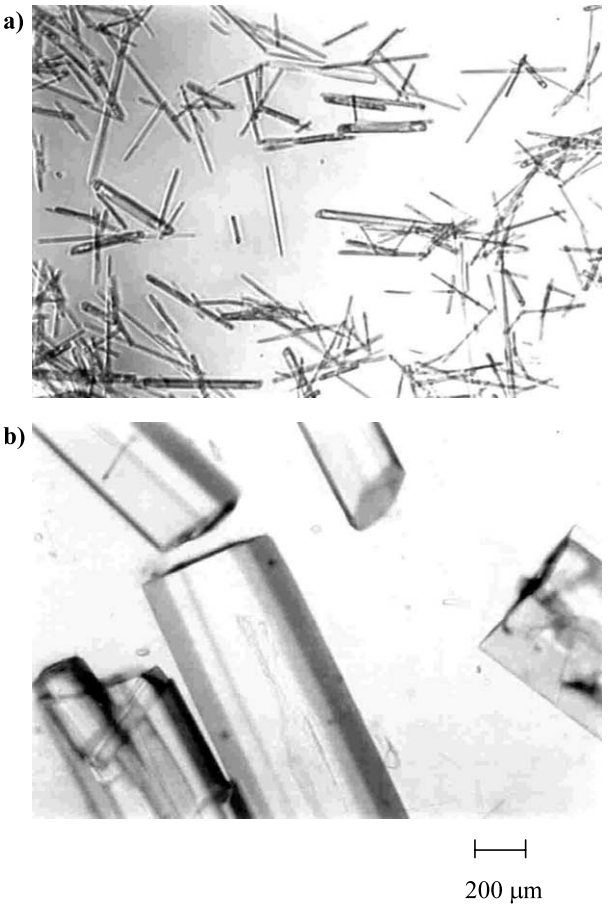

Fig. 2. Photographs of TX219 crystals: (a) formed without the use of the crystallization system but in the presence of glycine; (b) after 90 days of crystal growth in the crystallization system and in the presence of glycine (20% w/w).

photographs of these crystals observed with an optical microscope before and after optimization of the crystallization conditions. If Fig. 2a and b are compared, we note that the use of the crystallization system did not change crystals morphology (needles) but greatly improved crystals size in all dimensions. Fig. 2b shows some damaged crystal parts indicating that these elongated crystals are fragile and must be manipulated carefully. The selection of one of these crystals for single crystal X-ray diffraction analysis was carried out using an optical microscope.

3.3. Single crystal X-ray diffraction analysis and 3D-structure determination

For all non-hydrogen atoms, an anisotropical refinement was performed. The hydrogen atoms were localized on a difference Fourier map and adjusted to 0.96 Å to bonded atom. Their contribution was only introduced in the calculation but not refined.

The crystal data, the data collection and the structure refinement data from the structure determination are presented in Table 1 and the atomic coordinates are shown in Table 2. Further

Table 1
Crystal data and structure refinement data

Crystal data	
Empirical formula	$C_{23}H_{30}O_5 \cdot H_2O$
Formula weight	404.5 g
Temperature	298 K
Colour	Colourless
Wavelength	Mo αK (λ = 0.71073 Å)
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	$a = 8.5329(3)$ Å $b = 13.3480(7)$ Å $c = 19.0800(12)$ Å
Volume	2173.2(2) Å^3
Z	4
Calculated density	1.236 Mg m^{-3}
Absorption coefficient	0.088 mm^{-1}
Crystal morphology	Parallelepipedic (needle)
Cell parameters	From 4995 reflections
θ Range for data collection	0.998–27.485°
Data collection	
4962 Reflections	
3305 Reflections with $I > 2\sigma(I)$	
$\theta_{max} = 27.51°$	
$-11 \le h \le 11$	
$-17 \le k \le 17$	
$-24 \le l \le 24$	
Structure refinement data	
$S = 1.023$	
$R = 0.0743$	
$wR = 0.2006$	
4962 Reflections	
263 Parameters	
Calc. $w = 1/\sigma^2(F_0^2) + (0.1412P)^2) +$ 0.2202P where $P = (F_0^2 + 2F_c^2)/3$	

Table 2
Atomic coordinates and equivalent isotropic displacement parameters

Atom	x/a	y/b	z/c	Ueq (Å^2)
O1	0.3762(5)	−0.4743(3)	1.0974(2)	0.077(2)
O2	0.1617(6)	−0.3956(4)	0.8576(3)	0.103(3)
O3	0.1369(3)	0.1901(2)	0.86742(17)	0.0523(16)
O4	0.1178(5)	0.3202(3)	0.7942(2)	0.080(2)
O5	0.4909(5)	0.3142(3)	0.8299(3)	0.110(3)
O50	−0.0491(6)	0.0107(3)	0.7607(3)	0.086(3)
C1	0.3126(6)	−0.2048(3)	1.0860(3)	0.056(3)
C2	0.3770(6)	−0.2983(4)	1.1199(3)	0.062(3)
C3	0.3640(5)	−0.3893(3)	1.0731(3)	0.055(2)
C4	0.3409(6)	−0.3720(3)	0.9982(3)	0.055(2)
C5	0.3508(5)	−0.2820(3)	0.9678(2)	0.0433(19)
C6	0.3437(5)	−0.2709(3)	0.8916(2)	0.049(2)
C7	0.3657(5)	−0.1813(3)	0.8618(2)	0.050(2)
C8	0.3937(5)	−0.0873(3)	0.9018(2)	0.046(2)
C9	0.3168(5)	−0.0945(3)	0.9749(2)	0.044(2)
C10	0.3790(5)	−0.1882(3)	1.0121(2)	0.044(2)
C11	0.3389(7)	0.0008(4)	1.0173(3)	0.069(3)
C12	0.2866(7)	0.0958(4)	0.9770(3)	0.065(3)
C13	0.3680(5)	0.1035(3)	0.9061(3)	0.053(2)
C14	0.3310(5)	0.0067(3)	0.8640(2)	0.047(2)
C15	0.3836(7)	0.0293(4)	0.7910(3)	0.066(3)
C16	0.3359(7)	0.1391(4)	0.7800(3)	0.067(3)
C17	0.3064(5)	0.1840(3)	0.8539(3)	0.054(2)
C18	0.5449(6)	0.1195(4)	0.9136(4)	0.085(4)
C19	0.3127(6)	−0.3609(4)	0.8468(3)	0.059(3)
C20	0.3802(6)	0.2879(4)	0.8655(3)	0.070(3)
C21	0.3208(9)	0.3495(4)	0.9265(4)	0.091(4)
C22	0.0535(6)	0.2583(4)	0.8319(3)	0.061(3)
C23	−0.1161(7)	0.2495(5)	0.8462(4)	0.087(4)

details (bond lengths, bond angles, torsion angles, anisotropic thermal displacements and hydrogen positions) are available as supplementary material.

In Table 3, the parameters of the most significant potential hydrogen bonds are reported. The distances and angles indicate a particularly strong intermolecular hydrogen bond between O2–H2 and O50 from the water molecule incorporated in the structure. Considering this strong hydrogen bond and according to the result of the analysis of the possible cooperative hydrogen bond network (i.e. the (in)finite O–H···O–H···O–H chain) based on the utilization of PLATON package (Spek, 1990), it is possible to describe the structure as an infinite chain in the [001] direction. The other potential inter- and intramolecular hydrogen bonds reported in Table 3 are weaker and are not discussed here.

The molecular conformation of TX219 was obtained with ORTEP-II program (Johnson, 1976) and is shown in Fig. 3. The geometry of the core of the molecule appears relatively planar.

4. Discussion

4.1. Crystal growth

Among the different parameters tested for the optimization of the crystallization conditions, the use of the crystallization system, the viscosity of Cremophor® and the role of glycine appeared to be the most important.

It should be noted that, in a previous series of TX219 crystal growth experiments (data not shown), the presence of glycine in the crystallization environment made it possible to follow the progress of the crystals growth in real time with the optical microscope but crystals thickness and width were not suitable to perform single crystal X-ray diffraction analysis (see Fig. 2a). Glycine is

Table 3
Potential hydrogen bonds

Donor-H···Acceptor	D···A (Å)	D–H (Å)	H···A (Å)	D–H···A (°)
O2–H2···O50	2.748(9)	0.9794	1.7688	179.37
O50–H50b···O1	2.835(8)	0.97(3)	2.46(2)	102.5(16)
C2–H2a···O4	3.354(8)	0.970(13)	2.566(13)	138.4(10)
C4–H4···O2 (intra)	3.115(10)	0.97	2.5509	117.08
C12–H12a···O3 (intra)	2.754(8)	0.9697	2.3858	101.88
C16–H16b···O5 (intra)	2.838(11)	0.9706	2.3956	107.16
C21–H21b···O4 (intra)	3.079(11)	0.9698	2.5546	113.98

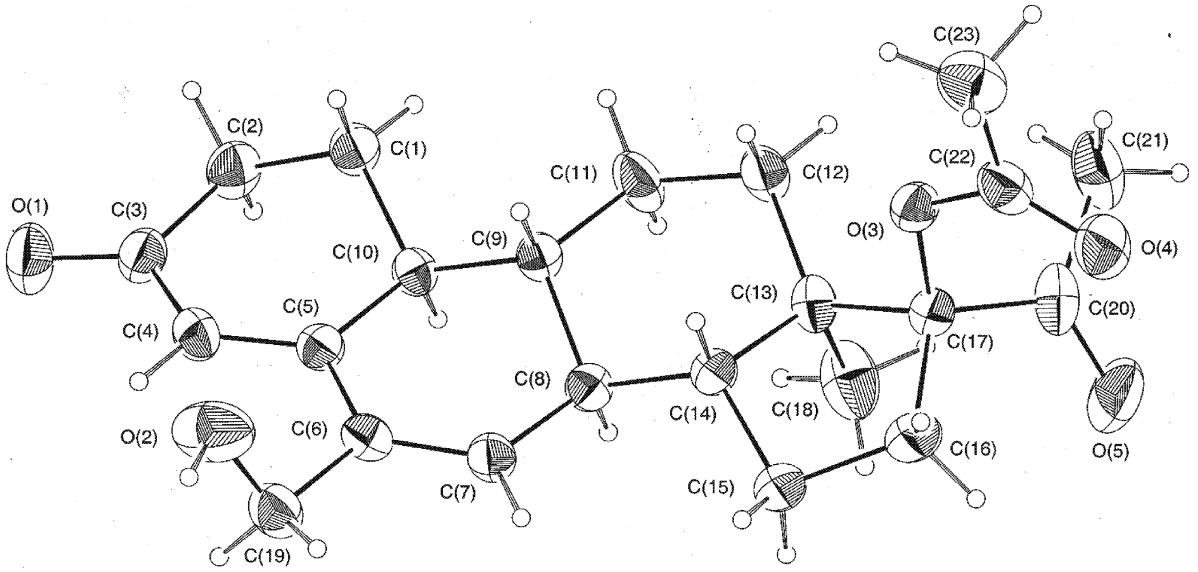

Fig. 3. The conformation of TX219 molecule. Displacement thermal ellipsoids are plotted at the 50% probability level.

a viscous liquid with a hydrophilic character. We determined the viscosity of pure glycine to be equal to 730 mPa s at 25 °C and for a shear rate of 50 s^{-1}. These two properties slowed the supply of water molecules through the interface to the drug solution and thus greatly improved the conditions for single crystal growth.

In addition, at room temperature, we noticed that the viscosity of Cremophor® RH40 was about two times higher than that of Cremophor® EL. This property could slow down the diffusion of water through the sintered glass filter to the drug solution. All of these parameters along with the presence, in the crystallization system, of a porous interface between the two solutions, involved in the crystallization process of TX219, contributed to the conditions for improving crystal size and quality.

4.2. Crystal structure

The establishment of the three dimensional structure of TX219 revealed the importance of water during the crystallization process of this compound since one water molecule is incorporated in the unit pattern.

If we consider the active part of the molecule which is situated along the carbon and oxygen chain O1–C3–C4–C5–C6–C19–O2, it is interesting to notice that the water molecule is incorporated in this area and is involved in a very strong intermolecular hydrogen bond O50···H2–O2 and a weaker one O1···H50–O50 (see in Table 3). Furthermore, as reported above, this very strong intermolecular hydrogen bond is also involved in the atomic arrangement of the molecules as an infinite chain in the [001] direction of the crystal.

In addition, if we examine the short intramolecular bonds analyzed by Platon package (data not shown), we can note several shorter intramolecular bonds in this active area of the molecule especially between C19 and H4, O2 and H4, H4 and H19a.

All these results are in agreement with the work of Cox et al. (2000) indicating that crystal formation of a drug is influenced by both inter- and intra-molecular hydrogen bonding. This implies that, in the present work, the presence of water may be crucial for recrystallization and for the atomic arrangement of the drug molecule. Even if, at the present time, we do not know if the anhydrous crystalline structure of this compound exists, it did certainly present different unit cell parameters and perhaps a different space group.

Even if the formation of such single crystals is reproducible with varying the operating conditions, it is difficult to know if we have obtained the most stable crystalline structure of this novel molecule. The hydrated form of TX219 determined in this study may belong to a pseudopolymorphism (Bauer, 1999). The possibility of obtaining other crystalline forms of this molecule, for example in the presence of other solubilizers, may be checked qualitatively, by powder X-ray diffraction analysis by comparing the positions of the high-intensity peaks on the powder diagram with the high-intensity peak positions calculated from the refined cell parameters determined in this study.

5. Conclusion

We found that TX219 can be solubilized either in Cremophor® EL or RH40 and that its solubility limit is largely sufficient for this compound to have a therapeutic activity.

The crystal growth study revealed the importance of the crystallization system used to improve crystal dimensions and quality and to perform single crystal X-ray diffraction characterization. We determined the crystal structure of this new potential drug molecule and observed the importance of the water molecule in the atomic arrangement of drug molecules, especially in the active area of this compound. Although single crystals of this compound presenting the same hydrated crystalline form were always obtained, this may be related to pseudopolymorphism. It would be of interest to determine if there are polymorphs of this promising drug substances. The search for polymorphic crystals is of great importance in any preformulation study since different crystal structures of the same drug may lead to different bioavailabilities.

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