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Short communication

Identification and proportion of the enantiomers of the antihypertensive drug chlortalidone in its Form II by high quality single-crystal X-ray diffraction data

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ABSTRACT

Chlortalidone (CTD) is a diuretic drug largely used as part of antihypertensive therapies. It is marketed as an equimolar mixture of its enantiomers in the racemic crystal phase named Form I, despite of the higher aqueous solubility of another crystal form. The latter, named Form II, was thought to contain both enantiomers as a racemic conglomerate, *i.e.*, in the form of a mixture of crystals, half of which consists solely of the (R)-enantiomer, the other half the (S)-enantiomer. The occurrence of both enantiomers in individual crystals of CTD Form II was demonstrated in this study. Spontaneous resolution does really occur upon crystallization, as presumed previously even without physical evidence of the (S)-enantiomer. Both (R) and (S)-enantiomers were successfully identified as two domains of a twinned by inversion single crystal of CTD Form II. A reliable Flack parameter of 0.14(4) allowed to determine the proportion of the enantiomers in the crystal, which is formed with 86% of the (R)-enantiomer and 14% of the (S)-enantiomer.

1. Introduction

Chlortalidone (CTD) is a diuretic drug largely used as part of antihypertensive therapies for more than six decades [1]. It is marketed as an equimolar mixture of its enantiomers in its racemic crystal phase named Form I (Fig. 1). Solid state characterization of Form I has begun in the first years of the 1990s [2]. However, its crystal structure was only elucidated in the end of the 2000s [3]. The second crystal form of CTD to be discovered was dated 2006 year [4], but its structure was elucidated in 2013 [5]. Form II has recently gained therapeutic relevance due to its higher solubility in water relative to commercial Form I [6]. Two other crystal forms of CTD are known, Form III, which is polymorphic variant of Form I [3], and Form IV, a racemic nonstoichiometric chloroform solvate of CTD [7].

Contrary to Form I (commercial), Form III, and IV, crystallizing in the centrosymmetric triclinic space group $P\bar{1}$ with one pair of enantiomers per unit cell (racemic crystal) [3,7], Form II has been crystallized in the non-centrosymmetric monoclinic space group $P2_1$ with two (R)-enantiomer units in its unit cell ($Z' = 1$) [5]. Single crystals of Form II suitable for X-ray diffraction structure determination were obtained from slow cooling of a solvothermally processed aqueous solution. Based on the crystal structure, one could think that Form II does contain only the pure CTD (R)-enantiomer. However, high performance liquid chromatography (HPLC) with enantioselective stationary phase and specific optical rotation measurements reveals that CTD Form II is racemic in water solution [5]. After dissolution of Form II in water, the resulting solution had a specific optical rotation of −0.045°, typical of racemate with a negligible (−)-enantiomeric excess, and two chromatographic peaks of similar areas using chiral-AGP column [5]. Based on that, and on the known racemization of CTD in aqueous solution [8,9], it was thought that both enantiomers crystallized in Form II as a racemic conglomerate [5,10,11], *i.e.*, an equimolar mechanical mixture of crystals, each one of which containing only

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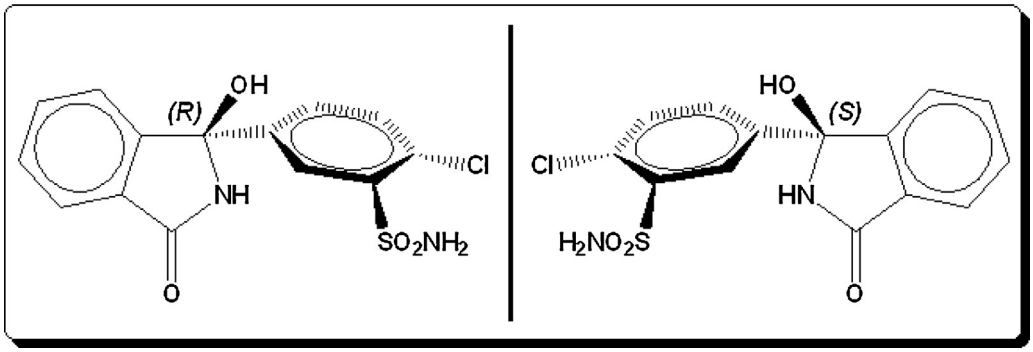

Fig. 1. Chemical structure of the CTD enantiomers.

one of the two enantiomers present in a racemate. Occasionally, these two different crystals could have grown together as one solid body, resembling a single crystal. This phenomenon is refereed as twinning. In this twinning case, the two different crystals (hereinafter named as domains) can have stuck together in a perfectly oriented way resulting in a diffraction pattern that looks like those of a single crystal from the pure enantiomers (with the same unit cell and symmetry data, but not with same scaled intensity data). If this orientation of the two domains into the solid body can be described by inversion symmetry, it is stated that the crystal is twinned by inversion. If twinned by inversion, the Flack parameter x is directly the molar fraction of the minor domain from an inversely-oriented two-domain-structure crystal. The molar fraction of the major domain is therefore equal to $(1-x)$ [10–13].

However, when determining the structure of Fom II, there was some doubt about its conglomerate nature because no crystallographic evidence of (S)-enantiomer could be found. Taken the (R)-enantiomer absolute configuration, Flack parameter was reliably refined to 0.01(4) on X-ray data with good Friedel coverage [12,13]. Therefore, there was only one domain in the measured crystal which is that of the (R)-enantiomer. Here, a crystal of Form II twinned by inversion and composed of two distinguishable enantiomer domains was isolated. The proportion of each domain has been determined through the Flack parameter whose value and its standard uncertainty is consistent with occurrence of two domains of *ca.* 86:14 proportion. Each domain can be interpreted as one crystal of the (R) and (S)-enantiomers, which have stuck together during crystallization. In this way, their proportion is directly the molar ratio of the enantiomers [10–13]. This study meant the proof of concomitant crystallization of both CTD enantiomers as a conglomerate in its Form II, besides an interesting example of a solid-state technique to identification and proportion ascertaining of enantiomers in non-enantiopure samples.

Table 1
High quality crystal data and refinement parameters for the crystal of CTD Form II.

		$CTD_{86:14}$
R:S proportion		86:14
Structural formula		$C_{14}H_{11}ClN_2O_4S$
fw		338.76
Cryst dimensions (mm^3)		$0.24 \times 0.18 \times 0.09$
Cryst syst		Monoclinic
Space group		$P2_1$
Z		2
T (K)		150.0(2)
Unit cell dimensions	a (Å)	8.1030(12)
	b (Å)	7.1284(11)
	c (Å)	12.3035(18)
	β (°)	103.868(6)
V ($Å^3$)		689.95(18)
Calculated density (Mg/m^3)		1.631
Mosaicity (°)		0.63
Absorption coefficient (mm^{-1})		0.448
Absorption correction		Multi-scan $T_{min}/T_{max} = 0.760$
Total time of data collect (h)		28.20
Number of frames collected		10,151
Average redundancy		7.390
q Range for data collection (°)		1.70–28.42
Index ranges	h	−10 to 10
	k	−9 to 9
	l	−16 to 16
Data collected		23,911
Unique reflections		3,438
Unique reflections with $I > 2\sigma(I)$		3,396
Symmetry factors (R_{int}/R_σ)		0.0308/0.0177
Completeness to θ_{max} (%)		99.7
F (000)		348
Parameters refined		211
Goodness-of-fit on F^2		1.040
Final R factors for $I > 2\sigma(I)$		$R_1 = 0.0230$ $wR_2 = 0.0623$
R factors for all data		$R1 = 0.0233$ $wR2 = 0.0625$
Largest diff. peak/hole ($e/Å^3$)		0.269/−0.206
Absolute structure	Flack parameter	0.14(4)
	Friedel pairs	1,576
	Friedel coverage (%)	91.7
CCDC deposit number		1053130

2. Experimental

2.1. Preparation of CTD Form II

As previously described [5], single crystals of Form II were prepared from dissolved raw CTD powder material heated under solvothermal conditions [14]. Non-twinned large crystals were isolated by polarized microscopy for the single-crystal X-ray diffraction (SCXRD) experiment.

2.2. Structure determination

Low temperature SCXRD data (150 K) for CTD form II were measured using a Bruker-AXS Kappa Duo diffractometer (Bruker AXS Inc., Karlsruhe, Germany) with an APEX II CCD detector (see Table 1 for data collect and refinement statistics summary). MoKα radiation from an IμS microsource with multilayer optics was employed. Diffraction images were recorded by ϕ and ω scans set using APEX2 software [15]. Data collect strategy was calculated without merging Friedel pairs to ensure good Friedel coverage. The APEX2 software was also employed to treat the raw dataset for indexing, integrating, reducing and scaling of the reflections. The crystallographic softwares SIR2004 [16] (structure solving) and SHELXL-97 [17] (structure refinement) were also used to deal with scaled data. The structure was solved through identification of all non-hydrogen atoms in asymmetric units directly from the Fourier synthesis of the structure factors after retrieval of their phase using the direct methods. Initial model was refined by full-matrix least squares