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THERMAL AND STRUCTURAL CHARACTERIZATION OF TWO POLYMORPHS OF THE BRONCHODILATOR TULOBUTEROL

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Abstract

Two polymorphs of the bronchodilator tulobuterol (2-chloro-α-[[(1,1-dimethylethyl)-amino] -methyl]benzenemethanol) with melting points differing by ~10 K were isolated and characterized by thermal analysis (HSM, TG, DSC), as well as powder and single crystal X-ray diffraction. Analysis of melting data for Forms 1 and 2 revealed a monotropic relationship, with ΔG_0, the Gibbs free energy difference at the melting temperature of the lower melting form, less than 1 kJ mol^{-1}. This small difference is reconciled with known structural features in the crystals of the two forms. The hydrogen bonding capacity of the tulobuterol molecule is fully utilised in both polymorphs in forming a common trimeric unit via three strong O–H···N interactions. Consequently only weak intermolecular forces characterize the packing of the trimers in the monoclinic polymorph (Form 1, $P2_1/n$, Z =12) and the triclinic polymorph (Form 2, P(–1), Z =6).

Keywords: β-adrenergic agonist, DSC, HSM, monotropy, polymorphism, TG, X-ray analysis

Introduction

Tulobuterol (2-chloro-α-[[(1,1-dimethylethyl)amino]methyl]benzenemethanol, Fig. 1) is a β-adrenergic receptor agonist, structurally related to terbutaline and currently marketed in the form of its hydrochloride salt [1]. In order to extend the chemistry and pharmaceutical applications of tulobuterol, we have converted the commercially available racemic hydrochloride into the racemic free base with the intention of investigating the polymorphism as well as the inclusion of the derived species in cyclodextrins. In this report, we focus on the polymorphism of the free base of tulobuterol, for which only a single melting point of 89–91°C has been reported [1].

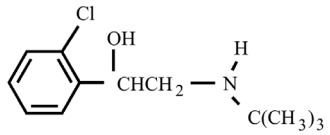

Fig. 1 Chemical structure of the tulobuterol molecule

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Two distinct, unsolvated crystal forms (polymorphs) were identified by thermal analysis and X-ray diffraction techniques. From the pharmaceutical viewpoint, it is desirable to establish whether such polymorphs are enantiotropically or monotropically related, as this determines the nature of possible transitions between them during manufacture or storage [2]. In this study, the thermodynamic relationship between the two polymorphs of tulobuterol was established from accurate melting points and enthalpies of fusion using the method of Yu [3], which also enables extrapolation of the transition temperature. From the available data, a semi-empirical Gibbs free energy *vs.* temperature diagram [4] was constructed. In addition, the salient features of the molecular and crystal structures of the polymorphs revealed by single crystal X-ray diffraction are described and related to the results of the thermal investigation.

The importance of thermal analysis in the characterization of drug polymorphs and solvates was reviewed earlier [5] and recent examples of such studies reported in this journal include detection of dimorphism in salbutamol laurate [6], thermal characterizations of pramocaine (free base and hydrochloride) [7], torasemide (mixed solvate) [8], as well as salts of drug substances [9].

Experimental

Materials and sample preparation

Racemic tulobuterol hydrochloride was obtained from Sigma-Aldrich (MO, USA). One molar equivalent of NaOH was added to an aqueous solution (0.55 M) of the hydrochloride salt and the resulting free base was extracted into dichloromethane. Subsequent solvent evaporation on a rotary evaporator yielded crystals of the free base, designated Form 1. A second polymorph, Form 2, was obtained by dissolving 20 mg of Form 1 in 3 mL water : methanol (1:1 *v*/*v*) at 60°C and allowing crystallization to occur by spontaneous evaporation at 25°C over a two-week period. Conditions for the isolation of Form 2 were critical and any deviations from these usually resulted in the crystallization of Form 1 exclusively or a mixture of Forms 1 and 2.

Analytical methods

Preliminary characterization was performed by HSM on a Linkam TH MS600 system hot stage microscope coupled to a Linkam TP92 temperature control unit. Thermogravimetry (TG) was performed on a PerkinElmer TG7 (PC7 series) instrument with sample masses in the range 1–5 mg. Differential scanning calorimetry (DSC) experiments were performed on a PerkinElmer DSC7 instrument with sample masses in the range 1–5 mg. All TG and DSC runs were recorded at a heating rate of 10 K min^{-1} over a temperature range of 30–100°C, using a dry nitrogen purge with a flow rate of 30 mL min^{-1}. Extrapolated melting points and enthalpies of fusion were obtained by averaging multiple measurements (n =4).

Single crystals were mounted on a Nonius Kappa CCD four-circle diffractometer and intensity data were collected with MoK_α X-rays (λ =0.71069 Å)

using suitable combinations of ϕ- and ω-scans. Unit cell parameters were initially determined at 294 K and each crystal was subsequently cooled to 173 K to optimise diffraction quality. For both crystals, unit cell dimensions refined using low-temperature intensity measurements were in accordance with those recorded under ambient conditions, confirming that no phase changes had occurred. Data-reduction, structure solution and least-squares refinement proceeded routinely [10, 11]. All H atoms were located and were generally placed in idealized positions in a riding model with U_{iso}=1.2 times those of their parent atoms. H atoms of the amine and hydroxyl groups were allowed to refine freely subject only to appropriate distance constraints. In the final refinements (against F^2) least-squares weights of the form $w=1/[\sigma^2(F_0^2)+(aP)^2+bP]$ where $P=[\max(F_0^2, 0)+2F_c^2]/3$ were employed.

Powder X-ray diffraction patterns were recorded on a Philips PW1050/25 vertical goniometer using Ni-filtered CuK_α-radiation (λ =1.5418 Å). Step scans of 2 s were carried out at 0.1° 2θ intervals in the 2θ-range 5–40°. FTIR spectra were recorded on a PerkinElmer 983 FTIR spectrophotometer over the range 4000–600 cm^{-1}. Samples were prepared as nujol mulls and the percentage transmission recorded *vs*. wavenumber.

Results and discussion

Thermal analysis

Crystals of Form 1 display monoclinic prismatic morphology while those of Form 2 are equant triclinic blocks (Fig. 2). The HSM micrographs show commencement of fusion of Form 2 at ~83°C and Form 1 at ~94°C. Thermogravimetric traces (not shown) yielded zero mass loss for samples of both crystal forms in the temperature range 30–100°C. DSC traces of Forms 1 and 2 (Figs 3a, b) show only a single endotherm corresponding to fusion in accordance with HSM observations. No indications of desolvation, phase transformations or interconversions were evident from

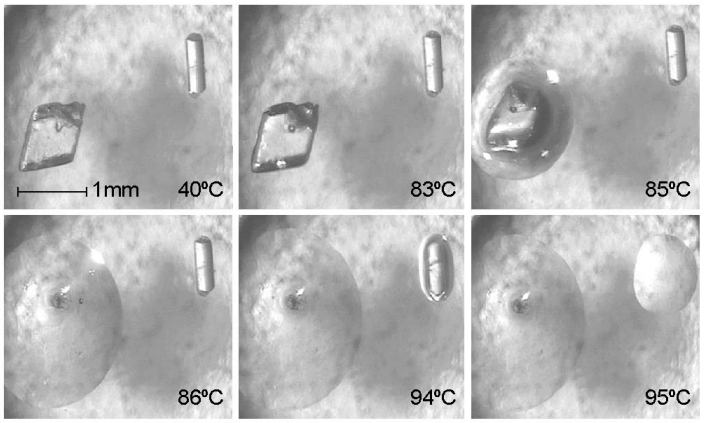

Fig. 2 HSM micrographs showing successive melting of Form]2 (left) and Form 1 (right) of tulobuterol

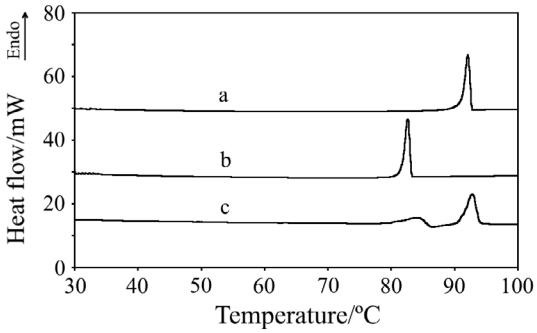

Fig. 3 DSC traces for a – Form 1, b – Form 2, c – Form 2 contaminated with Form 1

either HSM or DSC techniques when pure samples were investigated. In addition, the powder X-ray patterns (Fig. 4) are distinct, confirming that Forms 1 and 2 are polymorphs. The three most intense peaks occur at 2θ positions 10.1, 18.0 and 18.5° for Form 1 and at 9.2, 17.8 and 24.9° for Form 2.

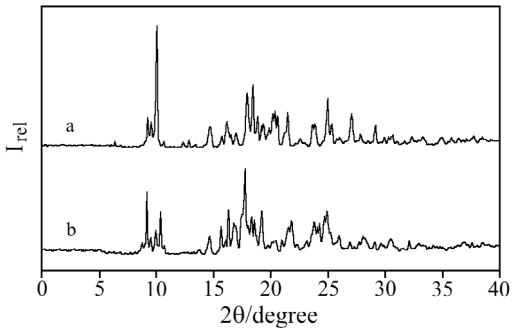

Fig. 4 PXRD traces of a – Form 1, b – Form 2

Table 1 lists the extrapolated onset melting points (T_f) and enthalpies of fusion (ΔH_f) derived from Fig. 3. On the basis of the Heat of Fusion Rule of Burger and Ramberger [12], since the higher melting form has the higher enthalpy of fusion, Forms 1 and 2 thus appear to be monotropically related under normal pressure. Form 1 is thus thermodynamically more stable than Form 2 at all temperatures up to the transition temperature (T_{tr}), which in this case is virtual, lying above the melting

Table 1 Thermal data[a] for polymorphs of tulobuterol

Form	t_f/°C	T_f/K	ΔH_f/J g^{-1}	ΔH_f/kJ mol^{-1}
1	90.9(0.2)	364.0(0.2)	119.0(1.3)	27.1(0.3)
2	80.0(0.1)	354.0(0.1)	111.5(0.6)	25.4(0.1)

[a]Data reported as mean (SD), $n = 4$

points of either polymorph. An estimate of T_{tr} was obtained using the treatment of Yu [3], which allows the calculation of the Gibbs free energy difference at the melting temperature of the lower melting form and extrapolation of ΔG to other temperatures. In particular, when ΔG is zero, the corresponding temperature is the required T_{tr}. Specifically, Yu showed that if the enthalpy, entropy and free energy changes for the conversion of the lower melting form (2) to the higher melting form (1) occurring at $T_{f,2}$ (Eq. 1), are denoted ΔH_0, ΔS_0, ΔG_0 respectively,

$$\text{Form 2 } (T_{f,2}) \rightarrow \text{Form 1 } (T_{f,2}) \quad (1)$$

then $$\Delta H_0=\Delta H_{f,2}-\Delta H_{f,1}+(C_{p,L}-C_{p,1})(T_{f,1}-T_{f,2}) \quad (2)$$

$$\Delta S_0=\Delta H_{f,2}/T_{f,2}-\Delta H_{f,1}/T_{f,1}+(C_{p,L}-C_{p,1})\ln(T_{f,1}/T_{f,2}) \quad (3)$$

and $$\Delta G_0=\Delta H_{f,1}(T_{f,2}/T_{f,1}-1)+(C_{p,L}-C_{p,1})\,[T_{f,1}-T_{f,2}-T_{f,2}\ln(T_{f,1}/T_{f,2})] \quad (4)$$

In these expressions, $C_{p,L}-C_{p,1}$ is the difference between the heat capacities of the supercooled liquid L and Form 1 at a temperature between $T_{f,1}$ and $T_{f,2}$, and is estimated to have an average value of $0.003(\Delta H_{f,1})$ [3]. Assuming a linear dependence of $\Delta G(T)$,

$$\Delta G(T) = \Delta G_0 - \Delta S_0\,(T-T_{f,2}) \quad (5)$$

the condition $$\Delta G(T_{tr})=0 \quad (6)$$

yields $$T_{tr}=\Delta H_0/\Delta S_0 \quad (7)$$

Application of these relationships to the data in Table 1 gives the following thermodynamic values for the transformation shown in Eq. (1): $\Delta H_0= -887$ J mol^{-1}, $\Delta S_0= -0.434$ J K^{-1} mol^{-1}, and $\Delta G_0= -733$ J mol^{-1}. The transition temperature is estimated from Eq. (7) as $T_{tr} \sim 2040$ K. (This should, however, be considered a nominal value with a large error owing to the sensitivity of T_{tr} to the errors in melting points and enthalpies of fusion. Using the SDs reported for the T_f values in Table 1, an error as high as ±500 K results for T_{tr}. An error of even larger magnitude in T_{tr} arises when the SDs in ΔH_f reported in Table 1 are taken into account). The small entropy difference implies only a small difference in the slopes of the *G vs. T* curves for Forms 1 and 2, consistent with the high value of T_{tr}. Extrapolated values of T_{tr} for monotropically related polymorphs exceeding that predicted here have been reported [3]. The monotropic relationship established for Forms 1 and 2 and the available thermal data allowed construction of the semi-empirical Gibbs free energy *vs.* temperature diagram shown in Fig. 5.

Several batches of Form 2 crystals exhibited the DSC behaviour shown in Fig. 3c, in which there is an initial endotherm (80–85°C) followed by a small exotherm (85–90°C) and a final endotherm (90–94°C). On HSM, the clear crystals commenced melting at ~80°C but rapidly recrystallized, as indicated by their becoming opaque, and finally melted at ~90°C (i.e. the *m.p.* of Form 1). It was suspected that contamination of these batches with Form 1 might have accounted for this behaviour. To test this hypothesis, a large single crystal, shown by single crystal X-ray

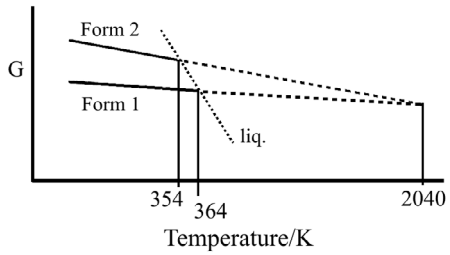

Fig. 5 Semi-empirical *G vs. T* diagram for Forms 1 and 2

analysis to be Form 2, was halved. One fragment, used as a control, yielded the same trace as in Fig. 3b, with only a single endotherm. The other fragment was deliberately contaminated by sprinkling grains of Form 1 on the surface (7% of total mass of analysed sample). This yielded a trace identical to that of Fig. 3c, supporting the assumption that Form 1, as a contaminant of Form 2, probably acted as a seed, promoting transformation of Form 2 to Form 1. This interpretation is consistent with the monotropic relationship inferred above, according to which the transition Form 2 $\rightarrow$ Form 1 is thermodynamically favoured. It was found that contamination at a level as low as 1% led to this behaviour. Using appropriate tests, heating rate variations and crystallite sizes were eliminated as possible factors for the dual behaviour of samples of Form 2.

Crystal structures of the polymorphs

Table 2 lists crystal data for Forms 1 and 2. The crystal morphologies described above (monoclinic prisms for Form 1, triclinic blocks for Form 2) are consistent with the crystal systems established by X-ray diffraction. The values of Z (12 for Form 1 and 6 for Form 2) indicated three molecules in the asymmetric unit of each polymorph. Taking into account the presence of both strong hydrogen bonding donor and acceptor groups in the tulobuterol molecule (Fig. 1), this finding suggested a crystallographic asymmetric unit comprising three molecules associated by hydrogen bonding. This was confirmed by the X-ray analyses and Fig. 6 shows the structure of the trimer as it occurs in Form 1 as representative, that in Form 2 being practically superimposable on it. A homodromic array of O–H···N hydrogen bonds (O···N range 2.751(2)–2.822(2) Å in Form 1, 2.765(2)–2.839(2) Å in Form 2) links the three molecules comprising the trimer. As the tulobuterol investigated was racemic, a trimer would necessarily contain two molecules with the same absolute configuration at the chiral centre and a third with the opposite configuration. Figure 6 shows the trimer containing the *S-, R-, R-* combination, the centrosymmetric space group requiring also the presence of a trimer *R-, S-, S-* in the crystal. (Steric considerations and simple modelling indicate that a trimer formed by three tulobuterol molecules of the same chirality is not feasible if advantage is to be taken of the formation of three stabilizing hydrogen bonds). The common occurrence of the trimeric motif in both polymorphs,

Table 2 Crystal data and refinement parameters for tulobuterol polymorphs

Parameter	Form 1	Form 2
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/n$	P(–1)
a/Å	10.802(1)	11.004(2)
b/Å	18.949(2)	11.896(2)
c/Å	19.649(1)	16.844(3)
α/°	90.0	91.28(3)
β/°	94.84(1)	94.76(3)
γ/°	90.0	115.32(3)
V/Å^3	4007.6(6)	1968.7(7)
Z	12	6
D_c/g cm^{-3}	1.132	1.152
μ/mm^{-1}	0.26	0.27
T/K	173(2)	173(2)
Reflections msd.	12617	10777
Data/restraints/parameters	6785/6/434	7041/5/439
R_1 [$I > 2\sigma(I)$]	0.0464	0.0398
wR2 [$I > 2\sigma(I)$]	0.1009	0.0921
S (goodness-of-fit)	1.015	1.029
Max. shift/e.s.d.	< 0.001	< 0.001
$\Delta\rho_{max}$/e Å^{-3}	0.30	0.40

with virtually identical molecular conformations and hydrogen bond geometries, reflects the inherent stability of this motif.

As the hydrogen bonding capacity of the tulobuterol molecule is fully utilised in trimer formation in both polymorphs, crystal assembly involves only softer (van der Waals, C–H···π) attractive interactions between the trimeric units. Figure 7 shows the

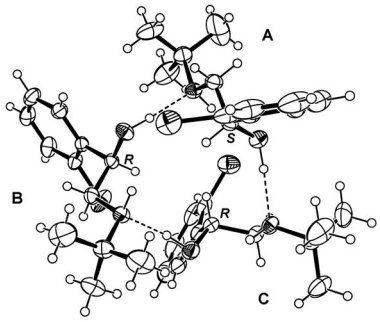

Fig. 6 Structure of the tulobuterol trimer in Form 1. Thermal ellipsoids are drawn at the 40% probability level

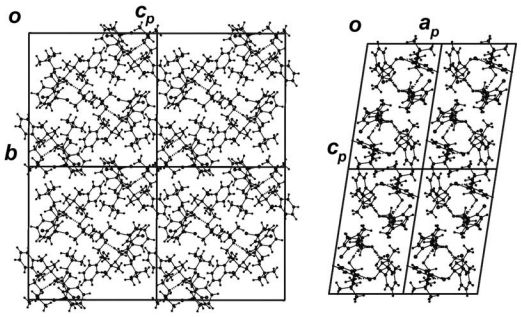

Fig. 7 Projections of the crystal packing arrangements in Form 1 (left) and Form 2 (right). The contents of four unit cells are drawn for each polymorph

crystal packing in the two forms. Four trimers occupy the unit cell in the monoclinic crystal (Form 1) while the triclinic crystal (Form 2) contains two trimers per unit cell. In the orientation shown for Form 2, the trimers are viewed normal to their hydrogen bonded planes. Detailed analysis of the intermolecular interactions revealed that the trimer in Form 1 engages in six C–H···π interactions with neighbouring trimers, while for Form 2 there are four interactions of this type. The low melting points recorded for the two polymorphs are consistent with the presence of only weak inter-trimer interactions.

FTIR spectra for Forms 1 and 2 (not shown) are indistinguishable, as expected from the above description of their molecular and crystal structures. In particular, in the range 3000–3600 cm^{-1}, which is often diagnostic for polymorphs containing –OH and –NH functions, only one peak appears in the spectra of both crystals. This occurs at 3288 cm^{-1} and is equally intense for both forms. This is readily accounted for by the observed uniform environments of the –OH and –NH groups in the trimers.

Finally, despite very different symmetry arrangements in Forms 1 and 2, the general uniformity of the inter-trimer interactions in the crystals is consistent with the relatively small differences in the values of the thermodynamic parameters established by thermal analysis.

Conclusions

The previously reported melting point of 89–91°C for tulobuterol [1] has been shown in this study to correspond with that of the thermodynamically stable member of a dimorphic pair, the less stable form melting at 81°C. Experimental solubilities of the two polymorphs have not been determined, but the small calculated value of ΔG_0 (<1 kJ mol^{-1}) indicates that they should not be significantly different. Nevertheless, from the pharmaceutical viewpoint, an in vivo study would be the most appropriate way to establish whether the polymorphism has any implications for bioavailability. Polymorphism could possibly have an impact in the context of cyclodextrin inclusion

of the free base when solid phases are employed for complex preparation, as in kneading and co-grinding procedures. This aspect is currently being investigated.

Regarding the occurrence of 'non-transforming' (Fig. 3b) and 'transforming' (Fig. 3c) crystals of Form 2, final proof for the hypothesis of surface contamination by Form 1 in the latter case was afforded by single crystal X-ray diffraction of specimens of both types of crystal. The resulting crystal structures were superimposable.

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