Preparation and Characterization of Two Crystalline Forms of 4-Amino-5-chloro-2-methoxy-N-[(2S,4S)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide (TKS159)

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For 4-amino-5-chloro-2-methoxy-N-[(2S,4S)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide (TKS159), two polymorphs, forms α and β, were prepared and characterized by means of X-ray powder diffraction, thermal analysis, infrared spectroscopy and 13C-NMR spectroscopy, both in the solution and solid phases. The X-ray powder diffraction analysis gave different patterns for forms α and β. In the thermogravimetry and differential thermal analysis profiles, form β exhibited characteristic endo- and exothermic peaks at 112.7 °C and 116.2 °C, respectively, due to the partial melting-induced phase transition to form α without accompanying weight loss, and these were followed by an additional endothermic peak at 138.2 °C due to fusion. For form α, only an endothermic peak at 137.8 °C due to fusion was observed. The IR spectroscopic analyses of forms α and β gave different absorption bands assigned to N–H and O–H stretching, N–H bending, and C=O stretching vibrations. From the data obtained by thermal analysis, form α was shown to be thermodynamically more stable than form β.

Key words : TKS159; polymorph; X-ray powder diffraction; thermal analysis; 13C-NMR spectroscopy; 4-amino-5-chloro-2-methoxy-N-[(2S,4S)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide

Polymorphism is a critical issue in pharmaceutical development, since physical and chemical properties are known to vary with crystalline forms.1) Polymorphs are regarded as thermodynamically different phases, which possess different thermal properties and spectroscopic profiles. Differences in thermodynamic properties can affect the stability, solubility, and bioavailability of pharmaceuticals. Thus, the polymorphism of various pharmaceuticals, e.g. indomethacin,2) fuzonazole,3) lomeridine,4) etc., has been investigated, and the choice of crystalline form of drug substance is one of the important aspects in drug development.

4-Amino-5-chloro-2-methoxy-N-[(2S,4S)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide (TKS159, I) developed by Teikoku Chemical Industries Co., Ltd., is a potential gastroprotective agent,5) and clinical study is ongoing. The chemical structure of TKS159 and atomic numbering used in this study are shown in Chart 1. The absolute configuration determined by single crystal X-ray diffraction analysis was reported in a previous paper.6) Crystals which were obtained by recrystallization from several solvents were characterized by various spectroscopic methods, and eventually two kinds of crystalline forms were clearly distinguished. The crystalline form that was recrystallized from aqueous ethanol, water, ethyl acetate, or methyl ethyl ketone was designated as form α, while that recrystallized from acetone was designated as form β. In this paper, we will report the preparation and characterization of the two polymorphs, forms α and β, of I. They were characterized by means of X-ray powder diffraction analysis, thermal analysis, infrared (IR) spectroscopy and 13C-NMR spectroscopy, both in the solution and solid states, and by elemental analysis.

Results and Discussion

First, the two crystalline forms were subjected to elemental analysis. The results of the elemental analysis for the two crystalline forms agreed with the calculated value without any solvents in crystals.

The X-ray powder diffraction patterns of form α and form β are shown in Fig. 1. Sharp and intense X-ray diffraction peaks of form α occurred at 2θ = 8.0, 13.3, 20.4, and 23.4°, as shown in Fig. 1a, while those of form β were observed at 2θ = 7.5, 9.9, 15.1, 22.9, 23.9, 24.5, and 26.4°, as shown in Fig. 1b. The distinctly different patterns observed for forms α and β are taken as definitive evidence in support of the existence of polymorphs.

The thermogravimetry–differential thermal analysis (TGA-DTA) thermograms of the two polymorphs are shown in Fig. 2. The single endothermic peak at 137.8 °C for form α in Fig. 2a is attributable to fusion. In contrast, form β showed closely located endo- and exothermic peaks at 112.7 and 116.2 °C, respectively, followed by an additional endothermic peak at 138.2 °C, as shown in Fig. 2b. Over the temperature range of 25–200 °C, the TG curves for both forms showed no weight loss. Since form β finally gave the same melting point as form α, the successive endo- and exothermic peaks
Fig. 1. X-Ray Diffraction Patterns of Forms \(\alpha\) (a) and \(\beta\) (b)

Fig. 2. TG and DTA Curves for Forms \(\alpha\) (a) and \(\beta\) (b) and Form \(\beta\) after Heating to 120\(^\circ\)C (c)

peculiar to form \(\beta\) are most probably attributable to the phase transition from form \(\beta\) to \(\alpha\), presumably initiated by the partial melting of form \(\beta\), as indicated by the endothermic peak at 112.7\(^\circ\)C, which is suddenly switched to an exothermic peak at 116.2\(^\circ\)C. A similar phenomenon has been reported for one of the polymorphic forms of indomethacin by Lin.\(^8\)

Additionally, when form \(\beta\) was heated to 120\(^\circ\)C, then cooled to room temperature, and re-heated again from 25 to 200\(^\circ\)C, the endo- and exothermic peaks between 110 and 120\(^\circ\)C completely disappeared, and only the endothermic peak at 138.1\(^\circ\)C appeared, as shown in Fig. 2c. This thermal profile of form \(\beta\) is irreversible. These observations strongly support a causative role of the phase transition.\(^8\)

The IR spectra of forms \(\alpha\) and \(\beta\) are shown in Fig. 3. Strong absorption bands due to the N–H and O–H stretching vibrations in the amino and hydroxyl groups are centered at 3421 and 3387 cm\(^{-1}\) for form \(\alpha\), as shown in Figs. 3a and 3d, whereas they shifted to 3453 and 3401 cm\(^{-1}\), respectively, for form \(\beta\), as shown in Figs. 3b and 3d. Absorption bands assigned to the N–H bending vibrations are centered at 1546 and 1637 cm\(^{-1}\) for form \(\alpha\). For form \(\beta\), these shift to 1532 and 1626 cm\(^{-1}\), respectively. The lower frequencies in the N–H and O–H stretching vibrations in the amino and hydroxyl groups, and the higher frequency in the N–H bending vibrations in the amide and amino groups are characteristic of the presence of a stronger inter- and/or intramolecular hydrogen bond.\(^9\)

For form \(\alpha\), the absorption band observed at 1626 cm\(^{-1}\) is characteristic of the C=O stretching vibrations in the amide group. For form \(\beta\), they are same as those for form \(\alpha\) at 1626 cm\(^{-1}\). The absorption band assigned to the C–H bending vibrations in the aromatic group is centered at 833 cm\(^{-1}\) for form \(\alpha\). For form \(\beta\), it shifts to 821 cm\(^{-1}\). It is considered that the different frequencies on the C–H bending vibrations are attributable to the different conformations of forms \(\alpha\) and \(\beta\) in crystals. It is also interesting to note that the IR spectrum for form \(\beta\) after heating to 120\(^\circ\)C, as shown in Fig. 3c, is practically identical to that of form \(\alpha\) shown in Fig. 3a, for which the phase transition is responsible.

The \(^{13}\)C-NMR spectra were obtained in CDCl\(_3\) solution and in the solid state, and the results are shown in Fig. 4. Analysis of the \(^{13}\)C-NMR spectra in the solution was facilitated by using distortionless enhancement by polarization transfer (DEPT), \(^{13}\)C–\(^{1}\)H correlated spectroscopy (COSY), and \(^{13}\)C–\(^{1}\)H correlated spectroscopy via long-range coupling (COLOC) techniques (data not shown). No peak for any solvent was observed in the \(^1\)H- and \(^{13}\)C-NMR spectra. Figs. 4b and 4c show the spectra of forms \(\alpha\) and \(\beta\), respectively. On the basis of the comparative study of the spectra obtained in
CDCl₃ solution and in the solid state, the principal peaks were assigned as shown in Table 1.

As can be seen from Fig. 4, notable peak broadening was observed, particularly for the signals of the aromatic C-4 and amide C-8. Since these carbons are incorporated in the hydrogen-bonding amino and amide moieties of I, the broadening may be attributable to the effects of the intermolecular hydrogen bonds in the crystals through the amino and carbonyl groups upon the magnetic environment of these carbons.

From comparison of the ¹³C-NMR spectra in solution and in the solid state, further insights into the conformational differences between forms α and β can be obtained. For easier comparison, deviations in the chemical shifts obtained in the solid state from the corresponding values observed in solution are plotted for the two crystalline forms in Fig. 5. It is noted that although the deviations from the solution-phase spectrum are significant for most carbons, practically the same chemical shifts as those in solution were obtained for C-2, C-7, and C-9, which are located near the intramolecular hydrogen bond between the amide N-H and the methoxy oxygen of I, as shown in the X-ray crystallographic study. This clearly indicates that the intramolecular hydrogen bond is not destroyed, even in CDCl₃ solution. In contrast, the chemical shifts of C-4, C-10, C-14, and C-15 depend critically on the crystalline form. Since these carbons are directly involved in the intermolecular hydrogen bonding network or are located nearby in form α, the different δ values observed for form β imply some drastic changes in the hydrogen bonding structure. In this context, it is interesting to note that form β, rather than α, gives chemical shifts which are more close to those observed in solution, as can be seen from Table 1 and Fig. 5. These facts indicate that the hydrogen bonding interactions are either much weaker or absent in form β. For more detailed comparative studies on the crystal Structures of forms α and β, the preparation of a single crystal of form β is currently underway.

Conclusion

Two polymorphs, forms α and β of 4-amino-5-chloro-2-methoxy-N-[(2S,4S)-1-ethyl-2-hydroxymethyl-4-pyrroloidinyl]benzamide (TKS159, I), were prepared and characterized by means of X-ray powder diffractionometry, thermal analysis, IR spectroscopy, ¹³C-NMR spectroscopy, and elemental analysis. From the data presented in this paper, particularly in thermal analysis data, form α is thermodynamically more stable than form β.

Experimental

Material. 4-Amino-5-chloro-2-methoxy-N-[(2S,4S)-1-ethyl-2-hydroxymethyl-4-pyrroloidinyl]benzamide (TKS159, I) was synthesized at Teikoku Chemical Industries Co., Ltd. (Osaka, Japan), according to the procedures reported previously (12).

Preparation of the Polymorphs. Polymorphs of I were prepared in the following manner.

Form α: 3.0 g of I was dissolved in 15 ml of a 1:1 mixture of water and ethanol by heating in a water bath at 85°C for 30 min, then the clear solution obtained was allowed to stand overnight at room temperature. The resulting crystals were collected by filtration, washed with 5 ml of acetone, and dried in vacuo at 50°C.

Form β: 3.0 g of I was dissolved in 15 ml of acetone by heating in a water bath at 60°C for 30 min, then the clear solution obtained was allowed to stand overnight at room temperature. The resulting precipitate was collected by filtration, washed with 5 ml of acetone, and dried in vacuo at 50°C.

X-Ray Powder Diffractionometry. X-Ray powder diffraction patterns were obtained using a Rigaku Geigerflex RAD-2C X-ray powder diffractometer under the following conditions: target, nickel filtered CuKα radiation, λ = 1.5418 Å; voltage, 40 kV; current, 20 mA; receiving slit, 0.15 mm; scanning speed, 4°/min; scanning range, 5°-30°/2θ.

Thermal Analysis. In TG-DTA, samples of 6.0 mg in open aluminum pans were heated at a rate of 5°C/min under a nitrogen purge, with an empty, open aluminum pan as the reference, in a TG-DTA apparatus (Seiko Instruments, model SSC-5000).

IR Spectroscopy. IR spectra in the solid state were measured in KBr.
disks over a range of 4400 to 550 cm⁻¹ on a Perkin-Elmer FT-IR spectrometer model 1650.

\[ ^{13} \text{C-NMR Spectroscopy} \] The \(^{13} \text{C-NMR} \) spectrum in CDCl₃ was recorded on a Bruker AMX-400 spectrometer using tetramethylsilane as an internal standard which was referenced to 0.00 ppm. The CP/MAS (cross polarization–magic angle scanning) \(^{13} \text{C-NMR} \) spectra were recorded on a Bruker MSL-200 instrument using the carbonyl carbon in glycine as an external standard which was referenced to 176.03 ppm.

References