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Preparation of Phenylbutazone Polymorphs and Their Transformation in Solution

NOBUYOSHI KANENIWA,* JUN-ICHI ICHIKAWA and TAKAHIRO MATSUMOTO

School of Pharmaceutical Sciences, Showa University, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142, Japan

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Polymorphism of phenylbutazone was investigated in detail. Pure α form of phenylbutazone could not be obtained by conventional methods, but it was found that the β form was transformed to the stable α form in ethanol solution at 4 °C. At 15 and 25 °C, the β form was transformed to a mixture of the α and δ forms, while at 35 °C, the α form in the mixture was converted to the δ form. These results showed that the preparation of a pure polymorphic form by means of recrystallization in solution is not necessarily straightforward. The dissolution behavior of each form in buffer solution was examined next. The transition temperatures of the pairs of α and δ forms and of α and β forms were 29.0 and 61.6 °C, respectively, in buffer solution as determined by means of the non linear van't Hoff model proposed by Grant *et al.* The heats of fusion of the α, β and δ forms were 8.74, 6.48 and 6.85 kcal/mol, and their melting points were 91.2, 93.3 and 101.4 °C, respectively. These results suggested that the pair of β and δ forms is in a monotropic relation and the pairs of α and β forms and of α and δ forms are in enantiotropic relationships.

Keywords——phenylbutazone; polymorph; recrystallization; transition; solubility; fusion heat; melting point; monotropy; enantiotropy

Introduction

It is believed that at least one-third of organic compounds have two or more kinds of polymorphic forms. Generally, a metastable form and an amorphous form have high dissolution rate. Therefore, they are suitable for clinical use, though they are less stable than a stable form. A stable form, however, is not necessarily more stable than a metastable form at a given temperature, since the relative stability of polymorphic forms in an enantiotropic system depends on the temperature of the system relative to the transition temperature.[1] Thus, it appears to be necessary to characterize the relative stability of polymorphic forms as monotropic or enantiotropic.

First, pure polymorphic forms must be prepared for characterization. Many methods for preparing polymorphic forms are known, and slight differences of preparation conditions often result in the formation of different polymorphic forms or their mixtures, because the existence of slight amounts of other forms may induce transformations by acting as seed crystals.[2]

There have been several reports on the polymorphism of the slightly soluble drug phenylbutazone.[3-10] Three polymorphic forms (α, β, and δ forms) have been prepared by recrystallization from various solvent systems[5-10] and the ε form has been prepared by a spray-drying method.[9] In this study, various methods for the preparation of pure polymorphic forms were examined and the relative stability of the polymorphs was characterized on the basis of solubility data and thermodynamic values in terms of monotropism or enantiotropism.

Experimental

Materials——Phenylbutazone (Lot No. 36607) supplied by Dolder Ltd., Basle, Switzerland, was used. All other chemicals were reagent-grade commercial products.

Preparation of Polymorphic Forms——The δ form was prepared according to the method described by Ibrahim *et al.*[5] Water was added to a 2-propanol solution of phenylbutazone until the cloud point, then the solution was warmed and allowed to cool. The β form was prepared according to the method described by Matsuda *et al.*[10] An acetone solution was added to 10 volumes of distilled water and stirred vigorously at room temperature. We could not obtain the pure α-form by the methods described by Matsuda *et al.*[9] and Müller.[6] However, we succeeded in obtaining it by adding an ethanol solution (1.5%(w/v)) of phenylbutazone to 1.5 volumes of cold distilled water. The mixture was stirred vigorously, and the resulting β form crystals were separated from the mother liquid as soon as possible, then the solution was kept for 144 h at 4°C. The crystals of α form that developed were removed by filtration and then dried under vacuum for 3 h at room temperature.

Identification of Polymorphic Forms——Each polymorphic form was identified by X-ray powder diffraction analysis (Nihon Denshi Co., Ltd., type JDX 7E; target, Cu; filter, Ni; voltage, 30 kV; current, 7 mA; time constant, 2 s; scanning speed, 1°/min), differential scanning calorimetry (DSC) (Shimadzu Seisakusho Co., Ltd., SC-20B; sample weight, 3 mg; sample cell, aluminum crimp cell; N_2 gas flow, 40 ml/min; heating rate, 10 °C/min), thermogravimetry (TG) (Shimadzu Seisakusho Co., Ltd; sample weight, 3 mg; N_2 gas flow, 40 ml/min; heating rate, 10 °C/min) and elemental analysis. The X-ray diffraction profiles (Fig. 1) and the DSC curves (Fig. 2) were identical with those reported previously.[10] The results of the TG curves and elemental analysis (Table I) suggested that the polymorphic forms were not solvated and did not decompose.

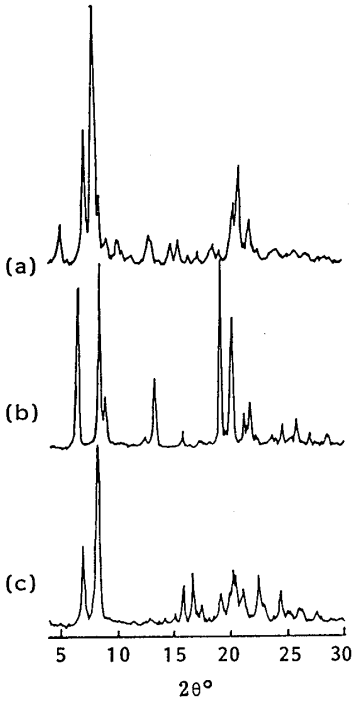

Fig. 1. Powder X-Ray Diffraction Patterns of Phenylbutazone Polymorphs

(a) δ form, (b) α form, (c) β form.

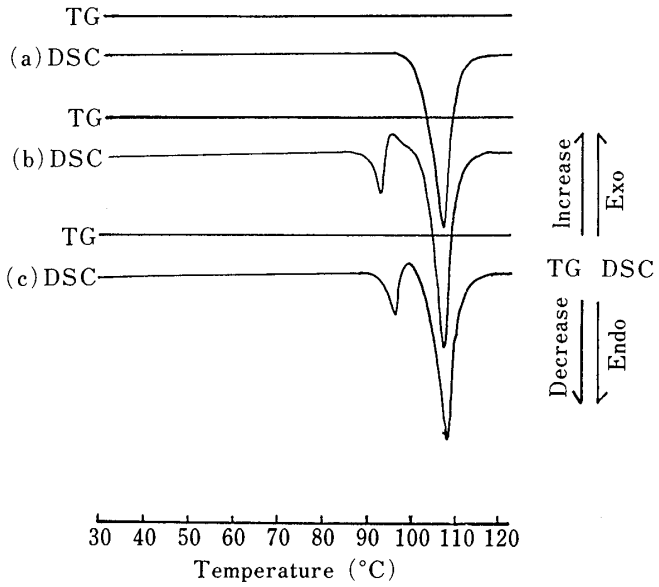

Fig. 2. DSC–TG Curves of Phenylbutazone Polymorphs

(a) δ form, (b) α form, (c) β form.

TABLE I. Elemental Analysis of Phenylbutazone Polymorphs

Sample	Calcd (%)			Found (%)		
	C	N	H	C	N	H
α Form	73.93	6.49	9.08	73.67	6.55	8.94
β Form	73.93	6.49	9.08	73.64	6.57	9.04
δ Form	73.93	6.49	9.08	73.89	6.55	9.06

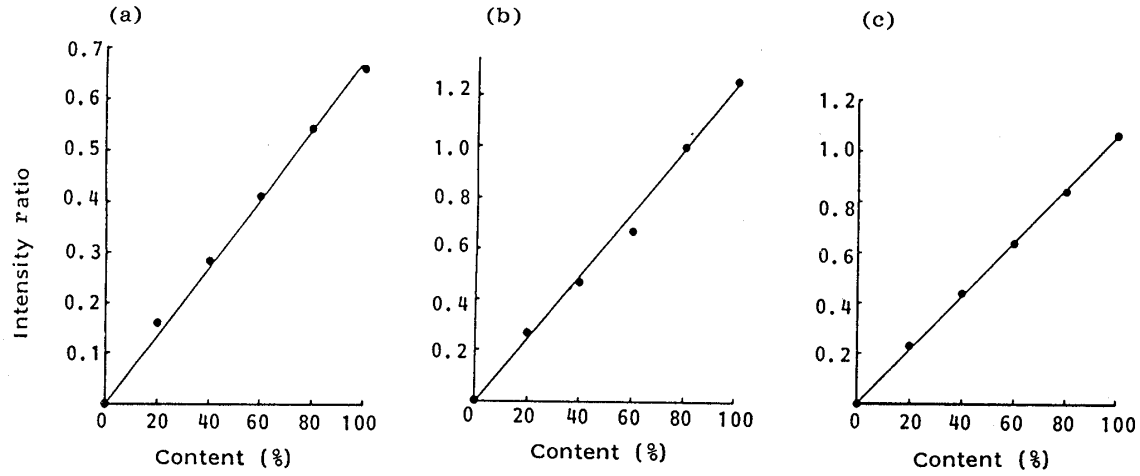

Fig. 3. Relation between Contents of the α, β, and δ Forms and the X-Ray Diffraction Intensity Ratio of Phenylbutazone with Respect to Internal Standard (LiF)

(a) δ form, (b) α form, (c) β form.

Measurement and Confirmation of Transformation of the β Form in Ethanol Solution——An ethanol solution of phenylbutazone (1.5% (w/v)) was added to 1.5 volumes of cold distilled water and the mixture was stirred vigorously, then kept at 4, 15, 25, or 35±0.5 °C. About 50 mg of crystals was collected by filtration at appropriate time intervals, then dried under a vacuum at room temperature. The X-ray diffraction pattern was measured to determine the content of each polymorphic form. Infrared (IR) spectra (Nihon Bunko Co., Ltd., IR-810 spectrophotometer) as a mull in Nujol, optical microphotographs (Olympus Co., Ltd) and a DSC instrument (Shimadzu Seisakusho Co., Ltd., SC-20B; sample weight, 3 mg; sample cell, aluminum crimp cell; N_2 gas flow, 40 ml/min; heating rate, 5 °C/min) were employed to confirm the transformation processes.

Measurement of the Contents of the α, β, and δ Forms in Mixtures——The X-ray diffraction profiles of physical mixtures (α and β forms or β and δ forms) and 20% LiF (internal standard) were measured. The measurements were done under the same conditions as mentioned above. Figure 3 shows plots of the ratio of the peak height at $2\theta = 5.1$, 13.3 and 16.5° due to the δ, α and β forms, respectively, and at $2\theta = 38.7°$ due to LiF *versus* the content of δ, α and β forms. Each plot gave a good straight line, and these calibration plots were used to determine the content of each polymorphic form.

Measurement of Solubility by the Dispersed Amount Method——A sample (3 g) of δ, α, or β form was rapidly placed in 250 ml of buffer solution (JP XI pH 6.8) in a 1000 ml round-bottomed flask (JP XI; Dissolution Test) maintained at 15, 25, 30, 35, 40 or 45±0.5 °C. Aliquots were taken by means of a glass syringe at suitable time intervals and immediately filtered through a 0.45 μm membrane filter (Millipore; HAWPO 01300). The filtrate was suitably diluted for spectrophotometric assay (Hitachi Seisakusho Co., Ltd., type 130) at 264 nm. The solubilities of δ and α forms or β form were regarded as the equilibrium or the maximum concentrations, respectively.

Measurement of Heat of Fusion and Melting Point——Heat of fusion and melting point were determined by using a DSC instrument (Shimadzu Seisakusho Co., Ltd., SC-20B) and corrected based on the measurement of indium as a standard sample. The measurement conditions were as follows: sample weight, 3 mg; sample cell, aluminum crimp cell; N_2 gas flow, 30 ml/min; heating rate, 20 °C/min (δ and β forms) or 40 °C/min (α form).

Results and Discussion

Transformation of the β Form of Phenylbutazone in Ethanol Solution

Reproducible methods, especially recrystallization methods, were required for the preparation of the pure polymorphic forms. The X-ray diffraction profile of form IV reported by Ibrahim *et al.*[5] was different from that of the α form reported by Müller.[6] even though the same recrystallization solvent and a similar recrystallization method were employed. In this study, it was found that the β form was transformed to the α form or the δ form depending on the suspension time and temperature during recrystallization in ethanol solution. Thus, the pure α form was independently prepared as described in the experimental section.

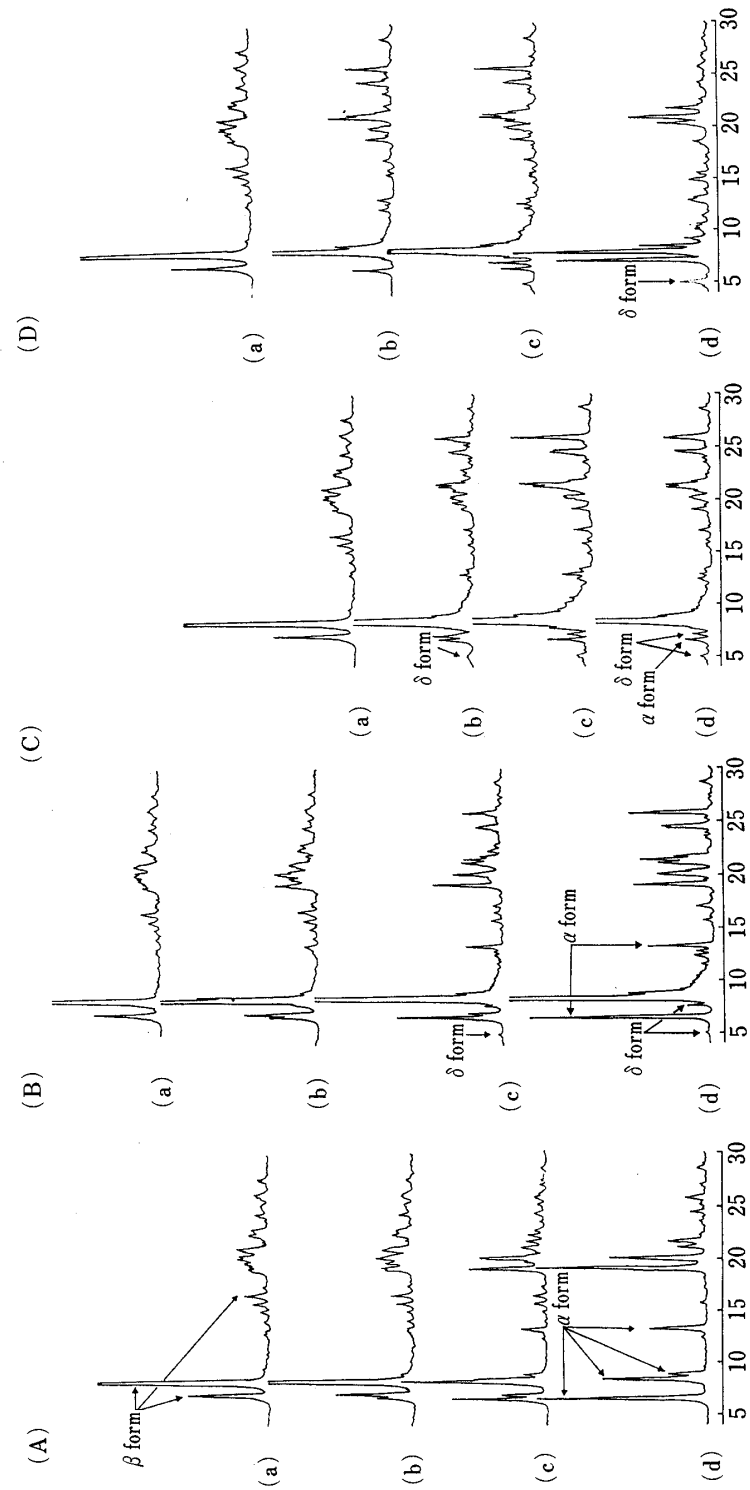

Fig. 4. Change with Time in the X-Ray Diffraction Patterns of the β Form in Ethanol Solution at Each Temperature

(A) 4 °C: (a) 0 h, (b) 24 h, (c) 48 h, (d) 120 h. (B) 15 °C: (a) 0 h, (b) 6 h, (c) 30 h, (d) 96 h. (C) 25 °C: (a) 0 h, (b) 3 h, (c) 24 h, (d) 120 h. (D) 35 °C: (a) 0 h, (b) 24 h, (c) 120 h, (d) 144 h.

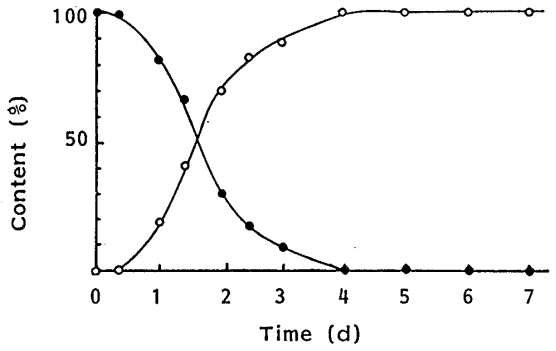

Fig. 5. Transformation of the β Form of Phenylbutazone in Ethanol Solution at 4 °C

○, α form; ●, β form.

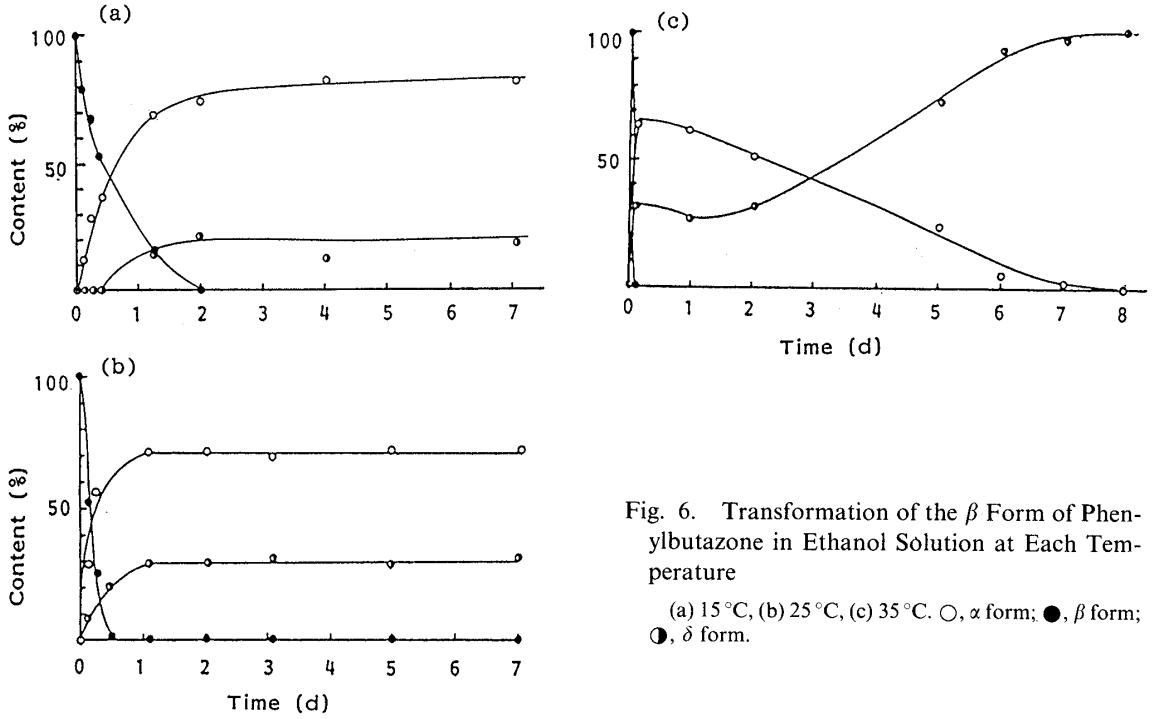

Fig. 6. Transformation of the β Form of Phenylbutazone in Ethanol Solution at Each Temperature

(a) 15 °C, (b) 25 °C, (c) 35 °C. ○, α form; ●, β form; ◑, δ form.

Figure 4 shows the changes of X-ray diffraction profiles of the β form at various suspension temperatures in ethanol solution, and the transformation processes determined by using the X-ray diffraction internal standard method are shown in Figs. 5 and 6.

In the suspension at 4 °C, the intensities of the peaks at $2\theta = 7.1$, 8.4, and 16.5° attributable to the β form decreased and simultaneously those of the peaks at $2\theta = 6.7$, 8.6, 9.0, and 13.3° attributable to the α form increased with suspension time. The X-ray diffraction pattern after 120 h was that of the α form. In the suspension at 15 and 25 °C, though the peaks attributable to the β form disappeared and those attributable to the α form appeared in the same way as at 4 °C, the peak at 5.1° attributable to the δ form also appeared after 30 h at 15 °C, or 3 h at 25 °C, as shown in Figs. 4B and 4C and Figs. 6a and 6b, though the peaks attributable to the α and δ forms overlapped. Furthermore, in the suspension at 35 °C, the peaks attributable to the α form disappeared after 144 h and those attributable to the δ form appeared. These results suggested that the β form was transformed to the α form when suspended in ethanol solution at 4 °C and to a mixture of α and δ forms at 15 and 25 °C, while at 35 °C, the α form was finally transformed to the δ form.

Figure 7 shows the changes in the IR spectrum of the β form in the suspension at various temperatures. There has been no detailed discussion about IR spectra in previous reports. In

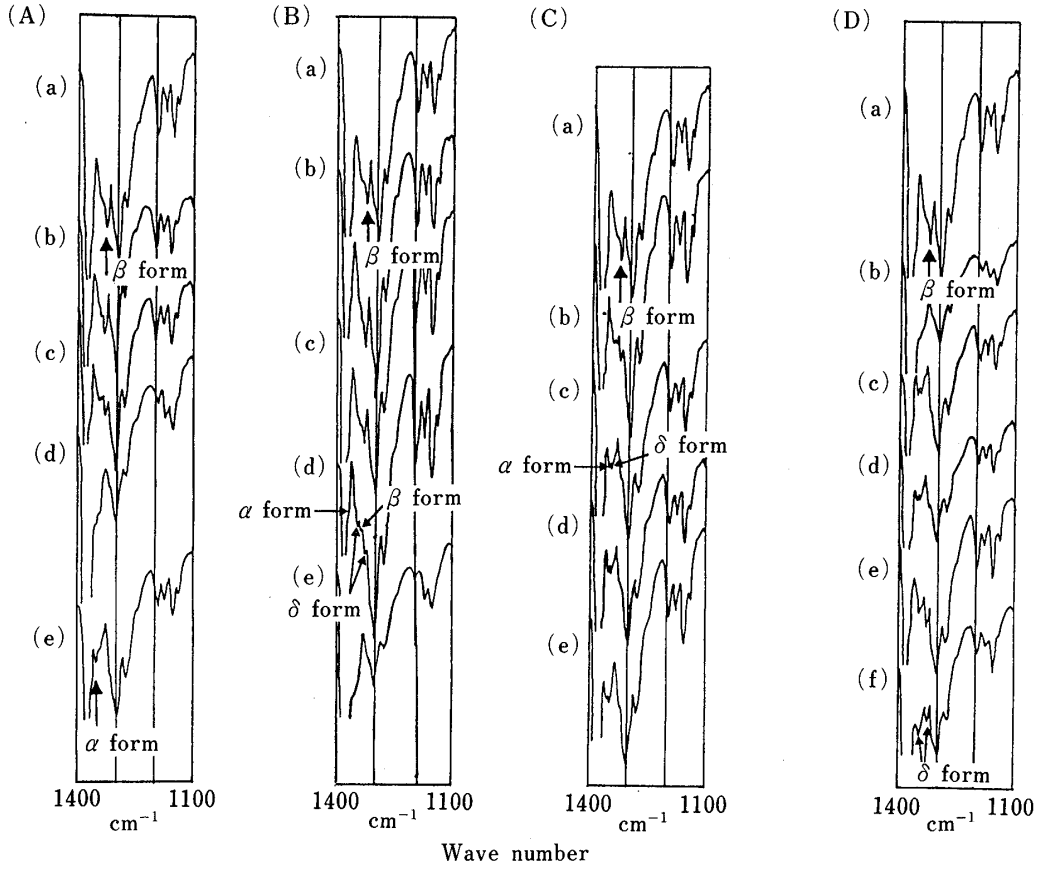

Fig. 7. Changes with Time in the IR Spectrum of the β Form in Ethanol Solution at Various Temperatures

(A) 4 °C: (a) 0 h, (b) 24 h, (c) 48 h, (d) 72 h, (e) 120 h. (B) 15 °C: (a) 0 h, (b) 6 h, (c) 9 h, (d) 30 h, (e) 96 h. (C) 25 °C: (a) 0 h, (b) 3 h, (c) 24 h, (d) 72 h, (e) 120 h. (D) 35 °C: (a) 0 h, (b) 3 h, (c) 24 h, (d) 48 h, (e) 120 h, (f) 144 h.

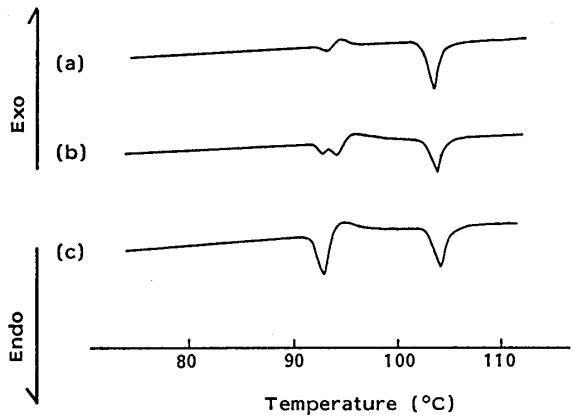

Fig. 8. Changes with Time in the DSC Curve of the β Form in Ethanol Solution at 4 °C

(a) 0 h, (b) 26 h, (c) 96 h.

this study, slight differences among polymorphic forms were observed in the region from 1300 to 1400 cm^{-1} and thus we attempted to use this region to confirm the transformation processes of the β form in ethanol solution, though assignment of the absorptions was difficult.

In the suspension at 4 °C, the peak at 1325 cm^{-1} attributable to the β form disappeared and a new peak appeared at 1355 cm^{-1}, attributable to the α form (Fig. 7A). At 15 °C, the peaks attributable to the β and α forms and those at 1320 and 1350 cm^{-1} attributable to the δ

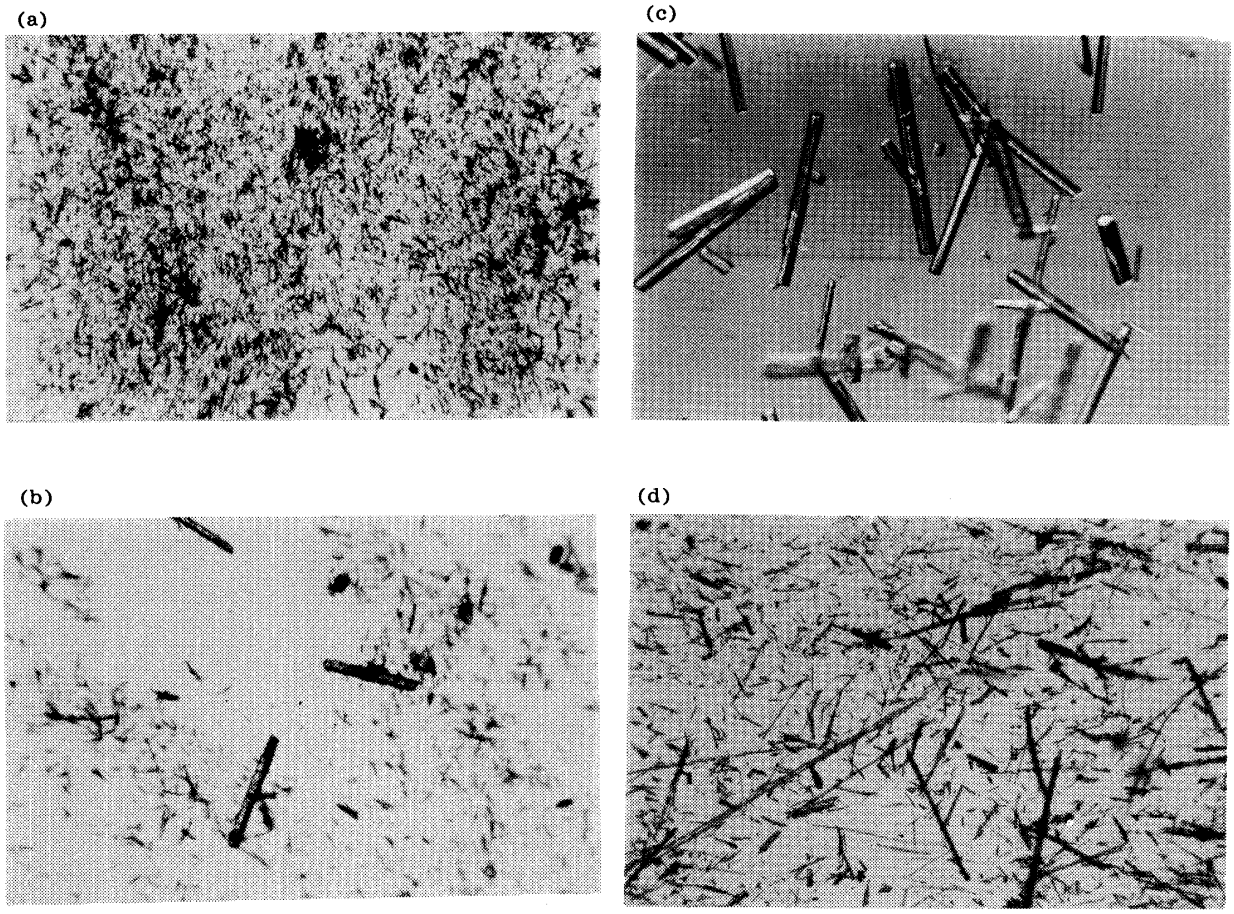

Fig. 9. Changes with Time in Optical Microphotographs of the β Form in Ethanol Solution at 4 °C

(a) 0 h, (b) 24 h, (c) 96 h, (d) δ form.

form overlapped after 30 h (Fig. 7B) and the peak attributable to the β form disappeared after 96 h. At 25°C, the peak attributable to the β form disappeared after 24 h and the peaks attributable to the α and δ forms overlapped (Fig. 7C). At 35 °C, only peaks attributable to the δ form were seen after 144 h, since the α form was transformed completely to the δ form.

Figure 8 shows the changes in thermal behavior of the β form in suspension at 4 °C. The DSC curves of the β form showed an endothermic peak at about 94 °C attributable to melting transition, an exothermic peak at about 95 °C attributable to crystallization to the δ form and an endothermic peak at 101°C attributable to fusion of the δ form. The DSC curve of the β form after 26 h showed two endothermic peaks at about 92 and 94 °C attributable to melting transitions of the α and β forms, respectively, while the DSC curve of the β form after 96 h was identical with that of the α form.

The optical microphotographs in Fig. 9 show changes in the appearance of the β form in suspension at 4°C. Needle crystals of the β form decreased with time and columnar crystals of the α form increased. These results were consistent with those of X-ray diffraction analysis and IR spectroscopy.

These results indicate that pure β form was transformed completely to the α form during recrystallization at 4 °C in ethanol solution and so it is necessary to separate the crystals from the mother liquid as soon as possible in order to obtain pure β form, whereas suspension for 144 h at 4°C was necessary in order to obtain pure α form.

The transformation mechanism in suspension appears to involve dissolution of the

soluble form and crystallization of the less soluble form from the solution phase.[11] These phenomena are complicated because the system is a three-component one, but differences in solubility among the polymorphic forms do seem to be important. It was considered that in suspension at below 25 °C, the more soluble β form will dissolve and the less soluble α or δ form will crystallize, while at 35 °C the crystallized α form will dissolve, and the less soluble δ form will crystallize out. To test this hypothesis, solubility measurements were made in buffer solution.

Measurement of Solubility

Figure 10 shows the dissolution curves of α, β and δ forms in the temperature range of

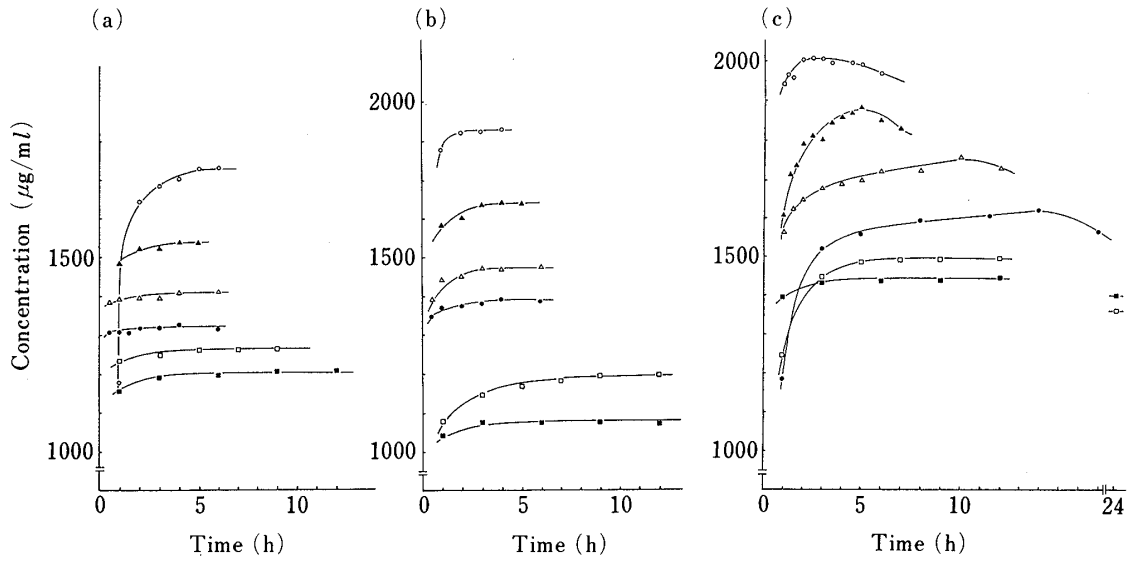

Fig. 10. Dissolution Curves for Phenylbutazone Polymorphs in Buffer Solution at pH 6.8 at Various Temperatures

(a) δ form, (b) α form, (c) β form.
■, 15 °C; □, 25 °C; ●, 30 °C; △, 35 °C; ▲, 40 °C; ○, 45 °C.

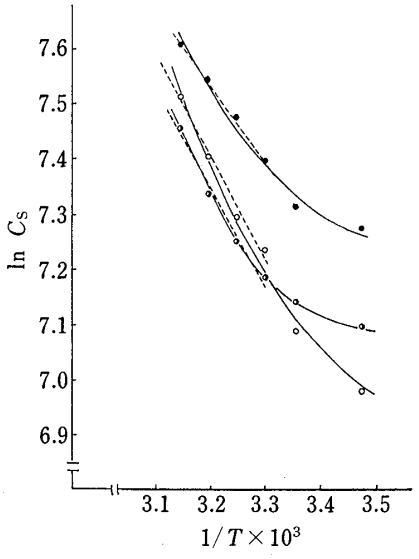

Fig. 11. The van't Hoff Plots of α, β and δ Forms of Phenylbutazone Polymorphs in Buffer Solution at pH 6.8

○, α form; ●, β form; ◑, δ form.

TABLE II. Solubilities of the δ, α, and β Forms of Phenylbutazone Polymorphs in Buffer Solution at pH 6.8

Temperature (°C)	Solubility (μg/ml)		
	δ Form	α Form	β Form
45	1727	1828	2008
40	1533	1639	1886
35	1410	1471	1762
30	1318	1386	1629
25	1264	1196	1497
15	1206	1072	1448

TABLE III. Thermodynamic Values of Phenylbutazone Polymorphs Derived from Classical Van't Hoff Plots

Sample	Heat of solution ΔH_{sol} (kcal/mol)	Heat of transition ΔH_t (kcal/mol)	Transition temperature (°C)
δ Form	3.43	0.17	−12.8
α Form	3.60	0.93	70.2
β Form	2.67		

TABLE IV. Result of Least-Squares Fitting of the Solubility Data in Table II to the Model of Grant *et al.*[12]

Sample	Calculated value			
	a	b	c	Transition temperature (°C)
δ Form	−45481.44	157.38	−520.915	29.0
α Form	−39148.67	140.30	−461.286	61.6
β Form	−39259.15	136.76	−451.093	

15—45 °C. The concentration of β form reached a maximum and then decreased gradually. It was confirmed by X-ray diffraction analysis that the β form was partially transformed to the δ form, whereas transformation of the α or δ form could not be observed. Thus, the solubilities of α and δ forms were estimated as the equilibrium concentrations and the solubility of β form as the maximum concentration. These results are listed in Table II. The solubility data are plotted in Fig. 11. The transition temperatures were estimated by extrapolation of the linear regions at 30—45 °C as shown by the broken lines in Fig. 11, according to the classical van't Hoff model, and the heats of transition were estimated from the differences in slope between these three straight lines. The results are shown in Table III. On the other hand, the model proposed by Grant *et al.*[12] to take account of the change in heat of solution was used to fit the data in the temperature range of 15—45 °C. The curves obtained by multi regression analysis are shown by the solid lines in Fig. 11 and the fitting parameters are listed in Table IV. The transition temperature of the pair of α and δ forms estimated by the classical van't Hoff model was −12.8 °C, whereas that estimated by the nonlinear van't Hoff model of Grant *et al.*[12] was 29.0 °C. It is evident that the transition temperature of the α and δ forms lies between 25 and 30 °C judging from actual solubility measurements, as shown in Fig. 11. The transformation process of the β form in ethanol solution can be discussed in the light of the results of these solubility experiments. Considering that the crystallized α form was transformed to the δ form at 35 °C, but remained stable at 4 °C in ethanol solution, the transition temperature from the α form to the δ form in ethanol solution appears to lie in the neighborhood of that in buffer solution. However, recrystallization from the β form to the α form was observed in ethanol solution, while the β form was transformed to the δ form in buffer solution. The crystallization of the α form in ethanol solution may be ascribed to the specific nature of the solvent ethanol. At 15 and 25 °C in ethanol solution, the presence of both α and δ forms can be ascribed to the small differences in solubilities between the α and δ forms.

Measurement of Heat of Fusion and Melting Point

The effects of heating rate on the thermal behavior of phenylbutazone polymorphs have been reported by Müller[6] and Tuladhar *et al.*[7] The heat of fusion could be calculated only when the endothermic peak attributable to melting of the original crystals could be observed

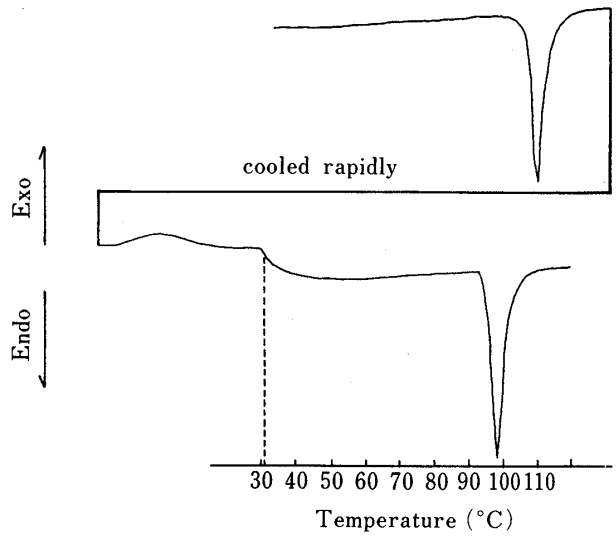

Fig. 12. The Recycling System on the DSC Instrument

Commercial phenylbutazone was melted (melting point 103 °C) and rapidly cooled, and then heated under isothermal conditions. After complete crystallization, the sample was heated at 20 °C/min.

TABLE V. Values of Heat of Fusion and Melting Point of Phenylbutazone Polymorphs

Sample	Heat of fusion (kcal/mol)	Melting point (°C)
δ Form	6.85	101.4
α Form	8.74	91.2
β Form	6.48	93.3

in the DSC curves. However, the DSC curves of α or β form included liquid-phase transition to the δ form as shown in Fig. 2 or 8, and the first endothermic peak area attributable to melting of the α or β form increased while that of the δ form decreased with increasing heating rate. The DSC curve of the α form shows only the endothermic peak attributable to melting of the α form at the heating rate of 40 °C/min, since the δ form had no time to crystallize, while that of the β form did not appear at the same heating rate, though the endothermic peak area attributable to the β form increased. Therefore, another method for the preparation of the β form was used in order to obtain only the endothermic peak attributable to melting of the β form. Müller[6] and Nagai *et al.*[4] have reported previously that the pure β form could be prepared by crystallizing the melt of phenylbutazone. Commercial phenylbutazone (δ form) was melted, rapidly cooled and then heated isothermally, and after complete crystallization the sample was heated at 20 °C/min. The resulting DSC curves are shown in Fig. 12. When heating was stopped at 70°C in the above system, the sample was identified as β form from the X-ray diffraction pattern and IR spectrum.

On the other hand, the DSC curves of the δ form showed a single endothermic peak attributable to melting at the heating rate of 5—40 °C/min. The heats of fusion and the melting points determined from the single endothermic peak attributable to melting of each polymorphic form in the DSC curve are summarized in Table V.

Burger and Ramberger[1] proposed the heat of fusion rule that if the higher melting form has the lower heat of fusion, the two forms are usually enantiotropic, and otherwise they are monotropic. Müller[6] suggested that the pairs of α and β forms and of α and δ forms were enantiotropic on the basis of vapor pressure curves, but it was not clear whether the β and δ forms were monotropic or not. Based on the above rule, the solubility data suggested that the pairs of α and β forms and of α and δ forms are enantiotropic (the transition temperatures of these pairs are 61.6 and 29.0 °C), while the pair of β and δ forms is probably monotropic, though the possibility that the melting and crystallization occurred simultaneously cannot be ruled out.

Conclusion

The complex polymorphic transformations of phenylbutazone in ethanol suspension were characterized in terms of solubility data and thermodynamic values. Care seems to be necessary in the preparation of pure polymorphic forms by recrystallization, especially when the transition temperature is in the region of room temperature.

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