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Studies on the Relationship between Physico-chemical Properties and Crystalline Forms of Tulobuterol Hydrochloride. I. Polymorphism of Tulobuterol Hydrochloride[1]

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The existence of four crystalline forms (forms I, II and III, and a hydrate) and an amorphous form of tulobuterol hydrochloride was confirmed by X-ray powder diffraction, infrared spectroscopy and thermal analyses (DSC and TG). The hydrate was found to be the monohydrate by elemental analysis and measurement of water content.

From the DSC measurement, it was found that forms I and II melted at 163°C and 170°C, and their heats of fusion were 5.15 kcal/mol and 4.76 kcal/mol, respectively. Form III, the amorphous form and the hydrate transformed into form II at 135°C, 90°C and 75°C, respectively. Activation energy for the dehydration of the hydrate determined by Kissinger's method was 56.1 kcal/mol.

No crystal changes were observed in the four crystalline forms when they were ground in a mortar or compressed at high pressure; however, after such mechanical treatments form I transformed into form II on being heated. The investigation of phase transitions of the four crystals showed that form II was the most stable among them.

Keywords——tulobuterol hydrochloride; polymorphism; X-ray powder diffraction; IR spectra; thermal analysis; thermodynamic properties; polymorphic transition by heating and mechanical treatment

A number of studies on polymorphism of various drugs have been presented in the literature.[2] Different polymorphs are known to possess different physicochemical properties such as solubility, dissolution rate, density, crystal shape, melting point and stability. Accordingly, the preparation of polymorphs of relatively insoluble drugs can often improve the bioavailability.[3]

Tulobuterol hydrochloride, α-[(*tert*-butylamino)methyl]-*o*-chlorobenzylalcohol hydrochloride (C-78), was synthesized by Koshinaka *et al.*[4] as a derivative of phenetylamine, and was reported to be an excellent bronchodilator with a prolonged and selective adrenergic β_2-stimulative action.[5] The metabolism in man has been discussed recently.[6] However, the relationship between the physicochemical properties and crystalline form of C-78 is little known.

In the present study, the crystalline forms of C-78 were investigated and four crystalline forms (forms I, II and III, and the hydrate) were confirmed. This paper also describes preparative methods for C-78 polymorphs and reports their thermodynamic properties. Furthermore, polymorphic transformations caused by heating and by mechanical treatments such as grinding and compressing were investigated by X-ray powder diffraction analysis, infrared (IR) spectrophotometry and thermal analysis with simultaneous measurements by differential scanning calorimetry (DSC) and thermogravimetry (TG).

Experimental

Materials——C-78 was prepared by the method reported by Koshinaka *et al.*[4] This sample is odorless, bitter-tasting and highly soluble in water.

Preparative Methods for Polymorphs——Form I: C-78 (20 g) was dissolved in hot isopropanol (100 ml) and allowed to recrystallize at room temperature. The rod-like crystals (Fig. 1,a) were collected by filtration, and dried at 60°C *in vacuo*. Form I also could be prepared from acetone, methanol, ethanol, *n*-propanol, chloroform, benzene and ethyl acetate in the same manner.

Form II: C-78 (20 g) was dissolved in hot isopropanol (100 ml) or acetone (100 ml), then hot isopropyl ether (400 ml) was added, and the mixture was allowed to cool to room temperature. The thin plate crystals (Fig. 1,b) obtained by seeding form II were collected by filtration, and dried at 60°C *in vacuo*. Form II was also obtained by spray-drying of aqueous solutions containing about 10% C-78. The spray-drying was carried out with a Niro Production Minor spray dryer equipped with a two-fluid nozzle assembly (inlet temperature, 350 ± 10°C; outlet temperature, 125 ± 5°C; feed rate, 300 ml/min).

Form III: C-78 (20 g) was dissolved in hot isopropanol (100 ml). The resultant solution was cooled to room temperature and added to isopropyl ether (1000 ml) with stirring. The minute thin plate crystals (Fig. 1,c), resembling those of form II, were collected by filtration and dried at 60°C *in vacuo*.

Amorphous Form: The amorphous form was obtained from aqueous solution by freeze-drying[7] and was stored in a P_2O_5 desiccator because of its marked hygroscopicity.

Hydrate: C-78 (20 g) was dissolved in hot water or 50% hot alcohol (10 ml), such as methanol, ethanol and isopropanol, and the solution was allowed to cool to room temperature. The large prismatic crystals (Fig. 1,e) thus obtained were collected by filtration and dried at room temperature. As mentioned below, this crystal was a monohydrate.

Characterization of Polymorphic Forms——Polymorphs of C-78 were identified by X-ray powder diffraction, IR spectroscopy and simultaneous measurements of DSC and TG. The instrument used for X-ray diffraction analysis was a Rigaku Geigerflex 2013 X-ray diffractometer (Ni filter, Cu-K_α radiation = 1.5418 Å). IR spectra were obtained in Nujol with a JASCO IRA-2 grating infrared spectrophotometer. DSC and TG were carried out on a Rigaku Thermoflex CN 8085-EI thermobalance and differential scanning calorimeter. Temperature corrections were made by the use of indium. N_2 gas and α-Al_2O_3 were used as a carrier gas and a reference material for all measurements, respectively. For the hydrate, the same amount of α-Al_2O_3 powder was blended to prevent the sample from dissolving in water produced during the dehydration.

The observation of crystal shape was carried out with a polarizing microscope.[8]

Determination of Thermodynamic Properties by DSC——The apparatus was the same as the one used for characterization:

(1) Measurements of Transition Temperature: Sample, form III, the amorphous form and the hydrate; sample weight, 3—13 mg; sample pan, solid aluminium pan; sensitivity, 4 mcal/s; heating rate, 0.625°C to 20°C/min; chart speed, 2.5 to 20 mm/min.

(2) Measurements of Heats of Fusion and Transition: Sample, five different forms of C-78; sample weight, 5—10 mg; sensitivity, 4 mcal/sec; heating rate, 5°C/min; chart speed, 5 to 40 mm/min. Areas under the fusion and transition peaks were measured by weighing the peak areas cut from photocopies of the thermographs.

(3) Measurement of the Activation Energy for Dehydration of the Hydrate: Sample weight, 3—13 mg; sensitivity, 4 mcal/sec; heating rate, 0.625°C to 20°C/min; chart speed, 2.5 to 20 mm/min. The activation energy was estimated by Kissinger's method[9] using the following equation:

$$-E = R(\ln\phi/Tm^2)/(1/T)$$

where E is the activation energy, Tm is the peak temperature of the DSC curve, ϕ is the heating rate and R is the gas constant.

Grinding and Compressing of Powder Samples——The grinding was done in a porcelain mortar and the compressing was done at 300 kg/cm² for 10 min using a JASCO hydraulic press for KBr tablets for IR spectroscopy.

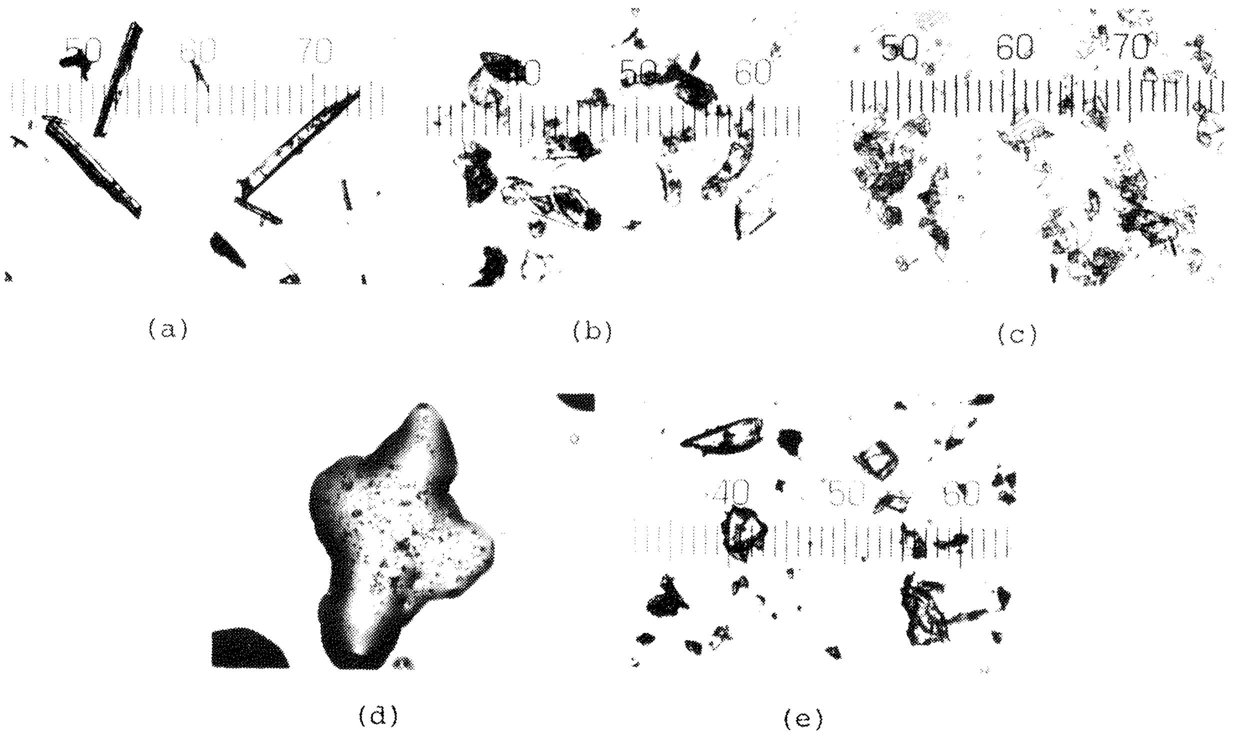
Results and Discussion

Characterization of Polymorphic Forms of Tulobuterol Hydrochloride

Photomicrographs of the five polymorphic forms are shown in Fig. 1. Form I was distinguished from the other forms by its characteristic rod-like crystal shape. With the other three crystal forms, a little difference in crystal habit was observed, as described in the experimental section and in Fig. 1. The amorphous form, when removed from the desiccator, deliquesced immediately due to its strong hygroscopicity and fine crystals appeared from the resultant solution.

The X-ray powder diffraction patterns and the IR spectra of the five different forms of C-78 are depicted in Figs. 2 and 3, respectively. The differences observed in the X-ray patterns and IR spectra are sufficiently distinct to characterize the various crystalline modifications.

The thermal behavior was determined by simultaneous measurements of DSC and TG. The solid and the dotted curves in Fig. 4 are DSC and TG curves, respectively. In the DSC



(a) (b) (c)

(d) (e)

Fig. 1. Photomicrographs of Polymorphs of Tulobuterol Hydrochloride

(a) form I (27.8 μm), (b) form II (27.8 μm), (c) form III (11.1 μm), (d) amorphous form, (e) hydrate (11.1 μm).
The value in parenthesis is the unit scale of measure in each case.

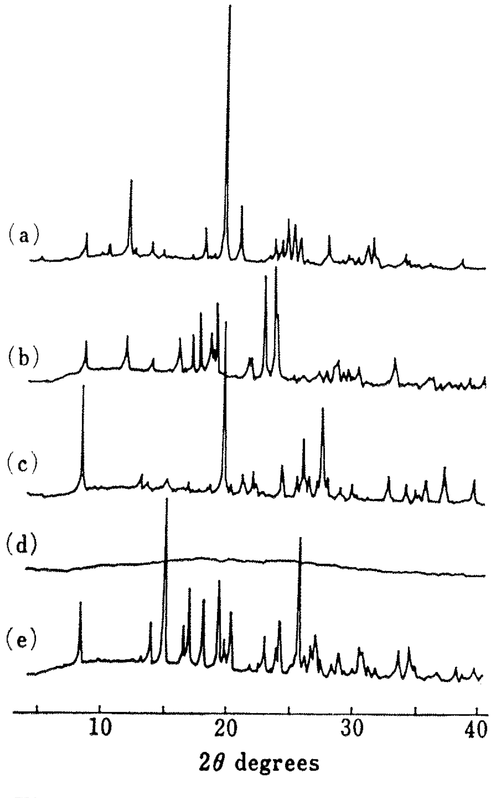


Fig. 2. X-ray Powder Diffraction Patterns of Polymorphs of Tulobuterol Hydrochloride

(a) form I, (b) form II, (c) form III (d) amorphous form, (e) hydrate.

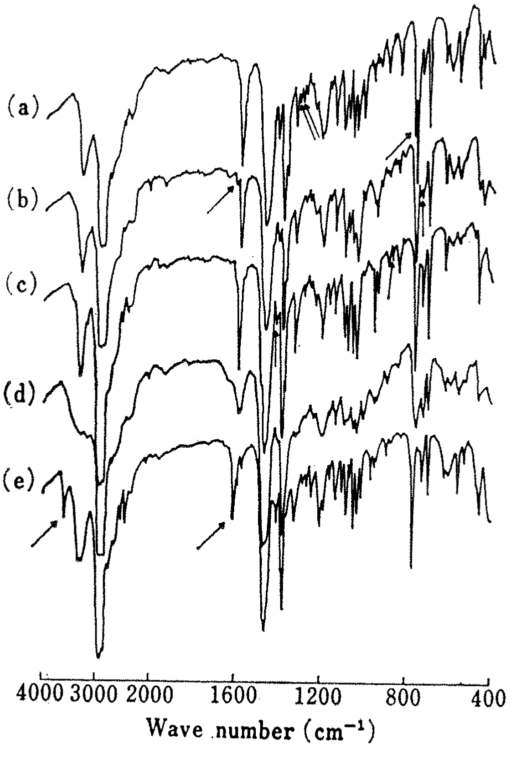


Fig. 3. Infrared Spectra of Polymorphs of Tulobuterol Hydrochloride in Nujol

(a) form I, (b) form II, (c) form III, (d) amorphous form, (e) hydrate.
Arrows indicate the characteristic peaks.

curves, forms I and II showed only one endothermic peak corresponding to the melting point at 163°C and 170°C, respectively, but form III gave a characteristic thermogram with two endothermic peaks at 149°C and 169°C, and an exothermic peak at 151°C. The endothermic peak at 149°C and the exothermic one at 151°C are attributable to the transformation from form III to II. The endothermic peak at 169°C corresponded to the melting point of form II. When treated on a hot plate, form III melted at about 148°C, yielding a transparent paste, then it solidified immediately. The resultant solid melted again at about 168°C. This observation suggested that the exothermic peak at 151°C might be attributable to the crystallization of form II from the melt of form III.

The DSC curve of the amorphous form showed an exothermic peak at 104°C and an endothermic one at 170°C. The peak at lower temperature corresponded to the crystallization into form II and the other peak to the melting point of form II.

In the TG curves of forms I, II and III, and the amorphous form, no change in weight was observed. These results indicated that the changes in the DSC curves were not due to decomposition or desolvation.

The elemental analysis data of forms I, II and III coincided well with the theoretical values, as shown in Table I. In contrast, a deviation from the theoretical values was found for the amorphous form because of its hygroscopic nature.

The DSC curve of the hydrate showed two endothermic peaks in the temperature range of 50—80°C and at 170°C, and an exothermic peak at about 93°C, suggesting that the former and the latter endothermic peaks corresponded to the dehydration of water and the melting point of form II, respectively, and that the small exothermic peak at 93°C represented the transformation of the anhydrate formed by dehydration into form II. In the TG curve, the weight loss due to dehydration commenced at about 50°C and the weight loss observed was approximately equal to the water content in Table I within the limits of experimental error. The results of elemental analysis and measurement of water contents by the Karl Fisher method, as shown in Table I, coincided closely with the theoretical values of the monohydrate.

The chemical identities of five different forms of C-78 were examined by thin layer chromatography (TLC), ultraviolet (UV) absorption measurement and nuclear magnetic resonance (NMR) spectroscopy. All of the *Rf* values on chromatograms(10) coincided with that of the standard sample and no foreign spot was detected. The UV(11) and NMR(12) spectra of the five forms were also the same. These findings suggested that polymorphic forms of C-78 had the same chemical nature in solution.

Thermodynamic Properties of Tulobuterol Hydrochloride Transition Temperature estimated by DSC——As shown in Fig. 4, it was found that form III, the amorphous form and the hydrate transformed into form II on heating. In order to determine their transition temperatures, DSC measurements were done at various heating rates. The transition temperatures were determined by plotting the temperature of transition against the heating rate, and extrapolating the curve approximately to the rate of zero, as shown in Fig. 5. Thus, the transition temperatures of form III and the hydrate to form II were estimated to be 135°C and 75°C, respectively. The transition temperature, *i.e.*, the crystallization temperature of the amorphous form to form II was found to be 90°C.

Determination of Heats of Fusion and Transition by DSC——The heats of fusion and transition were determined by measuring the areas of the fusion and transition peaks in the DSC curves. The heats of fusion of forms I and II were 5.15 ± 0.10 kcal/mol and 4.76 ± 0.12 kcal/mol, respectively. In the case of form III, the heats of fusion and transition into form II were approximately 1.78 ± 0.32 kcal/mol and -0.95 ± 0.16 kcal/mol, respectively. Furthermore, the heat of transition (*i.e.* crystallization) of the amorphous form into form II and that of transition of the anhydrate formed by dehydration of the hydrate into form II were determined to be -3.62 ± 0.08 kcal/mol and -0.77 ± 0.01 kcal/mol, respectively. These thermal

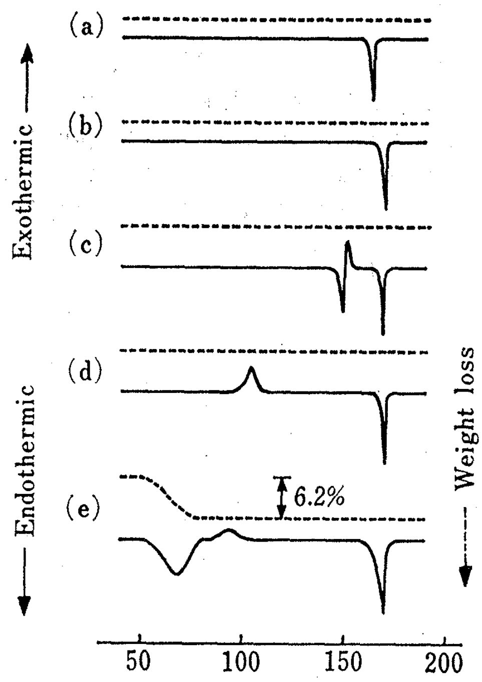


Fig. 4. Thermal Analyses of Polymorphs of Tulobuterol Hydrochloride

——, DSC curves; ----, TG curves. Heating rate: 5°C/min.
(a) form I, (b) form II, (c) form III, (d) amorphous form, (e) hydrate.

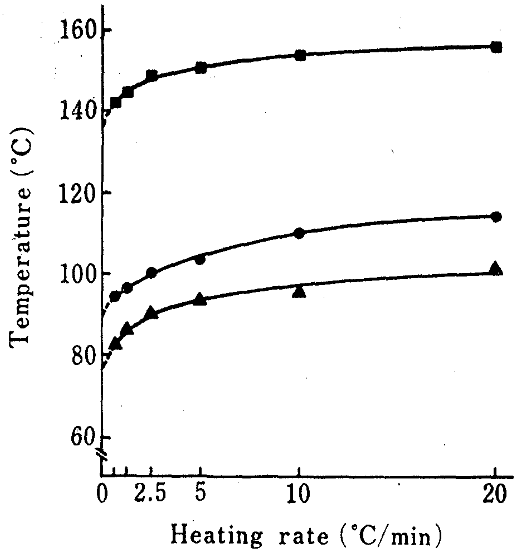


Fig. 5. Effect of Heating Rate in DSC on Apparent Transition Temperature of Polymorphs of Tulobuterol Hydrochloride

Heating rate: 0.625, 1.25, 2.5, 5, 10, 20°C/min.
—■—, form III → form II; —●—, amorphous form → form II; —▲—, hydrate → form II.

Table I. Composition Analyses of Polymorphs of Tulobuterol Hydrochloride

Form	Formula	Elemental analysis (%)						Water content (%)	
		Calcd			Found			Calcd	Found[a]
		C	H	N	C	H	N		
I	$C_{12}H_{18}ClNO \cdot HCl$	54.56	7.25	5.30	54.27	7.42	5.21	0	—
II					54.72	7.39	5.19	0	—
III					54.48	7.35	5.23	0	—
Amorphous					53.41	7.73	5.26	0	—
Hydrate	$C_{12}H_{18}ClNO \cdot HCl \cdot H_2O$	51.07	7.50	4.96	51.02	7.66	4.82	6.38	6.40

[a] Karl Fisher method.

data are listed in Table II.

The heats of fusion of forms II transformed from form III, the amorphous form and the hydrate, and prepared by spray-drying were 4.10—4.46 kcal/mol, *i.e.*, less than that of form II prepared by recrystallization from isopropanol–isopropyl ether mixture, as shown in Table II. It is considered that the disorder in their crystal lattices due to the rapid heat transformation resulted in the small heat of fusion.

Activation Energy for Dehydration——On heating above 50°C, the hydrate lost weight corresponding to one molecule of water and transformed into form II. The activation energy for the dehydration of the hydrate was determined by Kissinger's method. The reciprocals of dehydration temperatures (Tm) obtained at various heating rates (ϕ°/min) were plotted against log (ϕ/Tm^2) and a linear relationship was obtained (Fig. 6). From the slope, the apparent activation energy was calculated to be 56.1 kcal/mol.

Table II. Thermodynamic Values calculated for Polymorphs of Tulobuterol Hydrochloride by DSC Measurements[a]

Form	Temperature of transition to form II (°C)	Heat of transition to form II (kcal/mol)	Temperature of fusion (°C)	Heat of fusion (kcal/mol)
I	—	—	163	5.15
II	—	—	170	4.76[c] 4.46[d]
III	151(135)[b]	−0.95[f]	149 169[e]	1.78 4.39[e]
Amorphous	104 (90)[b]	−3.62	170[e]	4.36[e]
Hydrate	93 (75)[b]	−0.77	170[e]	4.10[e]

a) DSC measurements were carried out at a heating rate of 5°C/min and a sensitivity of 4 mcal/sec. Data are the means of three experiments.
b) Obtained by extrapolating the curves of Fig. 5 to a heating rate of 0°C/min.
c) Recrystallized from isopropanol–isopropyl ether mixture.
d) Obtained by spray-drying the aqueous solution.
e) Determined from the peaks corresponding to form II transformed from form III, the amorphous form and the hydrate.
f) A negative sign indicates an exothermic reaction.

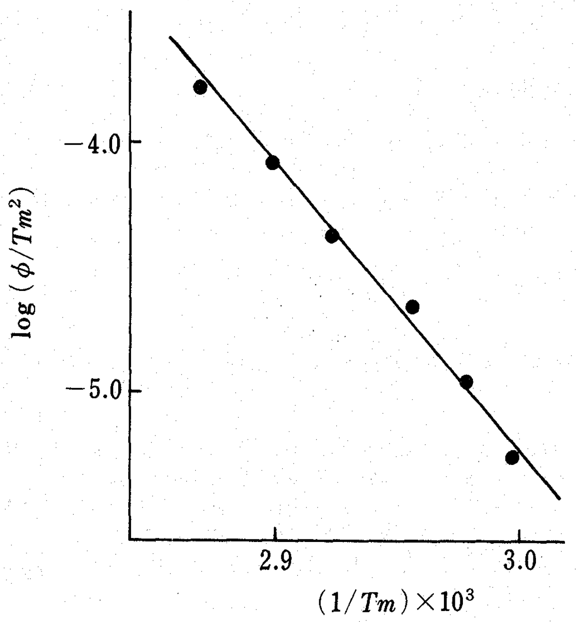


Fig. 6. Kissinger Plot for the Dehydration of the Hydrate of Tulobuterol Hydrochloride

Tm: temperature of peak (°K), ϕ : heating rate (°K/min).

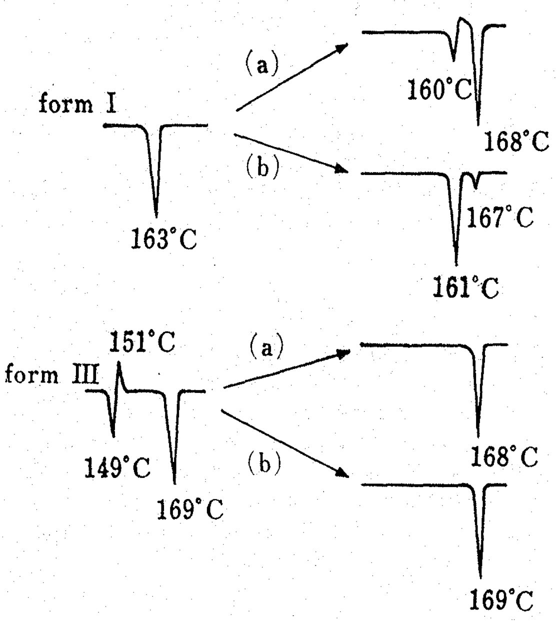


Fig. 7. Transitions of Forms I and III of Tulobuterol Hydrochloride through Grinding (a) and Compressing (b)

Polymorphic Transformation upon Heating and Mechanical Treatment

From the thermal behavior in the DSC curves in Fig. 4, it was found that form III, the amorphous form and the hydrate transformed into form II on heating, but form I did not. The difference of thermal behavior between forms I and III during heating depended on the molecular packing difference in the crystal structures. The X-ray analyses of crystal structures[13] proved that forms II and III were monoclinic, whereas form I was triclinic, and that the crystal density of form III was the smallest among them. Accordingly, the conversion from form III into II by heating was interpreted as a rearrangement into a more compact structure.

It is well known that polymorphic transformation of some drugs occurs during grinding or compressing.[14] Polymorphic transformation of C-78 through grinding in a mortar and compressing at high pressures was therefore investigated. With all crystalline forms, no changes occurred in the X-ray diffraction patterns or IR spectra through such mechanical treatment; however, form I transformed partly into form II upon subsequent heating; this was confirmed by thermal analyses (Fig. 7). Furthermore, the peaks at 149°C and 151°C on the DSC curve of form III disappeared and only one endothermic peak corresponding to the melting point of form II was observed. This finding indicated that the heat transition of form III into form II occurred more easily after grinding or compressing. These results might be attributable to strain or defects of the lattice produced by mechanical treatment.

A summary of the transformations among polymorphic forms of C-78 is given in Fig. 8. In conclusion, it was found that form II was the most stable.

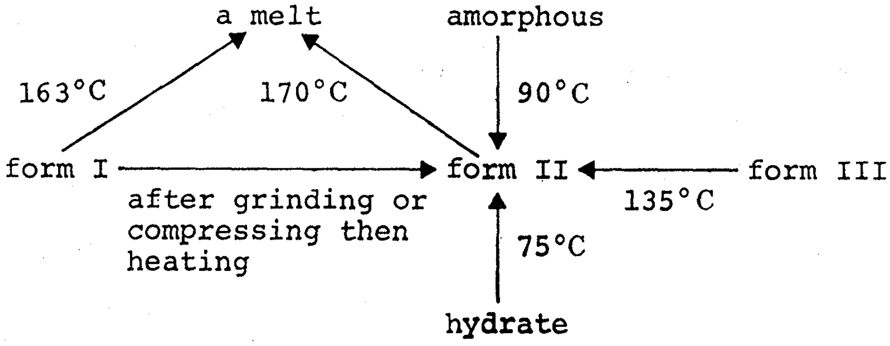


Fig. 8. Interconversion of Polymorphs of Tulobuterol Hydrochloride

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